
**FUNGI ASSOCIATED WITH MUNG BEAN (*VIGNA RADIATA*) SEEDS AND THEIR
CONTROL MANAGEMENT WITH *MONODORA MYRISTICA* AND *PIPER
GUINEENSE***

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SUMMARY

Mung bean (MB) (*Vigna radiata*) seed is a very important pulse infected with many fungi which causes reduced viability, germination, vigour and yield. Accurate diagnosis of seed-borne fungi and their severity is a crucial step towards assessing probable crop losses and taking appropriate management measures in mung bean production and protection program. Information seed-borne fungi of MB and its mitigation is lacking in Nigeria. Therefore, fungal pathogens infecting mung bean seeds (MBS), their effects on seed and its germinability were investigated. Also, effects of the botanicals *Piper guineense* and *Monodora myristica* on fungi were evaluated. Two MB varieties MN92 and MN94 from Agronomy Department, of Michael Okpara University of Agriculture, Umudike were taken to the laboratory for fungi isolation, identification, pathogenicity test and evaluation with *Monodora myristica* and *Piper guineense* for antifungal activity against seven seedborne fungi. Experimental design was complete randomized design with three replicates. Data were analyzed using ANOVA at $\alpha_{0.05}$. *Aspergillus tamarii*, *A. flavus*, *A. ochraceous*, *A. niger*, *Paecilomyces*, *Botryodiplodia theobromae*, *Fusarium* sp, *Rhizopus* sp, *Phomopsis* sp, *Penicillium chrysogenum*, *P. oxalicum* and *P. montanense*, were encountered in MBS. *Aspergillus* species were the most predominant, followed by *Penicillium* species while *Botryodiplodia theobromae* was the least in both MB varieties. *Aspergillus tamarii*, *A. flavus*, *A. ochraceous*, *A. niger*, *Botryodiplodia theobromae*, *Rhizopus* sp and *P. oxalicum* caused MBS rot. *Monodora myristica* and *P. guineense* at 20% concentration reduced fungi growth considerably up to 22.0-80% and 22.0-85%, respectively. Both plant products could be used to mitigate fungi contamination of Mung bean seeds.

Keywords: Mung bean, seed-borne fungi, seed germinability, Botanicals, antifungal

MUNG BEAN (*Vigna radiata* L. Wilczek) is an annual, semi erect to erect or sometimes twining, deep-rooted legume (Itoh *et al.*, 2006; Espin *et al.*, 2007). Mung bean looks more like a garden bean than like a soya bean plant. Pods mature in about 20 days after flowering (Itoh *et al.*, 2006).

Its short growth duration allows adaptation to many cropping systems (Shanmugasundaram *et al.*, 2009).

The crop is of ancient cultivation in India and is not found in a wild state. It is probably derived from *Phaseolus radiatus* L., which occurs wild throughout India and Burma, and is occasionally cultivated. It is a pulse species of the pan-tropical genus *Vigna* (Saranavakumar *et al.*, 2004) that is native to Asia but widely cultivated in Africa, