Journal of Agricultural Technology 2012 Vol. 8(2): 739-750
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Antifungal efficacy of *Capsicum frutescens* L. extracts against some prevalent fungal strains associated with groundnut storage

S.L. Soumya* and Bindu R. Nair

Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram, Kerala, India-695581, India

S.L. Soumya and Bindu R. Nair (2012) Antifungal efficacy of *Capsicum frutescens* L. extracts against some prevalent fungal strains associated with groundnut storage. Journal of Agricultural Technology 8(2): 739-750.

The antifungal potential of aqueous leaf and fruit extracts of *Capsicum frutescens* against four major fungal strains associated with groundnut storage was evaluated. These seed-borne fungi, namely *Aspergillus flavus, A. niger, Penicillium* sp. and *Rhizopus* sp. were isolated by standard agar plate method and identified by macroscopic and microscopic features. The minimum inhibitory concentrations (MIC) and minimum fungicidal concentration (MFC) of *C. frutescens* extracts were determined. MIC values of the fruit extract were lower compared to the leaf extract. At MIC, leaf extract showed strong activity against *A. flavus* (88.06%), while fruit extract against *A. niger* (88.33%) in the well diffusion method. Groundnut seeds treated with *C.frutescens* fruit extract (10mg/ml) showed a higher rate of fungal inhibition. The present results suggest that groundnuts treated with *C. frutescens* fruit extracts are capable of preventing fungal infection to a certain extent.

Key words: antifungal, aqueous extract, *Capsicum frutescens*, minimum inhibitory concentration (MIC), well diffusion, groundnut.

Introduction

Groundnut is the sixth most important oilseed crop in the world. FAO, 2009/2010 estimates showed that unshelled groundnut production accounts for 32.8 million tones, worldwide. Groundnut contains 48- 50 % oil, 26- 28 % protein, and is a rich source of dietary fiber, minerals, fats and vitamins such as B_1, B_2, B_6 and nicotinic acid. According to Sullivan (1984) groundnut seeds are highly susceptible to disease because they have a rich source of stored nutrients useful for numerous fungi such as *Rhizopus, Penicillium, Aspergillus and Fusarium.* Woodroof (1984) observed that groundnut samples removed from their shells were attacked by microorganisms and insects to a greater extent

* Corresponding author: S.L. Soumya; e-mail: sl.soumya@yahoo.com

than the samples intact within their shells. Ibrahim and Ebo (1986) are also of the opinion that if grains are dry harvested; the extent of damage by microorganisms will be less. Therefore it appears that the single most important environmental factor that influences the growth of endogeocarpic mycoflora during drying and curing in pod and kernel is moisture (Mc Donald & Harkness, 1964). Several other environmental factors within the storage facilities also influence the extent to which fungal growth and aflatoxin contamination occurs. Some of these factors are relative humidity and temperature. (Aliyu & Kutama, 2007). Diener & Cole (1982) observed that when the seed moisture exceeds 9% from the equilibrium humidity of 80% and a temperature of 30^0C, chances of invasion by *Aspergillus flavus* increases drastically. Panasenko (1967) reported that even constant relative humidity triggers the growth process. Affected nuts lead to serious economic loss.

Thus it is evident that fungi are significant destroyers of foodstuffs/ grains during storage, retarding their nutritive value. Fungi often produce mycotoxins, making the foodstuffs unfit for human consumption (Janardhana *et al*., 1998; Marin *et al*., 1999). Some mycotoxins target one organ at a time mostly the liver or kidney, where others have broad spectrum activity affecting several organs and tissues. For some mycotoxins, a concentration of many parts–per–million is required to elicit a toxic effect, while for other mycotoxins a concentration as low as one part–per–billion will elicit toxic effect. The main toxic effects are carcinogenicity, teratogenicity, nephrotoxicity, hepatotoxicity, reproductive disorders and immunosuppression (Lacey, 1988; Desjardins *et al*., 2000).

Even though effective and efficient control of seed–borne fungi can be achieved by chemical fungicides, the same cannot be applied to grains for reasons of pesticide toxicity (Ferrer and Cabral, 1991; Harris *et al*., 2001; Dukic *et al*., 2004). To store seeds/ grains for safe human consumption there is a need to search for alternative approaches that are cheaper and more eco-friendly. A National Academy of Sciences, 1986 report of pesticide residues on food indicated that fungicides pose more of a carcinogenic risk than insecticides and herbicides together. Since synthetic fungicides are suspect in our food chain, safer alternatives should be found immediately. Additionally, resistance by pathogens to fungicides has rendered certain fungicides ineffective, creating a need for new ones with alternative modes of action. Present activities to find both natural and synthetic fungicides focus on finding compounds that are safe to humans and the environment. Over the years much effort has been devoted to the search for new antifungal materials from natural sources for food preservation.

