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Use of Neem (*Azadirachta indica)* seed powder to treat groundnut seed-borne pathogenic fungi

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ABSTRACT

Plant seeds powder and extracts are being used to control the diseases since last several years. Extracts of the various plant parts like leaf, stem, root, fruit and seeds are found to be effective against seed-borne pathogenic fungi. The present study involved groundnut seed health test for seed-borne fungi and the evolution of Azadirachta indica seed powder as seed treatment bio-fungicides. These materials was used at the concentration of 0.1%, 0.5%, 1.0%, 2.0%, 5.0% 10.0% and control, while Apron plus was used at the recommended dose and two times the recommended dose the later being included for comparative purposes. The following fungi Aspergillus niger, Aspergillus flavus, and Rhizopus species were isolated from both untreated seeds. Treatment with Apron plus was found to be more effective than Azadirachta indica seed treatment which was found to significantly reduce the prevalence of Most of the isolated fungi the reduction being dosage dependent, Aspergillus niger is found to show resistant to neem (Azadirachta indica) seed powder. The application of plant material to control pathogenic fungi is receiving attention among scientist worldwide. The potential of Azadirachta indica seed powder to treat pathogenic seed borne fungi is promising. This plant seed powder can possibly be exploited in the management of seed-borne pathogenic fungi to prevent biodeterioration of seeds in an eco-friendly way.

INTRODUCTION

The use of plant material to treat groundnut seed-borne pathogenic fungi is a means of finding ways through bio-technology to treat stored groundnut pathogenic seed-borne fungi, with plant material neem (*Azadirachta indica*) seed powder.

Seed is the basic input in agriculture, the quality of seed used by farmers determines the "structure" of agriculture they practice. However for maximum gain in productivity the use of both improved varieties and improved integrated crop management practice is required. Not only do they contribute to the increasing productivity individually but they also act synergistically.

Groundnut is the sixth important oil seed crop in the world, it contains 48% - 50% oil and 26 - 28% protein, is rich in dietary fibers, minerals and vitamins. Groundnut is grown on 26.4 million hectare worldwide with a total production of 37.1 million metric tonnes and average productivity of 1.4 metric t/ha, (FAO 2003). Over 100 countries worldwide grow groundnut. The production of groundnut is concentrated in Asia and Africa 86-40% of the global area and 68-25% of the global production respectively: groundnut (*Arachis hypogea L.*) belong to the genus *Arachis* in sub tribe *stylosathine* of tribe *aeschynomenea* of family *leguminosae* (Nigam *et al.*, 1983).

Groundnut is first dried after harvesting; the primary objective of drying is to avoid *aflotoxin* contamination. Harvested plants should be staked for days to allow them to dry in the sun before stripping the pods then drying

should be continued until the moisture content is reduced to 6-8%. This can normally be achieved by drying the Pods in the sun for more days. Groundnut can easily store in bulk, clean jute, polyethylene or fiber bags, to ensure the best protection of groundnut should only be stored in bags or drum. Seed can be stored and conserved; it can be shelled or stored in unshelled form. Groundnut is best stored unshelled in cool dry conditions, it should be protected from rain and vermin (particularly rats, and mice). In Nigeria, shelled groundnuts are stored in sacks in covered warehouses and as pyramids in the open air.

Fungal diseases are known to cause great damages all over the world. Christesen and Kaufman (1974) reported that the fungi are the major causes of spoilage in grains and seeds and probably rank second to insects as a cause of spoilage and loss in all kinds of Agricultural products throughout the world. Different species of *Alternaria, Aspergillus, Ceratobasidium, Cercospora, Cochliobolus, Curvularia, Dreschslera, Fusarium, Gaeumannomyces, Microdochium, Penicillium, Pyricularia, Pythium, Rhizoctonia, Rhizopus, Sclerophthora, Trichoderma* and *Tricoconella* are most common associates of seeds all over the world, causing pre-and post-infections and considerable quality losses *viz.* seed abortion, seed rot, seed necrosis, reduction or elimination of germination capacity, seedling damage and their nutritive value have been reported (Miller, 1995; Janardhana *et al.,* 1998; Kavitha *et al.,* 2005). Seed treatment is the safest and the cheapest way of control of seed-borne fungal diseases and to prevent bio-deterioration of grains (Chandler, 2005; Bagga and Sharma, 2006).

Numerous fungi can invade the seeds damage the endosperm and embryo especially under moist cool condition (Schumutter 1990). The presence of fungi on seeds has been of concern to plant pathologists since various types of disease may be carried in seedlings and crops. Fungi present on seeds can initiate infection on seedlings and mature crops. There is thus a need to treat the seeds before sowing. Presently, seed treatment chemicals are used in stored grains, the rising cost of such chemicals and the other inputs necessitates a search for other alternatives which will not only be affordable and available but also environmentally friendly.

The species of *Aspergillus* are considerably involved in the deterioration of stored seeds; under certain circumstances they affect the seeds in such a way that they are made the seeds valueless for planting, *Aspergillus* also make the grains unpalatable or substances which are toxic to man and animals, the fungus when present in groundnut seeds produces *Aflotoxin* which is highly toxic, the species of *Aspergillus* are considerably involved in deterioration of stored grains and seeds (Christensen and Kaufman 1974). The most common means of controlling plant disease is by chemical compound, the control of seed-borne disease is mostly through the use of seed treatment chemical as Adrex T, Farnasan D, Apron plus, Vincent P, Stylar C, etc.

Some plants were found to have pesticide properties and these plants are being exploited for use in Agriculture pest control. One of these plants will be discuss in this paper neem (*Azadirachta indica)* seed powder.

The neem tree (*Azadirachta indica)* and its chemical products have been used for centuries in many facets for human utility—as an agrochemical, pesticide, food, heating source, wood, soil amendment and significant healing agent for purportedly over 100 diseases (Pamela 2009). The neem tree (*Azadirachta indica)*, a member of the Meliaceae family (Mahogany family), Neem populations are heterogenous in all respects, growing greatly to differences in soil and climate. The trees themselves are known to have genetic variation in height, branching type, leaf form, and colour (Muñoz-Valenzuela 2007).

Neem (*Azadirachta indica*) has impressive and far-reaching pesticide properties. Recently, neem (*Azadirachta indica*) has been suggested as an effective infertility agent in controlling populations of rodents, such as rats (Morovati *et al.,* 2008). It has also been shown to be effective in controlling food borne pathogens, making it a potential agent against food spoilage bacteria (Hoque *et al.,* 2007).

MATERIALS AND METHODS

For the purpose of this study, variety of seed used, was X-Dakar. The dishes were washed thoroughly using detergent and rinsed properly under running tap water, after which they were sterilized in air oven for a period of half (½) - 2 hours at temperature of 160^0C to keep them free from any contamination. The sterilized Petri dishes were then allowed to cool before pouring of media.

The experiment was replicated with each material containing 5 seeds/ Petri dish. It is then incubated at room temperature for 5 days after which the dish is to be examined, for seed-borne fungi using stereoscopic microscopic and compound microscope.

Seeds of X-Dakar were plated out on both 3 layers of whatman's filter paper (moisture with sterile distilled water), and PDA plates at the rate of 5 seeds/plate. There were 20 replicate plates per each method. Control-seeds are to be soaked in distilled water for 24 hours and the control treatment seeds are to be without any treatment. The plates were incubated at room temperature for 5 days after which the prevalence of fungal growth on the seeds were observed and recorded as percentage using this formula:

A/B.

Where
A = Number of seeds with fungal growth.
B = Total number of seed.

The different types of fungi present on the seeds (PDA plates) were also observed and sub-culture into fresh PDA plates and inoculated for 5 days to get pure culture of each which was used for identification. For identification a piece of mycelia was taken from each colony, transferred into water in the center of a clean slide, teased out and *lactophenol* cotton blue added and then covered with a cover slip. The preparation was passed over a flame to remove any air bubbles present. The slide was then observed under the microscope at x40 magnification and identified was made based on both the vegetative and reproductive features.

