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THE ANTIFUNGAL ACTIVITY OF AQUEOUS AND ETHANOL EXTRACTS OF *JATROPHA CURCAS* L. AGAINST *ASPERGILLUS NIGER* (VAN TIEGHEM) THAT CAUSE BLACK MOULD ROT OF ONION BULBS IN SOKOTO, NIGERIA

Bashir, L. U[1]., Gashua, I.B[2]., Isa, M. A.[3*] and Ali, A[4]
[1,4]Department of Biological Sciences, Yobe State University, Damaturu, Nigeria
[2]Department of Science Laboratory Technology, Federal Polytechnic Damaturu, Nigeria
[3]Department of Microbiology, University of Maiduguri, Nigeria
*Corresponding author: mustafaalhajiisa@gmail.com

Abstract

The antifungal activity of aqueous and ethanol extracts obtained from seed and leaf of *Jatropha curcas* were investigated using agar incorporation method *in vitro* against *Aspergillus niger*, a microbe known to be resistant to some chemical agents. Pathogenicity test revealed that *A. niger* was the pathogenic fungus that cause black mould rot of onion bulbs. The growth of *A. niger* was markedly suppressed by aqueous and ethanol extracts of leaf and seed, 65.7 and 57.0% at 160 mg/ml. The extracts at low concentration did not show considerable activity against the fungus except leaf ethanol extract 53.3% at 40 mg/ml.The *in vivo* study showed that aqueous extracts of seed and leaf reduced rot development, 59.4 and 54.4 % in onion bulbs. Highest rot inhibition 66.3 was obtained at 160 mg/ml seed extract.The result of the study suggest the potentials of *J. curcas* extracts as fungicidal agent that could be useful in management of black mould rot of onion bulbs caused by *Aspergillus niger*.

Key words: *Aspergillus niger*, Antifungal, *Jatropha curcas*, *In vitro*, *In vivo*.

Introduction

The onion (*Allium cepa* L.) is native to south west Asia or Mediterranean, considered important commercial vegetable crop in the world (Wani and Taskeen-Un-Nisa, 2011). Onion is an important vegetable crop in Nigeria based on consumption and economic value to farmers. The crop is grown for its bulbs which are used daily in every home for salad, seasoning and flavouring of foods. Onion has several medicinal uses; its use in the case of sun strokes is known worldwide (Rai and Yadav, 2006). Onion is a valuable ingredient in the diet due to its content of sugars, vitamins and minerals (Ole, *et al*., 2004). The crop is grown mainly in the northern part of Nigeria during the dry season (October to April). The onion farmers in Nigeria almost always store their onions after harvest for one to five months to ensure a continual supply through seasons when fresh produce were un-available. Fungi, especially moulds are important pathogens of fruits and vegetables particularly under tropical and sub-tropical conditions (Adebayo and Diyaulo, 2003). The importance of storage rots includes reduction in the quantity and quality of onion which affects the market value

(Dogondaji *et al*., 2005). Other important consequence often overlooked, is mycotoxin contamination of the affected materials (Muhammad *et al*., 2004).

Black mould disease caused by *Aspergillus niger* van Tieghem (An) is a limiting factor in onion (*Allium cepa* L.) production worldwide (Ozer and Koycu, 2004). *Aspergillus niger* also primary reported to survive between onion crops as a soil saphrophyte (on decaying organic matter) in or on onion bulbs or on cull onions in field or storage and being ubiquitous in occurrence, it attacks/infect bulbs of onion in field/storage, whenever they find injured tissues by producing various enzymes or toxins (Srinivasan and Shanmugam, 2006). Association of *A. niger* with onion seeds produced in hot (arid) climates and their transmission from soil and naturally contaminated seeds to onion seedlings and sets, have also been reported by Hayden and Maude, 1992 and causes 30 to 80% loss/spoilage of onion bulbs.

The fungicides are known to be highly effective in controlling various postharvest diseases of vegetables and fruits. Although effective, their continued or repeated applications may disrupt equilibrium of ecosystems, leading to dramatic disease outbreaks, widespread development of pathogens resistant to one or more chemicals, toxicity to non-target organisms and environmental problems (Lee, *et al*., 2009). Sometimes, they accumulate in the food chain as residues above safety limits (Lee, *et al*., 2008). A noticed decrease in pesticide efficacy, along with increased concern about the environmental effects of currently used fungicides have highlighted the need to develop alternative control strategies or innovative crop protection and postharvest methods of fruit and vegetable rot control with reduced use of conventional fungicides or without synthetic chemicals (Kim, *et al*., 2003). Research on plant-derived fungicides is now being intensified, as it became evident that these substances have enormous potentials to improve the future agrochemical technology. In fact, there are good reasons to suppose that secondary plant metabolites has naturally evolved to actively protect vegetable and fruit species from microbial pathogen attacks (Kim, *et al*., 2003).

Fungal contamination of onion bulbs especially black moulds constitute a menace in the production and storage of onion particularly in the tropics. Apart from toxins productions, presence of moulds in onion bulbs eventually leads to disease development, deterioration and reduction in market value. The objective of this study is to evaluate the antifungal potentials of *Jatropha curcas* L. aqueous and ethanol of seed and leaf extracts against *Aspergillus niger in vitro* and *in vivo*.

Materials and Methods

The study area

The study was conducted during the months of May to August, 2012 in the Department of Biological Sciences, Usmanu Danfodiyo University Sokoto. Sokoto State is one of the northern states where a large proportion of onion production and storage take place annually. The area is located in the north – western Nigeria (Longitude 3 – 9°East; latitude 10 – 14°North). It is characterized by long dry season (October to April) and a short rainy season (May to September). Average monthly temperature ranges from 21 to 35℃ and is lowest in December and January. Heat is more severe in March and April, the mean annual temperature is 27^{o}C (Ojanuga, 2006).

Preparation of Plant Materials

The leaves and seeds of *J. curcas* were collected from the permanent site of the Usmanu Danfodiyo University Sokoto, and identified in the herbarium of the institution. The seeds were sun dried and leaves were dried in an ambient laboratory conditions. The dried seeds and leaves were separately ground into powder in a blender (Philips, Mexico City, Mexico). The resultant powders were sieved in a fine sterile mesh (0.2 mm) to obtain the finest powder. One hundred gram (100 g) portion of each of the seeds and leaves powder were extracted separately with 500 ml of water and ethanol for 48 hours at room temperature. The extracts were filtered using a sterile muslin cloth. The filtrates were evaporated to dryness using a water bath at 40^{o}C. The residues were stored at 4^{o}C for subsequent use.(Sani and Aliyu, 2011).

Collection of Samples

Onion bulbs showing black discolouration and symptoms of rotting were randomly selected from different market stalls and local storage facilities in Sokoto metropolis, healthy fresh blemish free onions were also collected from fadama farms and were packaged in different sterile polythene bags and taken to Mycology laboratory of Department of Biological Sciences Usmanu Danfodiyo University for microbial analysis.

Isolation and Identification of Black Mould Rot Fungi

To isolate the pathogens responsible for the black mould rots on the affected onion bulbs, the bulbs were stripped of their outer dry scales and surface sterilized in 1% Sodium hypochlorite solution for 60 sec.(Dimka and Onuegbu, 2010) These were then rinsed in three successive changes of sterile distilled water and blotted dry with sterile filter paper. Small segments of tissues (3mm^3) from the margins of rotted lesions were cut out with a sterile scalpel and plated on potato dextrose agar (PDA) in 90mm Petri – dishes. The plates were incubated at room temperature (28 ± 3℃) for 7 days. Developing fungal colonies were sub – cultured continuously on fresh PDA plates to obtain pure culture of the isolates. Fungal isolates, *Aspergillus niger* were identified based on cultural and morphological characteristics (Barnett and Hunter, 1998; Koneman et al., 2006).

Pathogenicity Test

Fresh, healthy blemish free onion bulbs were stripped of their outer scales washed with tap water, rinsed with distilled water and surface sterilized with 70% ethanol. Cylindrical discs were removed from the bulbs with a sterile 4 mm cork borer. A disc of a five days old culture of the isolated *Aspergillus niger* was transferred into hole created in the bulbs. The plug was carefully placed and the wounded area sealed with Vaseline to prevent extraneous infection. The inoculated bulbs were placed in separate air tight containers and incubated for 14 days at room temperature (28 ± 2^{o} C). The same procedure was used for the control except that discs of uninoculated PDA were placed in the holes created in the bulbs (Amienyo and Ataga, 2006). Three replications were prepared for treatment and control. After incubation period, the onion bulbs were examined for infection and disease development. The causal agents were re-isolated from the infected bulbs and compared with the original isolates.

Antifungal Testing

Potato dextrose agar (PDA) was prepared and autoclaved before the addition of extracts. Seed and leaves extracts were mixed with the molten agar at (45^{o}C) to final

concentrations of 5, 10, 20, 40 and 160 mg/ml and poured into Petri dishes. Each plate was swirled carefully until the agar began to set. Blank plates containing only PDA served as control. The prepared plates were inoculated with plugs obtained from actively growing margin of fungi plates and incubated at 25^{o} C for 7 days (Aliero *et al*., 2006). The diameter of the fungal growth was measured and expressed as percentage inhibition of three replicates. (Baretto *et al*., 1997; Quiroga *et al*., 2001):

$$\% \text{ Inhibition} = \frac{\text{Growth of fungal colony in control- growth of fungal colony in extract}}{\text{Growth of fungal colony in control}} \times 100$$

Effect of Plant Extracts on Rot Development

The method of Udo *et al*. (2001) was used to determine the effect of extracts on rot development. Fresh blemish free, healthy onion bulbs were washed with water, surface sterilized with 70% ethanol solution and rinsed in five changes of sterile distilled water. The onion bulbs were soaked in different concentrations of 0.5 L. of (5, 10, 20, 40, 160 mg/ml) of *Jatropha curcas* seed and leaf extracts and were allowed to stand in the solution for 3 min. In the control, bulbs were soaked in sterile distilled water for 3 min. . Cylindrical discs were removed from the bulbs with a sterile 4 mm cork borer. A disc of a five days old culture of the isolated fungi was transferred into hole created in the bulbs. The plug was carefully placed and the wounded area sealed with Vaseline to prevent infection. Three replicate were maintained for each treatment and control. The inoculated onion bulbs were placed in a sterile containers and incubated at room temperature (28 ± 2^{o}C) for 14 days.

After the incubation period, the tubers were incised horizontally with sterile knife. The length of rotted portion from each hole was measured over the total surface length with a metre rule. Fungi toxicity were determined in form of percentage growth inhibition and was calculated according to the formula of Okigbo and Nmeka (2005).

$$\text{Growth inhibitio (\%)} = \frac{(LC - LT)}{LC} \times 100$$

Where LC = average length of unrotted portion of control and LT = Average length of unrotted portion with treatment.

All data collected were statistically analyzed for significant difference ($P<0.05$) by analysis of variance (ANOVA) and means were separated using Duncan Multiple Range Test (DMRT). (Snedecor and Cochran, 1967).

Results and Discussion

The causal agent of black mould rot of onion showed similar symptoms as described earlier by different workers on onion bulbs in storage (Dag and Sing, 1982; Quadri *et al*., 1982; Joentaek *et al.*, 2001). The result showed that A. niger as causal organism of black mould rot of onion as shown in pathogenicity test. The primary symptoms was black discolouration of tissues, infected bulbs showed blackening at the neck, streaks or spots of black colour appeared on or beneath the first and second outer scale. In advance stage of infection the entire bulb appears black and become shriveled.

The *in vitro* efficacy of aqueous and ethanol of *Jatropha curcas* seed and leaf extracts were presented in table 1 and 2. The result showed that significant percentage inhibition 65.7 and

55.6 % leaf and seed extracts at 160 mg/ml respectively. The result revealed that both leaf and seed extract at 5 mg/ml and 10 mg/ml do not exhibit antifungal activity against the tested fungi. The investigation also showed that antifungal activity was enhanced with increase in concentration of extracts, this also support the earlier investigation by (Banso and Adeyemi, 2007; Varaprasad et al., 2009) that medicinal plant with tannin content possesses remarkable toxic activity against bacteria and fungi and may assume pharmacological importance. Amienyo and Ataga (2007) reported significant (P=0.05) mycelial growth inhibition of A. niger with extracts of A. cordifolia and A. sativum which is in agreement with this investigation.

Table 1: Percentage Inhibition of *J. curcas* Water Extracts on *Aspergillus niger*

Conc. (mg/ml)	Seed extracts	Leaf extracts
5	$0.0^{d} \pm 0.0$	$0.0^{d} \pm 0.0$
10	$0.0^{d} \pm 0.0$	$5.4^{cd} \pm 1.6$
20	$13.9^{c} \pm 4.7$	$23.0^{bc} \pm 6.7$
40	$38.7^{b} \pm 5.1$	$39.1^{b} \pm 12.4$
160	$55.6^{a} \pm 2.6$	$65.7^{a} \pm 0.9$

^{a,b,c} means in a column with different superscripts are significantly different (P<0.05)
Values are means ± standard error of 3 replications

Table 2: Percentage Inhibition of *Aspergilus niger* by Ethanol Extracts of *J. curcas*

Conc. (mg/ml)	Seed extracts	Leaf extracts
5	$11.1^{e} \pm 0.6$	$12.8^{e} \pm 1.8$
10	$15.4^{d} \pm 1.0$	$32.6^{d} \pm 0.7$
20	$21.1^{c} \pm 2.0$	$39.3^{c} \pm 1.8$
40	$33.0^{b} \pm 0.5$	$53.3^{b} \pm 3.2$
160	$57.0^{a} \pm 1.0$	$65.7^{a} \pm 0.2$

^{a,b,c} means in a column with different superscripts are significantly different (P<0.05)
Values are means ± standard error of 3 replications

The *in vivo* investigation showed a significant reduction in black mould rot development which is presented in (Figure: 1). the degree of protection of onion bulbs from rot development by different concentration of *J. curcas* seed and leaf extract varied and was significant (P<0.05). The highest reductions in rot development were observed 66.3 and 59.4% at 160 and 40 mg/ml seed extracts respectively. Moderate inhibition was observed 54.3% at 160 mg/ml leaf extracts.

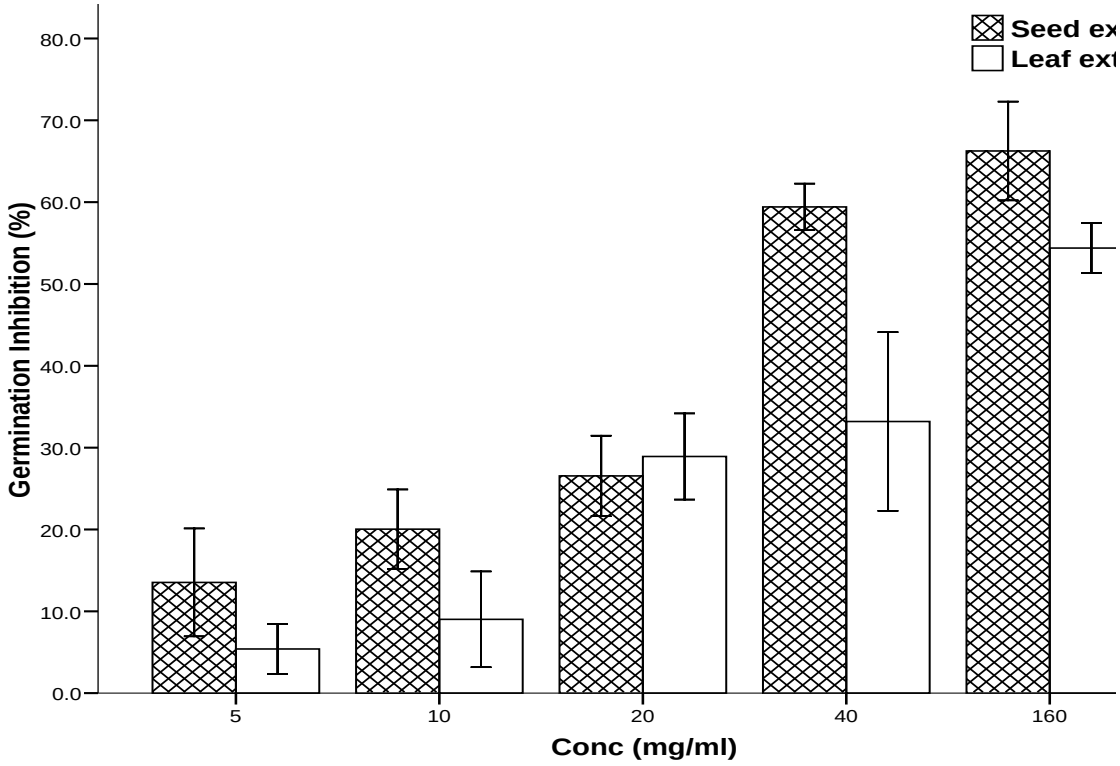

Figure 1. The *in vivo* investigation

The antifungal activities of of some plant extracts in controlling different pathogens have been reported by several workers (Tewari and Nayak, 1991; Amadioha, 2000; Okigbo and Ajalie, 2005). In this study it was observed that, the inhibition is due to fungitoxic activities of J. curcas plant extracts which agrees with report of other workers (Qasem and Abu-Blan, 1986; Okigbo and Nmeka, 2005). It is noteworthy that the active principles present in plants were influenced by many factors which include the age of the plant, plant part used, extracting solvent, methods of extraction and time of harvesting plant materials. (Amadioha and Obi, 1991; Okigbo and Ajalie, 2005; Okigbo *et al*., 2005).

Conclusion

The present investigation revealed that *A. niger* was the cause of black mould rot of onion bulbs, Fungitoxic compounds were present in *J.* curcas seed and leaf since extracts inhibit growth of fungi tested *in vitro* and *in vivo.* From this findings it can be suggested that *J. curcas* extracts can be used as protective biofuncides in management of plant disease which is economically viable. Thus, it is required that further investigation including toxicological evaluation and isolation of antifungal moieties is imperative.

References

Adebayo L. O. and Diyaolu, S. A. (2003). Mycology and spoilage of cashew nuts. *African Journal of Biotechnology*. 2:369-373.

Aliero, A. A., Grieson, D.S. and Afolayan, A. J. (2006). Antifungal activity of Solanum pseudocapsicum. *Research Journal of Botany*.1(30:129-133.

Amadioha A.C. 2000. Fungitoxic effectsof some leaf extracts against Rhizopus oryzae causing tuber rot of potato. *Archphytopathology Pflan*.4: 1-9.

Amadioha, A.C. and Obi, V.I. (1999). Control of Anthracnose disease of cowpea by Cymbopogon citrates and Ocimum gratissimum . *Actaphytopathology Entomology Hungerica.* 34. (1-2): 85-89.

Amienyo, C. A. and Ataga. A.E. (2006). Post-harvest fungal diseases of sweet potato(Ipomoea batatas {L} Lam) tubers sold in selected markets in Rivers State, *Nigeria. Sci. Afr*. 5(2): 95-98.

Banso, A. and Adeyemo, S.O. (2007). Evaluation of antibacterial properties of tannins isolated from Dichrostachys cinera. *Afr. J. Biotechnol*. 6 (15):1785-1787.

Baretto, M., Straker, C.J., Crichley,A.T. (1997). Short note on the effect of ethanolic extracts of selected South African sea weeds on the growth of commercially important plant pathogens, Rhizoctonia solani Kuhn and Verticullium sp. *South African Journal of Botany*. 63(6):521-523.

Barnetth H.L. and Hunter, B. B. (1998). *Illustrated Genera of Imperfect Fungi*. Colliar Macmillan, London. 200-219.

Dang, J.K and Singh, J.P. (1982). Fungal rot of stored onion bulb in Harayana. *Harayana Agric. Univer. J. Res*. 12: 513

Dimka, S. O.N. and Onuegbu, B. A. (2010). Mycoflora of copra and effect of brining on some properties of copra in Nigeria. *Agriculture and Biology Journal of North America*: 2151-7525.

Dogondaji, S.D., Baba, K.M., Muhammad, I. and Magaji, M.D. (2005). Evaluation of onion storage losses and implication for food security in Sokoto Metropolis. *Bulletin of Science Association of Nigeria*. 26: 10 – 14.

Hayden, N.J. and Maude, R.B. (1992). The role of seedborne Aspergillus niger in transmission of black mould of onion. *Plant Pathol*. 41: 573-581.

JoonTaek, L., Dong, W. B. SeunHee, P., Chang, K. S., Youn, S. K. and Hee Kyu, K.(2001). Occurrence and Biological control of Post harvest Decay in onion caused by Fungi. Plant *Pathol. J*. 17: 141-148.

Kim, M. K., Choi, G. J. and Lee, H.S.(3003). Fungicidal Property of Curcuma longaL. Rhizome-Derived Curcumin against Phytopathogenic Fungi in a Greenhouse. J. *Agric. Food Chem*. 51: 1578–1581.