The principle aim of the present work is to test the antifungal activity of aqueous *Capsicum frutescens* extracts against important seed–borne fungi of groundnut based on different *in–vitro* screening methods.

Materials and methods

Groundnut Samples: Shelled raw groundnuts purchased from the local markets.

Plant Material

Fresh, disease free leaves and fruits of *Capsicum frutescens* collected from a local home garden.

Isolation and identification of seed-borne fungi

Fungi were isolated from groundnut seeds using the standard agar plate method (I.S.T.A., 2003). Four replications of 20 seeds were placed on Sabouraud's Dextrose Agar (SDA) medium at the rate of 5 seeds per plate without surface sterilization. Another batch of seeds were surface- sterilized by soaking in 0.1% mercuric chloride solution for 2 minutes and washed in three successive changes of sterile distilled water. The seeded plates were incubated at 28^0C for 4 days. Following incubation, each fungal colony that grew on these plates was picked up and transferred onto a clean glass slide for identification. Fungi were macroscopically and microscopically identified on the basis of their typical structure and basic characters as suggested by Barnett (1960) and Melone and Masket (1964). Colony characteristics of fungi such as color and shape were identified using a binocular microscope. Observations were recorded after 24 hours, 48 hours, 72 hours and 4 days, time intervals. Each of these identified fungal strains was streaked separately on SDA slants and sub cultured.

Preparation of crude aqueous extracts of Capsicum frutescens L.

The freshly collected leaves and fruits of *Capsicum frutescens* were dried under shade at normal room temperature for an appropriate time period. The dried plant parts were powdered separately and preserved in air tight containers for further use. About 10 g of the powder was blended with 100 ml distilled water separately. The mixture was heated for a continuous period of 4 hours in a soxhlet apparatus at 40^0C, filtered in a double layer of muslin cloth and reduced to half its original volume by a rotary evaporator. The sample was

completely evaporated in the hot air oven and the solid residue was weighed. The residue obtained was stored in refrigerator for further analysis.

Antifungal activity screening

The efficacy of the extracts to inhibit fungal growth was tested using three methods such as liquid dilution method, well diffusion method and aqueous extract bioassay.

Liquid dilution Method for detection of MIC and MFC

MIC (Minimum inhibitory concentration) is the minimum concentration of the extract required to inhibit/arrest the growth of all microorganisms in the culture and MFC (Minimum fungicidal concentration) is the minimum concentration of the extract required for the complete death of fungal strains present in the culture. Liquid dilution method was used to find out the extract concentration to be used for further analysis (Rios *et al*., 1988).

Indofil was used as reference drug and measured at 620 nm using the spectrophotometer (Beekman Du-40). The test preparations were incubated at 28^0C. Each extract was assayed in triplicate. The MIC of each extract was determined by measuring the absorbance of the preparations at 620 nm with the multi-plate reader (Thermo Labsystems Multiscan Ascent^{TM} with Ascent software) against the corresponding control. The lowest concentration which gave a zero absorbance reading was taken as the MIC of the test extract.

The MFC was determined by adding 50µl aliquot of the preparations which does not show any growth at log phase after incubation during the MIC assays into 200µl of broth. These preparations were incubated at 28^0C for 48 hours. The concentration required to kill each microorganism was regarded as the lowest concentration of extract which did not produce an absorbance at 620 nm.

Well Diffusion Method

Well diffusion method of Khyade *et al* (2009) was used. The medium used was sterile Sabouraud's Dextrose Agar (SDA) medium. With the help of a micropipette, 100µl of extract at each concentration (T) was loaded in one well. The second well was used for the negative control, DMSO (D) and the third for positive control, Indofil (C). Plates were incubated at 37^0 C for 48 hours and the zone of inhibition was measured. The experiment was performed in triplicate and the average diameter of inhibition was calculated. The percentage inhibition was calculated by using the formula;

Percentage of inhibition=Zone of diameter of plant extract in mmZone of diameter of positive drug in mm ×100

Statistical Analysis

Data including the percentage inhibition values of four fungal strains against *C.frutescens* leaf and fruit extracts were subjected to statistical analysis. Three replicates were conducted for each experiment. Analysis was done using SPSS Version 7.5 and the statistical tool applied was analysis of variance (ANOVA) with Duncan's multiple range tests.

Aqueous extract bioassay

Aqueous extract bioassay was used to test the efficacy of the crude extracts on the groundnut seed, (Shafique *et al*., 2007). Groundnut seeds were soaked in 10 mg/ml and 5 mg/ml of leaf and fruit extracts for 24 hours. As a control, the seeds were soaked in distilled water. All the treated seeds were placed on sterile SDA medium and plates incubated at 28^0C. Each treatment was replicated thrice. After 48 hours of incubation, fungal species found growing on the surface of seeds were identified and their percentage inhibition was calculated by applying the formula;

Percentage of inhibition=No. of seeds on which fungus appearsTotal no. of seeds ×100

Results and discussions

Isolation and identification of seed-borne fungi

In the present study, based on the macroscopic and microscopic features, four fungal strains were identified from the groundnut samples such as *Aspergillus flavus, A. niger, Penicillium* sp. and *Rhizopus* sp. *Aspergillus flavus* had markedly higher incidence in the surface sterilized samples than the un-sterilized ones. The four fungal strains were later isolated and cultured on agar slants.

These fungi were listed to be the common seed–borne fungi of groundnut by Emechebe (1981) and Lumpungu *et al.*(1989). *Aspergillus* was noticed to be the predominant fungus in the studied samples. The order of occurrence of fungi in unsterilized samples was *A. niger* >*A. flavus* > *Rhizopus* sp. >*Penicilium* sp. Mukherjee *et al*, (1992) also reported *A. flavus* and *A. niger* to be the predominant storage fungi of groundnut seeds. Christensen (1973) is of the opinion that *Aspergillus*, *Penicillium* and *Rhizopus* were responsible for seed damage during storage and also reduction in germination potential.

Surface sterilization with 1% mercuric chloride significantly reduced the incidence of *A. niger*, *Rhizopus* and *Penicillium*. The results showed that microbial contamination could be eliminated to a certain extent by chlorine disinfestations. However, surface sterilization with 1% mercuric chloride had no effect on *A. flavus*. The presence of such aflatoxigenic fungi in surface-sterilized samples demonstrates that a simple clean up precaution before consumption would never safeguard the consumers from the risk of contamination. Consumers of fresh peanuts (non-processed) are exposed to the risk of high mycotoxin intake. The contamination may also result indirectly from consumption of animal products such as milk from livestock exposed to contaminated feed (Bankole and Adebanjo, 2003).

Biological control processes such as competitive exclusions of toxigenic fungi using different *Aspergillus* mutants are of tremendous utility to the control of aflatoxin accumulation both in pre- and post-harvest seeds (Wilson *et al*., 1986; Cotty and Bayman, 1993). Chemical fungicides are also used to control fungal contamination. However, it is very difficult to implement profitably and accurately such control measures as they are expensive, especially in the case of farmers of the developing world. World Health Organization (WHO) banned many agriculturally important pesticides which are known to cause pollution problems, due to wide range of toxicity against non–target organisms including humans, (Barnard *et al*., 1997). Some of the developing countries are still using these pesticides for agriculturally important products despite the fact that their pre–harvest and post–harvest technologies have resulted in many toxic epidemics. Generally toxic synthetic fungicides are used for improving seed quality and not to prevent bio-deterioration of grains or seeds during storage (Harris *et al*., 2001). Even then, there is an urgent need to search for alternative methods for prevention of bio-deterioration of seeds and grains during storage without any toxicity to the consumer. Under such circumstances, the development of a naturally derived bio-fungicide has great value. Now the need for natural antifungal compounds is at the very high level.

Antifungal Activity Screening

Many species of *Capsicum* are used as herbal medicines for a variety of ailments of probable microbial origin. Harbant *et al*. (2011) showed the *C. annum* extracts at very low concentrations could completely inhibit the growth of *Aspergillus niger*. Mixtures of *C. annum* and *Terminalia chebula* extracts could inhibit the growth of *Aspergillus parasiticus* and *A. flavus* (Gali *et al*., 2010). Iorrizzi *et al*. (2002) and Curtis *et al*. (2004) suggested that among the different plant extracts screened, those from *Allium* and *Capsicum* sp. showed high levels of antimicrobial activity towards plant pathogens. According to

Soetarno *et al*., (1997), *C. frutescens* is known to possess antibacterial and antifungal properties. CAY-1, a novel saponin, from *Capsicum frutescens*, has shown antifungal activities against several fungi. CAY-1 was found to be active against 16 different fungal strains, including *Aspergillus fumigatus*, and acted by disrupting the membrane integrity of fungal cells (De Lucca *et al*., 2006).

Liquid Dilution Method

Aqueous leaf and fruit extracts of *Capsicum frutescens* were tested against four seed-borne fungi to determine their MIC and MFC. The results of the treatments are shown in Table 1.

Table 1. MIC and MFC values of aqueous extracts of *Capsicum frutescens*

Treatment	Fungal strains	MIC values (mg/ml)	MFC values (mg/ml)
Aqueous leaf extract	*Aspergillus flavus*	10	10
	Aspergillus niger	20	20
	Penicillium sp.	5	10
	Rhizopus sp.	5	10
Aqueous fruit extract	*Aspergillus flavus*	5	20
	Aspergillus niger	10	10
	Penicillium sp.	1.25	20
	Rhizopus sp.	5	20
Indofil	*Aspergillus flavus*	0.039	0.078
	Aspergillus niger	0.039	0.156
	Penicillium sp.	0.019	0.039
	Rhizopus sp.	0.019	0.039

n = 3

From the table it can be seen that compared to leaf extract; fruit extract had lower MIC values against all tested fungi. Lowest MIC value of 1.25 mg/ml was observed for *C. frutescens* fruit extract against *Penicillium* sp. The MFC values were higher than the MIC values. MIC and MFC values (extract concentrations) were found to vary according to the fungal strains involved. Liquid dilution method clearly indicated that the MIC values were comparatively lower for the aqueous fruit extract (Table 3).

Indofil was used as the positive control. The study showed that MIC values were very low for the positive control when compared to the plant extracts therefore it appears that very low concentration of chemical fungicide (Indofil) was required for fungal inhibition. However, it is known that these synthetic fungicides are harmful to living organisms even at very low concentrations. Direct consumption and biomagnification serve to affect the

food chain at all levels. On the other hand, the naturally derived fungicides are believed to have low or no side-effects. They are eco-friendly, are easily available and thus have tremendous applications. Exploitation of naturally available chemicals from plants, which retards the reproduction of undesirable microorganisms, would play a more prominent role in the development of future commercial pesticides for crop protection strategies with special reference to the management of plant diseases (Verma & Dubey, 1999; Gottileb *et al.,* 2002).

Well Diffusion Method

The efficacy of the extracts against the fungal strains for well diffusion method is presented in Table 2.

Table 2. Antifungal activity screening of aqueous extracts of *Capsicum frutescens* by well diffusion method

Extracts	Fungal strains	Concentration (mg/ml)	Zone of diameter of positive control (mm)	Zone of diameter of plant extract (mm)	% of inhibition ± SEM
Aqueous leaf	*Aspergillus flavus*	10	25	22	88.1±2.15^{a}
	Aspergillus niger	20	38	30	79.3±5.89^{bc}
	Penicillium sp.	5	16	3	20.5±7.60^{d}
	Rhizopus sp.	5	45	32	69.0±2.60^{c}
Aqueous fruit	*Aspergillus flavus*	5	24	20	79.2±1.71^{a}
	Aspergillus niger	10	36	31	88.3±1.15^{a}
	Penicillium sp.	1.25	5	1	32.9±21.21^{b}
	Rhizopus sp.	5	39	30	77.2±2.87^{a}

Similar superscripts indicate homogenous sets; n =3

The well diffusion assay showed that the leaf extract of *C. frutescens* could inhibit the growth of *A.flavus* to a greater extent at a lower concentration (88% at 10 mg/ml) compared to *A.niger* (79% at 20 mg/ml). However, the fruit extract of *C. frutescens* could inhibit *A.niger* to a greater extent but at a higher concentration (88% at 10 mg/ml) compared to *A.flavus* (79% at 5 mg/ml). From this it could be understood that *C. frutescens* fruit extract was more effective against *A.flavus* and *A.niger* at a comparatively lower concentration.

Effect of aqueous extracts on groundnut seeds

Results of the previous experiments showed that the MIC values ranged from 1.25 mg/ml to 20 mg/ml. Treatment with extracts at low concentration could inhibit the growth of only some fungi while extracts at high concentrations could eliminate all rapidly growing fungi. However, treatment

with high concentration of extracts is not suitable for a food crop. In this context, extracts at concentrations of 5 mg/ml and 10 mg/ml were used for the aqueous extract bioassay.

The efficacy of aqueous extracts against seed–borne fungi on groundnut seeds is presented in Table 3.

Table 3. Effect of aqueous Extracts on groundnut seeds

Extracts	Concentration (mg/ml)	% inhibition of fungal strains
Aqueous leaf	10	16.67±1.37[b]
	5	_
Aqueous fruit	10	83.4±3.46[a]
	5	50±2.15[a]

similar superscripts indicate homogenous sets; ` –` indicates no inhibition; n = 3

Treatment of groundnut seeds in *in-vitro* conditions with aqueous fruit extract (10 mg/ml) inhibited the growth of all fungi (83.4%). Percentage inhibition was found to decrease with a reduction in the extract concentration. The only fungal strain that could resist the treatment was *Aspergillus*. Leaf extract could not inhibit fungal growth to any appreciable degree (16.6%). Thus the present investigation revealed that *C.frutescens* fruit extract was more effective in the inhibition and spore germination of the tested fungi.

The chemical constituents should be determined for a proper assessment of the antifungal activity exhibited. Reports showed that, the chemical constituents responsible for the pungency in Capsicum are capsaicinoids. The capsaicinoids are composed of 12 different compounds, of which, the two major compounds are capsaicin and dihydrocapsaicin (80-95%). Zhang *et al* (2003) found the potential of capsaicin in inhibiting the growth of adult T- cell leukemia cells. There is difference of opinion regarding the antimicrobial properties of capsaicin. Kurita *et al*. (2002) suggested that capsaicin is a major antimicrobial factor. Cichewicz *et al*. (1996) observed that the pure capsaicin and dihydrocapsaicin had no antifungal properties. The antifungal property exhibited by species of *Capsicum* is probably due to certain other compounds. Lectins from *C. annum* and *C. frutescens* are reported to exhibit antifungal and sugar-binding characteristics (Sanatombi *et al*., 2007).

Previous studies conducted in our lab revealed that secondary metabolites like alkaloids, flavonoids, quinones, terpenoids and saponins were concentrated in both leaf and fruit extracts of *C. frutescens*. However, phenols were detected only in the fruit extracts. It has been reported that phenolic compounds have a wide antimicrobial spectrum (Dorman and Deans, 2000; Elgayyar *et al*., 2001). Nychas (1995) indicated that phenolic compounds could denature enzymes

responsible for spore germination or interfere with amino acids involved in germination. Phenols are reported to be fungistatic (Huang and Chung, 2003; Okwu, 2007). The antimicrobial potency of the plant may be attributed to the single or combined effect of any of these bioactive compounds. Further studies are required to identify and isolate the specific compounds responsible for the antifungal activity exhibited by these extracts of *C. frutescens.*

From the results of the study, it may be suggested that, treating groundnuts with *C. frutescens* fruit extracts at least for a period of 24 hours, may prevent the growth of seed–borne fungi. However, cytotoxicity has been associated with *C. frutescens* fruit extract (Anon, 2007). The bioactive compounds if identified and isolated may be used as seed treating biofungicides after a proper toxicological analysis.

Acknowledgement

We acknowledge our sincere gratitude to Professor and Head, Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram for the facilities provided.

Reference

Aliyu, B.S. and Kutama, A.S. (2007). Isolation and identification of fungal flora associated with groundnut in different storage facilities. Science World Journal (2): 34-36.

Anonymous, (2007). Final report on the safety assessment of capsicum annuum extract, capsicum annuum fruit extract, capsicum annuum resin, capsicum annuum fruit powder, capsicum frutescens fruit, capsicum frutescens fruit extract, capsicum frutescens resin, and capsaicin International Journal of Taxicology 26 Suppl 1:3-106.

Bankole, S.S. and A. Adebanjo (2003). Mycotoxins in food in West Africa: Current situation and possibilities of controlling it. African Journal of Biotechnology 2: 254–63.

Barnard, C. Padgitt, M. and Uri, N.D. (1997). Pesticide use and its measurement International Pest Control 39: 161-164.

Barnett, H.L. (1960). Illustrated genera of Imperfect fungi. 2nded. Burgess Publishing Company Minneapolis 15, Minn.

Christensen, C.M. (1973). Loss of viability in storage microflora. Seed Science and Technology 1: 547-562.

Cichewicz, R.H.,Thorpe, P.A. (1996). The antimicrobial properties of chilli peppers (Capsicum species) and their uses in Mayan medicine. Journal of Ethnopharmacology 52: 61-70.

Cotty, P.J., Bayman, P. (1993). Competitive exclusion of a toxigenic strain of *Aspergillus flavus* by an atoxigenic strain. Phytopathology 83:1283 -1287.

Curtis, H., Noll, U., Stormann, J. and Slusarenko, A.J. (2004). Broad spectrum activity of the volatile phytoanticipin allicin in extracts of garlic (*Allium sativum* L.) against plant pathogenic bacteria, fungi and Oomycetes. Physiological and Molecular Plant Pathology 65: 79-89.

De Lucca, A.J., Boue, S., Palmgren, M.S., Maskos, K. and Cleveland, T.E. (2006). Fungicidal properties of two saponins from *Capsicum frutescens* and the relationship of structure. Canadian Journal of Microbiology 52(4), 336-342.

Desjardins, A.E., Manandhar, G., Plattner, R.D., Maragos, C.M., Shrestha, K. and Mc Cormick, S.P. (2000). Occurrence of *Fusarium* species and Mycotoxins in Nepalese Maize and wheat and the effect of traditional processing method on mycotoxins levels. Journal of Agricultural and Food chemistry 48: 1377-1383.

Diener, U.L. and Cole, R.J. (1982). Aflatoxins and other mycotoxins in peanuts. In: Peanut Science and Technology (eds. Pattee & Yoakum, Texas, USA) American Peanuts Research and Education Society, 486- 519.

Dorman, H.J.D. and S.G. Deans (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of Applied Microbiology 88:308-316.

Dukic, N.M., Bozin, B., Sokovic, M. Simin, N. (2004). Antimicrobial and antioxidant activity of *Melissa officinalis*,L. (Lamiaceae) essential oil. Journal of Agricultural Food Chemistry 52: 2485-2489.

Elgayyar, M., F.A. Draughon, D.A. Golden, and J.R. Mount (2001). Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. Journal of Food Protection 64:1019-1024.

Emechebe, A.M. (1981). A checklist of diseases of crops in the Savanna and semi acid areas of Nigeria, Samara Miscellaneous paper No.100, Institute of Agricultural Research, Ahmadu Bello University, Zaria, Nigeria, 3-6.

Ferrer, A. and Cabral, R. (1991) .Toxic epidemics caused by alimentary exposure to pesticides: a review of food additives contamination 8: 755-776.

Gali, K.K., Reddy D.S.R., Yagnika, S., Nischala T. and Jacob A.S. (2010). Exploitation of aqueous plant extracts for reduction of fungal growth and detoxification of aflatoxins, KMITL Science and Technology Journal 10 (2) : 52-62.

Gottileb, O.R., Borin, M.R. and Brito, N.R. (2002). Integration of ethnobotany and phytochemistry: dream or reality? Phytochemistry 60: 145-152.

Harbant S., Ghassan F. and Mohd Syarhabil (2011) Anti-fungal activity of *Capsicum frutescence* and *Zingiber officinale* against key post-harvest pathogens in citrus. International Conference on Biomedical Engineering and Technology IPCBEE vol.11.

Harris, C.A., Renfrew, M.J. and Woolridge, M.W. (2001). Assessing the risk of pesticide residues to consumers: recent and future developments. Food Additives and Contamination 18: 1124-1129.

Huang, J.W. and Chung, W.C. (2003). Management of vegetable crops diseases with plant extracts.Advances in Plant diseases management 37:153-163.

Ibrahim, M.H and Ebo, K.C. (1986). Mycoflora of stored crops from six northern states in Nigeria. National stored product Research Institute Technical Report, No.1: 26.

Iorizzi, M., Lanzotti, V., De Marino, S., Zollo, F., Blanco-Molinas, M., Macho, A. and Munoz, E. (2002). New glycosides from *Capsicum annum* l. var. *acuminatum*. Isolation, structure determination, and biological activity.Journal of Agriculture and Food Chemistry 49: 2022-2029.

ISTA, (2003). International rules for seed testing proceedings of the international seed testing association, Seed science technology 21: 25-30.

Janardhana, G.R., Raveesha, K.A. and Shetty, H.S. (1998). Modified atmosphere storage to prevent mould –induced nutritional loss in maize. Journal of Science and Agriculture 76: 573-578.

Khyade, M.S. and Vaikos, N.P. (2009). Antibacterial evaluation and phytochemical analysis of *Wrightia tinctoria* R. Br. Leaves. Pharmacologyonline, 2, 808-813.

Kurita, S., Kitagwa, E., Kim, C.H., Momose, Y. and Iwahashi, H. (2002). Bioscience, Biotechnology, and Biochemistry vol.66, 3: 532-536.

Lacey, J. (1988). The microbiology of cereal grains from areas of Iran with a high incidence of oesophageal cancer. J. Stored Products Res. 24: 39-50.

Lumpungu, K. Baelenge, B. and Bitijula, M. (1989) The effect of groundnut seed coat on the development of pathogenic fungi. Tropicitura 7 (4): 128-131.

Marin, S., Homedes, V., Sanchis, V., Ramos, A.J. and Magan, N. (1999). Impact of *Fusarium moniliforme* and *F.proliferatum* colonisation of maize on calorific losses and fumonisin production under different environmental conditions.Journal of Stored Product Research 35: 15-26.

Mc Donald, D. and Harkness, C. (1964). Growth of *Aspergillus flavus* and Production of Aflatoxin in Groundnuts. IV. Tropical Science 6: 12-27.

Melone, J.P. and A.E. Maskett (1964). Seed-borne fungi. Proc. Intl. Seed Test. Assoc., 29: 179-384.

Mukherjee, P.S., Nandi, S.K. and Nandi, B. (1992). Deteriorative changes in groundnut seeds in storage. Journal of Mycopathological Research, 30(2): 113-119.

Nychas, G.J.E. (1995). Natural Antimicrobials from Plants. In: New Methods of Food Preservation, Gould, G.W. (Ed.). Blackie Academic Professional, London, pp: 58-89.

Okwu, D.E. and Morah, F.N.I. (2007). Isolation and characterization of flavanone glycoside 4,5,7 Trihydroxide flavanone Rhmnoglucose from Garcina kola seed .Journal of Applied Sciences. 7 (2): 155- 164.

Panasenko, V.T. (1967). Ecology of Mycoflora. Botanical Review 33: 189-215.

Rios, J.L., Recio, M.C. and Villar, A. (1988). Screening methods for natural products with antimicrobial activity: A review of the literature, Journal of Ethnopharmacology 23: 127-149.

Sanatombi, K. and Sharma, G.J. (2007). Micropropagation of *Capsicum frutescens* L. using axillary shoot explants, Scientia Horticulturae 113, 96-99.

Shafique, S., Javaid, A., Bajwa, R. and Shafique, S. (2007). Effect of aqueous leaf extracts of allelopathic trees on germination and seed – borne mycoflora of wheat. Pakistan Journal of Botany 39 (7): 2619-2624.

Soetarno, S., sukrasno, Yulinah, E and Sylvia (1997). Antimicrobial activities of the ethanol extracts of Capscium fruits with different levels of pungency. Journal of Medical Sciences 2 (2): 57-63.

Sullivan, G.A. (1984). Seed and Seedling diseases In: Compendium of Peanut Disease (eds. Porter, Smith & Kabana). St. Paul, Minnesota, USA, American Phytopathological Society: 37-38.

Verma, J. and Dubey, N.K. (1999). Prospective of botanical and microbial products as pesticides of tomorrow. Current Science 76: 172-179.

Wilson, D.M., Mc Millian, W.W and Widstrom, N.W (1986). Use of *Aspergillus flavus* and *A. Parasiticus* colour mutants to study aflatoxin contamination. In: (Eds. Llewellan, Rear and Barry) Biodeterioration Vl. International Biodeterioration Society, London.

Woodroof, J.G. (1984). Peanuts: Production, Processing, Products. 3rd ed.West Port, Conn. AVI Publishing.

Zhang, J., Nagasaki, M., Tanaka, Y. and Morikawa, S. (2003). Capsaicin inhibits growth of adult T- cell leukemia cells. Leukemia Research 27 (3), 275-283.

(Published in March 2012)