For this study neem (*Azadirachta indica)* seed powders as well as the chemical Apron plus were used. The plant material was used at the rate of 0.1%, 0.5%, 1.0%, 2.0%, 5.0 %, and 10%. The above concentrations were prepared by dissolving 1g, 5g, 10g, 20g, 50g and 100g of the material in one liter of distilled water. Respectively Apron plus was tested at the recommended rate 2kg of seed to 10g and two times the recommended rate (X2). For each concentration, there were 3 replicate plates. The control consists of untreated seeds which were plated into PDA plates. All plates incubated for 5 days at room temperature after which the seeds were observed for fungal growth and the prevalence recorded. Different fungi were isolated into fresh PDA to obtain pure cultures, from these pure cultures, slides were prepared and identification of the fungi was made.

RESULTS AND DISCUSSION

The antifungal activity of neem (*Azadirachta indica)* seed power against seed borne fungi is presented in table 1. The result shows that the groundnut variety X Dakar seeds are highly infected with seed-borne fungi, using the filter paper method, 95% of the seeds had seed-borne fungi, and with the PDA method 100% of the seeds had fungal growth. The following fungi were isolated from the seeds *Aspergillus niger, Aspergillus flavus, Rhizopus* species, and one unidentified species as shown in table 2.

TABLE 1. Prevalence of fungi on seed before treatment with chemical and *Moringa oleiofera* seed powder

Ground nut Variety	Untreated with Filter paper	Untreated with PDA	Distilled water treatment	Zero treatment With PDA
X-Dakar	94%	100%	31%	100%

TABLE 2: Prevalence of fungi on seed after treatment using PDA and *Moringa oleiofera* seed powder at different concentration

Ground nut variety	Fungal colony	Zero treatment	0.10%	0.50%	1.00%	2.00%	5.00%	10.00%	Apron plus	Apron plus(x2)
X-Dakar	*Aspergillus niger*	100%	100%	100%	100%	93.30%	100%	100%	52.0%	49.0%
	Aspergillus flavus	100%	93.3%	86.7%	80.0%	80.0%	53.3%	53.3%	4.0%	1.0%
	Rhizopus species	100%	53.3%	13.3%	13.3 %	6 .7%	3.3%	0%	0%	0%
	Unidentified species	100%	43.3%	6.7%	3.3%	0%	0%	0%	0%	2.0%

The prevalence of fungi on seeds that have been treated with neem (*Azadirachta indica)* seed powder at the test concentration compares with that under control and Apron plus treated seeds, shows that *Aspergillus niger, Aspergillus flavus, Rhizopus* species and other unidentified species were also isolated using neem (*Azadirachta indica)* seed powder, but the prevalence were significantly reduced compared with these of untreated seeds except for *Aspergillus niger*. The result also reveals that the effectiveness of neem (*Azadirachta indica)* seed powder was dosage dependent, the higher the dosage the greater the reduction in prevalence of the fungi on the seed.

The difference in concentration of *Azadirachta indica* did not reduce the prevalence of *Aspergillus niger*. For *Aspergillus flavus* the concentration *Azadirachta indica* in which further increase reduce the prevalence, *Rhizopus* species was found to be very sensitive to *Azadirachta indica* seed powder it is also dose dependent the prevalence was 0.1% (53.3%) and 0.5% (13.3%), 1.0% (13.3), 2.0% (6.7), 5.0% (3.3). For the unidentified species it shows to

be sensitive to *Azadirachta indica* seed powder as it reduce the prevalence and it shows no growth at the concentration of 0.5%. Apron plus was found to be effective than the *Azadirachta indica* seed powder in the control of the unidentified species.

The control of fungi has been achieved previously by the use of chemical fungicides such as Apron plus, Farnansan D, Styler C, Aderex T. the cost of this chemicals is high and their availability cannot be guaranteed, environmentally not friendly they also tend to be poisonous to the consumers. Those factors limited their use at the peasant farmer's level under which most of the groundnuts in Nigeria is produced. The effect of these chemicals on the environment and on non-target organism is also another disadvantage.

Azadirachta indica seed powder was found to be effective for the control of *Aspergillus niger, Aspergillus flavus* and *Rhizopus* species. This is an important finding in view of the fact that neem (*Azadirachta indica)* from which the seed powder was prepared.

There is a lot of scope for the use of plant materials, the potentials of neem (*Azadirachta indica)* in this aspect is emerging. The use of plant materials for controlling plant disease is a fertile ground for research. Secondly, the technology of preparation is simple, pounding and soaking the required concentration in water and applying on stored ground nut. Another significance of this finding is the potential of this preparation to serve as an alternative to Apron plus, which is a fungicide recommended for seed treatment. These fungicides, though not expensive is not available in the remote rural area.

Rhizopus species cause rust of seed in the field under high temperature and humid condition, (Neegard, 1986). Thus the effectiveness was highly sensitive to neem (*Azadirachta indica)* seed powder is a very welcome development, all that is needed to get rid of *Rhizopus* species from groundnut seeds is 5.0% neem (*Azadirachta indica)* seed power which is very low and will be very economical. These results show that the neem (*Azadirachta indica*) seed powder is well endowed with biologically active compounds that some pathogenic fungi are sensitive to them. Thus it can be used as a natural source for the control of pathogenic fungi

Many reports revealed that, plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides (Varma and Dubey, 1999; Harborne, 1998; Gottlieb *et al.*, 2002). Even though effective and efficient control of seed borne pathogenic fungi can be achieved by the use of synthetic fungicides, the same cannot be applied to grains for reasons of pesticide toxicity (Wodageneh *et al*., 1997; Harris *et al*., 2001). Thus, there is a need to search for alternative approaches to store seeds, grains/cereals for human consumption without toxicity problems that are eco-friendly and not capital intensive.

REFERENCES

[1] Bagga, P.S. and Sharma, V.K, *J. Mycol. Plant Patho*l. **2006**, 59, 305–308.
[2] Christensen, H.H and Kaufman, C. M, *An Association Cereal Management:* **1974**, Pp159.
[3] Chandler, J., *Int. J. Pest Control*, **2005**, 42, 2, 257– 260.
[4] Food Agriculture Organization, (FAO), *Groundnut Seed Control Manual*, **2003**, Pp 15.-16.
[5] Gottlieb, O.R., Borin, M.R. and Brito, N.R., *Phytochemistry* **2002**, 60, 2, 145–152.
[6] Harris, C.A., Renfrew, M.J. and Renfrew, M.W., *Food Additive. Contam* , **2001**, 18, 12,1124–1129.
[7] Harborne. J.B., *Phytochemical methods*: A guide to modern techniques of plant analysis. 3rd ed. Chapman & Hall Pub., London, UK, **1998**, pp.7–8.
[8] Hoque, M. Bari, ML, Inatsu, Y, Juneja, VK, Kawamoto, S., *Foodborne Pathog Dis.*, **2007**, 4, 4, 481-488.
[9] Janardhana, G.R., Raveesha, K.A. and Shetty, H.S, *J. Sci. Food Agric*, **1998**, 76, 4, 573– 578.
[10] Kavitha, R., S.Umesha and Shetty, H.S., *Seed Res*, **2005**, 33, 2, 187–194.
[11] Morovati, M. Mahmoudi, M. Ghazi-Khansari, ., Khalil Aria, A. Jabbari, L., *Turk J Zool*, **2008**, 32, 155-162.
X BN]
[12] Munoz-Valenzuela S, Ibarra-López, AA, Rubio-Silva, LM, Valdez- Dávila, H. Borboa-Flores, J., *Neem tree morphology and oil content*. *Issues in new crops and new uses.* ASHS Press, **2007**.
[13] Miller, J.D., *J. Stored Product Res*, **1995**, 31,1, 1–16.
Nigram, F.R., *Field Crop disease*. Von No strand Rein hold, New York, **1983**, 465-499.
[14] Neegard, P., A source of hunger or source of life. *Species lecture series 94, Danish government institution of seed pathology for the developing countries*. Copenhagen Denmark, **1986**, Pp176.
[15] Pamela, P., Neem the Wonder Tree: Its Pesticide and Medicinal Applications. *Master in Chemical and Life Sciences. University of Maryland,* **2009**.
[16] Schumutter, H., *Annual Review of Entomology*,**1990**, 35, 271-298.

[17] Varma, J. and Dubey, N.K. *Curr. Sci.* **1999**, 76, 2, 172–179.
[18] Wodageneh, A. and Wulp, H.V.D, *Pestic. Information*, **1997**, 23, 1, 33–36.