Koneman, E., Procop, G., Shreckenberger, p., Wood, G., Janda, W., Allen. S. and Winn,C.W. (2006). *Konneman color Atlas and Diagnostic Microbiology*. Lippincoth Williams and Wikkins London.1535pp.

Lee, Y.S., Kim, J., Shin, S.C., Lee, S. G. and Park, I. K.(2008). Antifungal activity of Myrtaceae essential oils and their components against three phytopathogenic fungi. *Flavour Fragr*. J. 2 23: 23–28.

Lee, Y.S.., Kim, J., Lee, S.G., Oh, E., Shin, S.C. and Park, I.K.(2009). Effects of plant essential oils and components from Oriental sweet gum (Liquidambar orientalis) on growth and morphogenesis of three phytopathogenic fungi. *Pestic. Biochem. Phys*. 93. 138–143.

Muhammad, S. Shehu, K. and Amusa, N. A. (2004): Survey of the market Diseases and aflatoxin contamination of tomato (LycopersiconescolentusMill.) Fruits in Sokoto, Northwestern Nigeria. *Nutrition and food science*, 34 (2):72-76

Ojanuga, A. G. (2006) *Agroecological zones of Nigeria Manual*. National Special programme for food security/FAO.Abuja.Nigeria.124 pp.

Okigbo, R.N.and Nmeka, I.A. (2005). Control of Yam tuber rot with leaf extracts of Xylopia aethiopica and Zingiber officinale. *Afr. J. Biotechnol*. 4(8): 804-807.

Okigbo, R.N.and Ajalie, A. N. (2005). Inhibition of some human Pathogens with tropical plants extracts Chomolinaena odorata and citrus aurantifolia and someantibiotics. *International Journal Molecular of Medical Advanced Sciences* (Pakistan).1(1):34-40.

Ole, H., Torben L., Lars, P.C., Ulla, K., Nazmul, H. And Shakuntala, H.A. (2004): Contents of Iron Zinc, and β - ca-rotene in Commonly consumed vegetables in Bangladesh. *Journal of Food Composition and Analysis*.Vol. 17: 587 – 595.

Ozer, N. and Koycu, N.D. (2004). *Seed-borne fungal diseases of onion and their control.* Academic publishers, Dordrecht.

Qasem, J.R. and Abu-Blan, H. A. (1996). Fungicidal activity of some common weeds extracts against different pathogenic fungi. *Journal of Phytopathology*. 144: 157-161.

Quadri, S.M.H., Srivastava, J.K., Bhonde, S.R., Pandey, U.B. and Bhagchandani, P.M. (1982). Fungicidal bioassays against some important pathogen of onion. *Pesticides*, 16:11-16.

Quiroga, E.N., Sampiepto, A.R. and Vattuone, M.A. (2001). Screening for antifungal activity of selected medicinal plants. *Journal of Ethnopharmacology*. 74:89-96.

Rai, N. and Yadav, D.S. (2005). *Ad. in veg. prod*. Research Book Centre. Ch. 2.2: onion. New Delhi India.

Sani, H.D. and Aliyu, B.S. (2011). In vitro antibacterial activity of Anogeissus leocapus DC (Stem bark) extracts against Escherichia coli and Staphylococcus aureus. *Bayero Journal of Pure and Applied Sciences*. Vol .4(2):56-59.

Snedecor, D.G.W. and Cochran,W.G. (1967). *Statistical Methods, 6th Ed*. Iowa State University Press, U.S.A. 593pp.

Srinivasan, R. and Shanmugam, V. (2006). Post-harvest management of black mould rot of onion. *Indian Phytopathol*. 59:87-93.

Tewarri S.N.and Nayak, N. (1991). Activity of four plants leaf extracts against three fungal pathogens of rice. *Tropical Agriculture*. (Trinidad). 68:373-375.

Udo, S.E. Madunagu, B.E.and Isemin, C.D. (2001). Inhibition of growth and sporulation of fungal pathogens on Sweet potato and yam by Garlic extract. *Nigeria. J. Bot*. 14: 35-39.

Vasaprasad, B., Prasanth, K.K., Naidu, K.C. and Somasekhar, P. (2009). Antifungal activity of selected plant extracts against phytopathogenic fungi Aspergillus niger. *Indian Journal of Science and Technology*. Vol.2 (4):87- 90.

Wani, A.H and Taskeen-Un-Nisa (2011). Management of black mould rot of onion. *Mycopath.* 9(1):43-49.