A peer-reviewed journal
PSM Microbiology
2016 | Volume 1 | Issue 2 | Pages 79-84
ISSN: 2518-3834

www.psmpublishers.org

Research Article **Open Access**

Inhibitory Effects of Organic Extracts against *Aspergilus flavus* and their Comparative Efficacy upon Germination of Infested Rice Seeds

Waqar Islam[1]*, Manzoor Ahmed[2]

[1]College of Plant Protection, Fujian Agriculture and Forestry University, Fuzhou 350002, China.
[2]Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan.

Received: 10.Sep.2016; Accepted: 20.Oct.2016; Published Online: 26.Nov.2016
***Corresponding author: Waqar Islam; Email: ddoapsial@yahoo.com**

Abstract
Aspergilus flavus is the most common seed born fungus that deteriorates the seed quality and minimizes the export value of rice all over the world. As the excessive use of chemicals worldwide has proved lethal environmentally so the present study was carried out to evaluate different organic extracts against fungal growth and their effects in improving the germination percentage of infested rice seeds. The experiment was carried out in-vitro using four different concentrations (2, 4, 6, 8%) of organic extracts as compared to control in a completely randomized design and all the data was subject to statistical analysis. Regarding mycelial growth, higher concentrations of plant extracts were significantly better as statistical results revealed that the organic extracts of Neem and Brassica were proved better and comparative to each other followed by activity of Diver (Mineral Oil), Onion and Garlic extracts. Similarly, the performance of Brassica and Neem extracts were recorded better regarding improvement in germination percentage of infested rice seeds as compared to Diver, Onion and garlic extracts. Hence we suggest avoiding the dangerous chemicals and use the organic extracts to manage the particular fungus efficiently and safely.
Keywords: Aspergillus, Organic, Efficacy, Rice, Extracts.

To cite this article: Islam, W., Ahmed, M., 2016. Inhibitory Effects of Organic Extracts against Aspergilus flavus and their Comparative Efficacy upon Germination of Infested Rice Seeds. PSM Microbiol., 01(2): 79-84.

INTRODUCTION

Aspergillus flavus is saprotrophic and pathogenic fungus in nature having diverse distribution all around the globe (Machida, 2010; Ramírez-Camejo, 2012). The fungus is associated with pre as well as post-harvest losses in legumes, grains and nuts by producing huge quantity of mycotoxins (George, 2005). The particular fungus is also involved in causing diseases to mammals and human beings such as Aspergilosis (Amaike, 2011). *Aspergillus flavus* is characterized by yellowish to greenish spore powdery mass production upon legumes and grains surface giving downy or powdery texture (Bennett, 2010). Fungus grows by production of hyline and septate hyphea which penetrate in the target surface by production of degenerative enzymes (Ehrlich, 2005). Matt like surface can be seen via microscope in which colorless conidiophores are produced by asexual manner (Alexopoulos, 1996). Sexual reproduction only occurs when opposite mating type strains are cultured artificially or in nature (Horn, 2009). *Aspergillus flavus* affects large number of grain commodities including rice grains (Pitt, 2000).

Rice (*Oryzae sativa* L.), is highly nutritive commodity in south Asian countries as these countries consume rice as staple food (Smith and Bruce, 2000). The particular commodity play very important role in economy of Pakistan as the country is ranked second in rice exporters list (Zahid, 2005). Including *Aspergilus flavus*, Rice crop and its grains are stressed by more than fourty diverse species of various bacteria and fungi (Khan, 2009). *Aspergilus flavus* deteriorates the rice quality and is most commonly isolated fungus according to several researchers (Khan, 2000; Javed, 2002; Arshad, 2009; Habib, 2012; Ishfaq, 2015). The polluted seeds may fail to germinate, transmit disease from seed to seedling and from seedling to growing plants (Fakir, 2002). The particular fungus is also involved in association with many seed born fungus to result in complex disease. The other fungus that are involved with this are *Pyricluaria, Alternaria, Bipolaris, Curvularia, Rhizopus, Mucor, Schlerotenia, Fusarium* etc and all these play vital role in rice qualitative and quantitative losses (Wahid, 2001). Due to unavailability of durable resistance against fungus, the most common practice for its eradication is the use of synthetic chemicals (Habib, 2012). Even considering the worth of synthetic chemicals for their

quick action, ready availability and success against the target fungus, there are some bad aspects also such as they have vigorous residual effects, high priced, hazardous during handling and are extremely dangerous to its surrounding human beings and environment (Perry, 2014). So finding the alternatives than the chemicals is the best solution. Coping this, the direction goes towards the use of organic extracts which are environmentally safe and least hazardous to human beings. Organic extracts of different Plants have been documented successful against inhibition of different fungi associated with different seed borne diseases (Arshad, 2009; Habib, 2012; Naher, 2012). So the following studies were conducted to evaluate some locally available organic extracts to manage the test seed born fungus.

MATERIALS AND METHODS

Isolation, Purification and Fungus Identification

Collection of fungal infected discolored grain samples was done from different areas of Kala Shah Kaku in 2015 and were immersed in 70% Ethanol solution for one minute. Afterwards, rinsing of these samples was performed twice in sterilized water leading to drying by placing upon blotter paper and then placed upon PDA (Potato starch = 20 g ; Agar Agar = 20 g ; Dextrose = 20 g ; Distilled Water = 1 liter) in Petri plates. All the plates were incubated at 27 ± 2^0C for a week (Nghiep and Gaur, 2005). After the appearance of the colony growth, observation of all the petri plates was carried out via low power stereo microscope. Visual identification of *Aspergilus flavus* was done by consulting the available literature and morphological features of its colony growth, color, sporulation type (Barnett and Hunter, 1990; Mathur, 2003) (Figure 1). The particular fungus was isolated and purified culture was prepared for further experimentation.

Fig. 1. Identification of spore and mycelial growth of the *Aspergillus flavus*

In-vitro evaluation of different Organic Extracts against *Aspergillus flavus*

The experimentation was done via completely randomized design (CRD) with the involvement of four replications under laboratory conditions. Organic Extracts named as Diver (Mineral Oil 97% EC), Neem extract (*Azadirachta indica*), Onion extract (*Allium cepa*), Brassica extract (*Brassica campestris*) and Garlic extract (*Allium sativum*) were evaluated for their inhibitory effects upon colony growth of *Aspergilus flavus* via poisoned food technique comparing with control check. 2, 4, 6 and 8% concentrations of organic extracts were used as treatments against the fungus. To prepare these concentrations, One gram of fresh leaves from each botanical was taken, grinded and sterilized in 100 ml of sterilized water i.e. 1% organic extract solution. Before pouring the PDA media into the petri plates, one ml of each organic extract concentration was added. Afterwards, approximately 12-15 ml of sterilized PDA medium was poured in each sterilized Petri plate (9 cm). These plates were allowed to solidify, leading to placing of 0.7 cm discs of 10 days old purified culture of *Aspergilus flavus* and incubation at 27 ± 2 ^{0}C. Data regarding radial colony diameter was recorded after one week of incubation when the fungal colonies growth covered the plates labeled for control check. Data was recorded from the bottom side of petri plates by computing the inhibition in colony growth diameter by using following formula (Sundar, 1995).

$$\text{Percent inhibition} = \frac{X - Y}{X} \times 100$$

Where,

X = Growth of test fungus in control in cm.

Y = Growth of test fungus in treatment in cm

Evaluation of Organic Extracts for germination test

Randomized application of all the organic extracts (Diver, Neem Extract, Brasicca Extract, Garlic Extract, and Onion Extract) was done as compared to control. Infected rice seeds were dipped in *Aspergilus flavus* suspension (1.50×10^5/ml). Four concentrations (2, 4, 6, 8 %) of Organic extracts were prepared for seed dressing purposes. Two hundred fungal infected rice seeds were soaked in organic extracts for 24 hours while keeping the untreated seeds as control check. Sowing of these treated seeds was done in petri plates by placing fifty seeds per plate via using standard blotter paper technique. Four petri plates were used for each organic extract concentration and labeling of these plates was done according to standardized method. Percentage germination of rice seeds was determined by using following formula.

$$\%\text{age Germination} = \frac{\text{Number of germinated seed}}{\text{Number of seed sown}} \times 100$$

Statistical Analysis

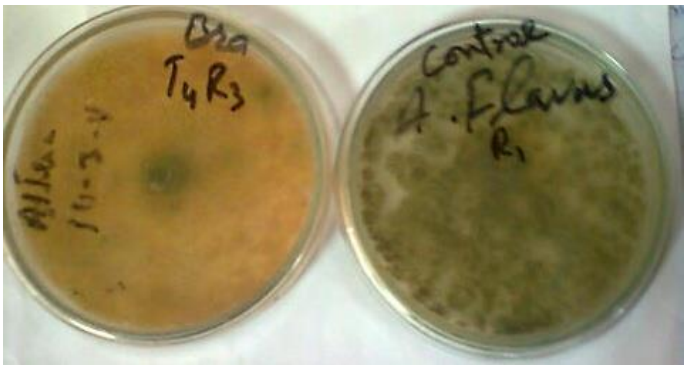
Statistical analysis was carried out for all the recorded observations by determining all the possible interactions through analysis of variance and comparison of all the treatment means was done by LSD test at 5% level of probability (Steel, 1997).

RESULTS

Evaluation of various organic extracts against mycelial growth of *Aspergillus flavus*

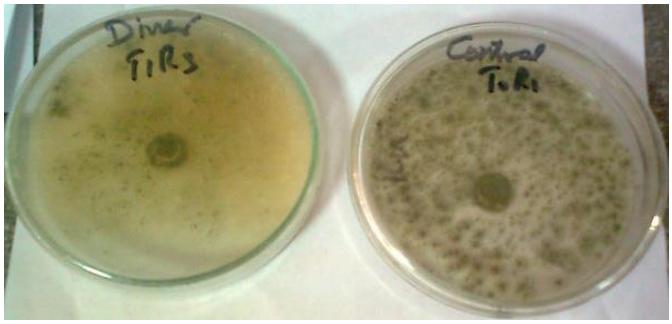
In-vitro evaluation of five organic extracts was carried out against colony growth of *Aspergillus flavus* through standard poisoned food technique. The data exhibited significant effects regarding decrease in mycelial growth of the fungus by organic extracts compared to the control. Higher concentration levels of organic extracts recorded better results such as on average basis at 8% concentration level, the mean mycelial colony growth diameter for all the extracts was 48.77% (Figure 2 a-e).

a. Neem Extract at 8%

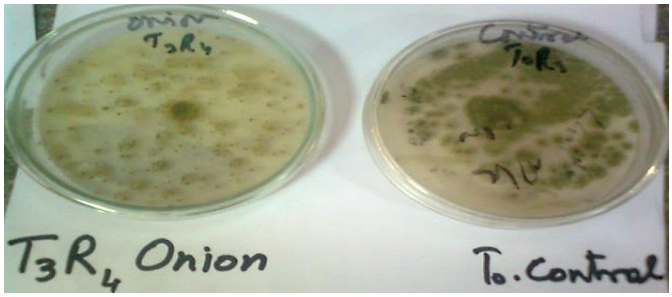


b. Brassica Extract at 8%

c. Garlic Extract at 8%



d. Diver (Minrol oil) at 8%



e. Onion Extract at 8%

Fig. 2 (a-e). Growth inhibition of *Aspergilus flavus* at 8% concentration of Organic Extracts

While 6% concentration exhibited 35.36% followed by 4% (21.71%) and 2% (11.88%) compared to control (100%). Similarly, all the organic extracts performed different but shown significant reduction in mycelial growth of the fungus. Highly significant results were observed regarding Neem extract showing percent mycelia inhibition as 49.13% followed by Brassica extract (44.67%), Diver (42.77%), Onion extract (41.35%) and Garlic extract (39.71%). Overall performance of organic extracts revealed that Neem Extract provided highly significant results in decreasing mycelia growth at 8% concentration level by showing inhibition percentage as 55.95% while Garlic extracts performed below power by showing 41.78% as compared to control (100%). Neem extract also exhibited better results at 6% concentrations (43.75%) followed by 4% (31.20%) and 2% (14.77%) while Garlic extracts yielded least effective results by exhibiting 28.50% at 6% concentration followed by 17.50 (4%) and 10.80% (2%) comparing to control(100%)(Figure 3).

Evaluation of Organic Extracts on germination percentage of rice seeds

The impact of organic extracts on the germination percentage of rice seeds was studied using standard blotter paper technique. Results revealed that organic extracts provided significant results by improving the germination percentage of the infected seeds as compared to control (Figure 4). Higher concentration of all the extracts proved significantly better as 8% concentration level provided better results regarding all the extracts averagely i.e. 77.66% followed by 6% (69%), 4% (63.83%) and 2%

(59.17%) as compared to control (53.33%). Overall, all the organic extracts improved the germination percentage of infected rice seeds up to certain levels. For example, Brassica extract and Neem extract gave higher significant germination percentage at 8% (83%) and (81%) followed by Diver (69%), Onion extract (61%) and Garlic extract (58.50%) while 2% concentration of all the extracts resulted in minimized seed germination where Brassica extract recorded germination percentage as 63% followed by Neem extract (62%), Diver (52%), Onion Extracts (51%) and Garlic extract (50.50%) respectively (Figure 5).

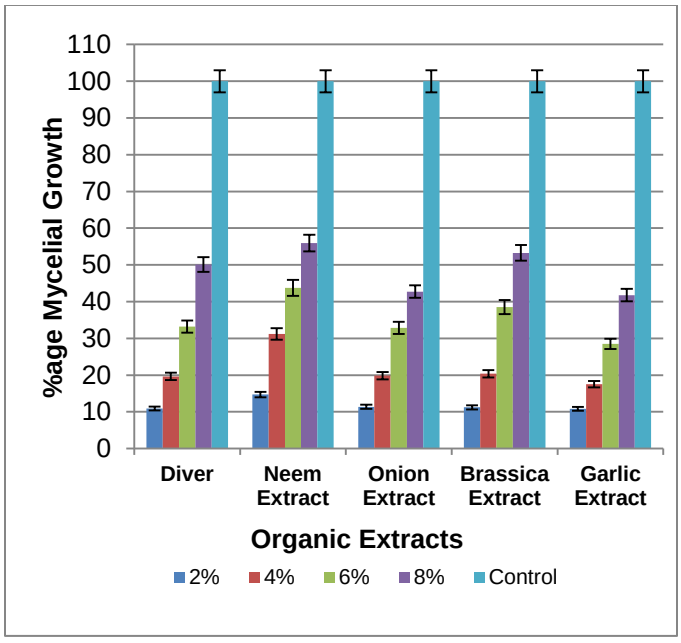


Fig. 3. Efficacy of various organic extracts against mycelial growth of *Aspergillus flavus*.

Fig. 4. Organic extracts and their effects upon germination of infested rice seeds

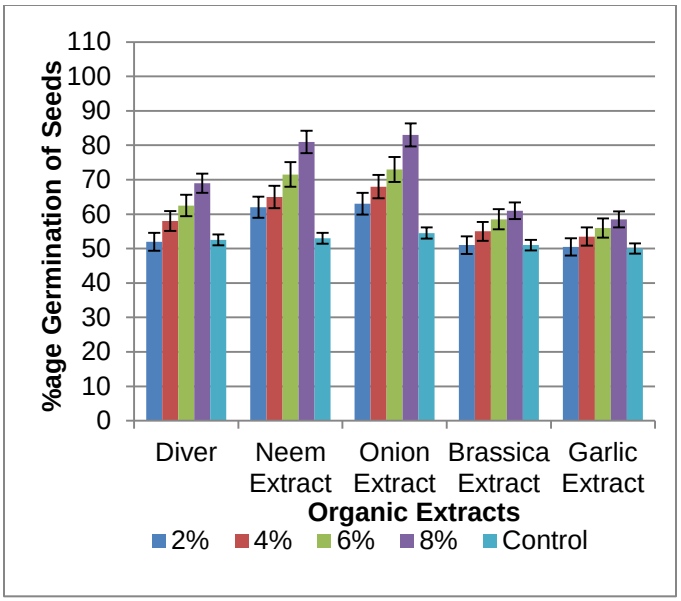


Fig. 5. Evaluation of various organic extracts on germination percentage of rice seeds

DISCUSSION

Aspergilus flavus is associated with many other seed born fungi and results in yield and quality losses of rice commodities (Khan, 1990). As durable resistance against the fungus does not exist so applications of synthetic chemicals or organic extracts seems convenient. Among the two, organic extracts have advantage as they are safe to use and can be made locally. Previously, Essential oils extracted from various plants have shown significant antifungal properties (Arshad, 2009; Habib, 2012; Omidpanah, 2015). So accordingly, we tried to evaluate some organic extracts against *Aspergilus flavus*. Our results were confirmatory with the previous studies showing inhibitory effects of *Azadirachta indica* against mycelia growth of *A. flavus* and *A. parasiticus* (Bhatnagar, 1990). We found that Neem Extract performed excellent in curtailing the mycelial growth of *A. flavus* and recorded 55.95% mycelial growth at 8% concentration level. Our results are in connection with the results of Sitara (2008) who documented the successful minimization of Aspergilus at similar concentration levels of Neem extracts and Mustard oil. We used 2, 4, 6 and 8% concentrations for evaluation of various organic extracts against the test fungus which got support from the Reports of Locke (1995) who explained the management of *Alternaria alternata, Aspergillus spp.* and *Fusarium oxysporum* through application of 2-10% Neem extracts. 2.5% concentration of Neem extract reduced the myclial growth of all species of *Aspergillus* (Niaz and Kazmi, 2005). We found that higher concentration of all the extracts proved significantly better as 8% concentration level provided better results regarding all the extracts averagely i.e. 77.66% followed by 6%

(69%), 4% (63.83%) and 2% (59.17%) as compared to control (53.33%). These studies are encouraged by the findings of Udomsilp (2009) who recorded 11.11% decrease in growth of *A. flavus* when he applied cassia (*Acacia farnesiana*) and Frankincense oil (*Boswellia carteri*) *in-vitro* at 0.4, 0.6, 0.8, 1.0 and 2.0 % concentrations. Furthermore findings of Tijjina (2014) connect with ours who mentioned 4.31cm colony growth of *Aspergilus flavus* by application of 4% Garlic extract.

We also tested organic extracts for seed dressing of infected rice seeds and recorded the germination percentage of seeds. We found that all the organic extracts improved the germination percentage of infected seeds up to certain levels. We recorded that mineral oil Diver shown 69% germination of infected seeds which are coordinating with the findings of Nguefack (2008) who resulted that Diver improved 13% germination capacity of fungal infected seeds. Our results exhibited that Neem extract rigorously improved the germination of infected seeds by showing 81% germination of infected seeds at 8% doze level. These are closed to the reports of Ghorbanian (2008) who have documented the success of Neem extract in improving infected seed germination. We also explained that seed dressing by onion extract show considerable results in germination of infected seeds and listed 61% germination rate. Our results match closely to Kim (2010) who recorded more than 50% germination of onion extract treated infected seeds. Our results explain that Garlic and Neem extracts shown 58.5% and 81% fungal infested seed germination at 8% concentration which are nearer to the findings of Ahmad (2013) who mentioned 68.39% and 66.90% germination of infested rice seeds by application of Garlic and Neem extracts respectively at 1:1 ratio.

CONCLUSION

Excess use of chemicals all over the world has increased the toxic effects in the surrounding environment so in search of alternatives, we studied some organic extracts which proved successful in minimizing the growth of *Aspergilus flavus* and also improved the germination percentage of infected rice seeds.

ACKNOWLEDGEMENT

We acknowledge the reviewers for their critical analysis of the manuscript and are also thankful to all those who supported us in writing up these research findings.

CONFLICT OF INTEREST

The authors verify having no interest in competition and have no conflicts of interest

REFERENCES

Agrios, George N., 2005. Plant Pathology: Fifth Edition. Elsevier Academic Press. p. 922.

Ahmed, M., Hossain, M., Hassan, L., Dash, C.K., 2013. Efficacy of Different Plant Extract on Reducing Seed Borne Infection and Increasing Germination of Collected Rice Seed Sample. Univ. J. Plant Sci., 1(3): 66-73.

Alexopoulos, C.J., 1996. Introductory Mycology. John Wiley & Sons, Inc. p. 869.

Arshad, H.M.I., Khan, J.A., Naz, S., Khan, S.N., Akram, M., 2009. Grain discoloration disease complex a new threat for rice crop and its management. Pak. J. Phytopathol., 21 (1): 31-36.

Ashfaq. M., Shaukat, M.S., Akhter, M., Haider, M.S., Mubashar, U., Hussain, S.B., 2015. Comparison of fungal diversity of local and exotic rice (Oryza Sativa l.) Germplasm for their seed health. J. Animal & Plant Sci., 25(5):1349-1357

Barnett, H.L., and Hunter, B.B., 1990. Illustrated genera of imperfect fungi. Macmallian Publishing Company, New York, U.S.A. 4: 1-218.

Bennett, J.W., 2010. "An Overview of the Genus Aspergillus" From: Aspergillus: Molecular Biology and Genomics Machida and Gomi, Eds. Caister Academic Press. ISBN: 978-1-904455-53-0

Bhatnagar, D., Zeringue, H.J., and Cormick, S.P., 1990. Neem leaf extracts inhibit aflatoxin biosynthesis in Aspergillus flavus and A. parasiticus. In Proceedings of the USDA neem workshop Beltsville, Maryland: US Department of Agriculture. pp. 118–127.

Ehrlich, K.C., Montalbano, B.G., Cotty, P.J., 2005. Divergent regulation of aflatoxin production at acidic pH by two Aspergillus strains. Mycopathologia., 159(4):579-581.

Ezuka, A., Kaku, H., 2000. A historical review of bacterial blight of rice. Department of Genetic Resources II and I. Bull. Nalt. Inst. Agrobiol. Resour, 15: 1-207. Smith, A. and D. Bruce, 2000. The Emergence of Agriculture. Scientific American library, A division of HPHLP. New York, USA

Fakir, G.A., Hossain, I., Ahmed, M.U., Doullah M.A.U., Alam, M., 2002. Quality of farmers Boro and T. Aman rice seeds collected before sowing from Bogra, Rajshahi and Rangpur districts of Bangladesh. A paper presented in the review and planning meeting of the Rice Seed Health Improvement (SHIP), PETRRA project held on 17-18 April at BRRI, Gazipur, Bangladesh.

Ghorbanian, M.R., Abyaneh, M., Allameh, A.S., Ghahfarokhi, M., Qorbani, M., 2008. Study on the effect of neem (*Azadirachta indica*) leaf extract on the growth of Aspergillus spp and production of aflatoxin by it at different incubation times. Mycoses., 51(1):35-39.

Habib, A., Javed, N., Sahi, S.T., Waheed, M., 2012. Detection of seed borne mycoflora of different coarse and fine rice varieties and their management through seed Treatments. Pak. J. Phytopathol., 24(2):133-136.

Horn, B.W., Moore, G.G., Carbone. I., 2009. "Sexual reproduction in Aspergillus flavus". Mycologia. 101 (3): 423–429. doi:10.3852/09-011

Javed, M.S., Wahid, A., Idrees, M., Gill, M.A., Saleem, A., 2002. Seed mycoflora studies in rice. Pak. J. Phy., 14(2): 132-134.

Khan, S.A., Anwar, S.A., Bhutta, A.B., 1990. Studied on seeed borne fungi, Bacteria and nematodes of rice in the Punjab. Pak. J. sci. Ind. Res., 33 (11): 489-492.

Khan, J.A., Jamil, F.F., Gill, M.A., 2000. Screening of rice varieties/ lines against bakanae and baterial leaf blight (BLB). Pak. J. Phytopathol., 12: 6–11.

Khan, J.A., Arshad, M.I., Jamil, F.F., Hasnain, S., 2009. Evaluation of rice genotypes against bacterial leaf blight (BLB) disease. Pak. J. Phytopathol., 21: 26–30.

Kim. J. G., 2010. Inhibitory effect of green onion on the growth of Aspergillus spp. Apl. Micro. Bio. and Biotch.457-462.

Locke, J.E., 1995. Fungi. In: The Neem Tree, source of Unique National Products for Integrated pest Management, Medicine, Industry and Other proposes. (Ed.): H. Schmutterer, V.C.H. Weinheim, Germany. pp. 118-125.

Machida, M., Gomi, K., (2010). Aspergillus: Molecular Biology and Genomics. Horizon Scientific Press. p. 157.

Mathur, S.B., Kongsdal, O., 2003. Common laboratory seed health testing methods for detection fungi, Published by the ISTA, Switzerland. 1:393-399.

Naher, L., Tan, S.G., Yusuf, U.K., Ho, C.L., Abdullah, F., 2012. Biocontrol Agent Trichoderma harzianum Strain FA 1132 as An Enhancer of Oil Palm Growth. Pertanika J. Trop. Agric. Science., 173 – 182.

Nghiep, H.V., Gaur, A., 2005. Efficacy of seed treatment in improving Seed quality in rice. Omon rice., 13: 42-51.

Nguefack, J., Leth, V., Lekagne Dongmo, J.B., Trop, J., Amvam Zollo, P.H., Nyasse, S., 2008. Use of three essential oils as seed treatments against seed borne fungi of rice. American-eurasian J. Agric. And Environ. Sci., 4(5): 554-560.

Niaz, I., Kazmi, S.A.R., 2005. Neem seed coat oil fractions on stored grain fungi. INT. J. Biol. Biotech., 2(3): 705-706.

Omidpanah, S., Sadeghi, H., Sarcheshmeh, M.M., Manayi, A., 2015. Evaluation of antifungal activity of aqueous extracts of some medicinal plants against Aspergillus flavus, pistachio aflatoxin producing fungus in vitro. Drug Dev. Therap., 6(2). DOI: 10.4103/2394-6555.162446

Perry, L., Adams, R.D., Bennett, A.R., Lupton, D.J., Jackson, G., Good, A.M., Thomas, S.H., Vale, J.A., Thompson, J.P., Bateman, D.N., Eddleston, M., 2014. National toxicovigilance for pesticide exposures resulting in health care contact – An example from the UK‘s National Poisons Information Service. Clinical Toxicol., 52, 549-555.

Ramírez-Camejo, L. A., Zuluaga-Montero, A., Lázaro-Escudero, M. A., Hernández-Kendall, V. N., Bayman, P., 2012. "Phylogeography of the cosmopolitan fungus Aspergillus flavus: Is everything everywhere?". Fungal Biol., 116 (3): 452–463.

Sitara U., Niaz, I., Naseem, J., Sultana, N., 2008. Antifungal effect of essential oils on in vitro growth of pathogenic fungi. Pak. J. Bot., 40(1): 409-414.

Smith, A., Bruce, D., 2000. The Emergence of Agriculture. Scientific American library, A division of HPHLP. New York, USA

Steel, R.G.D., Torrie, G.H., 1997. Principles and Procedures of Statistics: A Biochemical Approach, 3rd ed. Mc Graw-Hill, New York, U.S.A.

Sundar, A.R., Das, N.D., Krishnaveni, D., 1995. In-vitro Antagonism of Trichoderma spp. against two Fungal Pathogens of Castor. Indian. J. Pl. Prot., 23: 152-155.

Tijjani, A., Adebitan, S.A., Gurama, A.U., Aliyu, M., Haruna, S.G., Mohammad, G.U., Mus’ab, I., 2014. Invitro and Invivo Efficacy of Some Plant Extracts for the Control of Tomato Fruit Rot Caused by Aspergillus Flavus. Int. J. Sci. Res. Pub., 4(4): 1-5.

Udomsilp, J., Piyo, A., Khun, P.K., Thobunluepop, P., 2009. Antifungal properties of essential oils from Thai medical plants against rice pathogenic fungi. As. J. Food Ag-Ind. Special Issue., S 24-30.

Wahid, A., Javed, M.S., Indrees, M., Gill, M.A., 2001. Association of F. solani to rice seeds in the Punjab, Pakistan, Abstracts Published in 3rd Nat. Conf. Pl. Path. 1-3. October, 2001 at NARC, Islamabad. 41pp.

Zahid, M.A., Akhtar, M., Sabir, M., Anwar, M., Jamal, A., 2005. Genotypic and phenotypic correlation and path analysis in coarse grain rice. Proceeding of the International seminar in rice crop. Oct 2-3. Rice Research Institute, Kala Shah Kaku. 29.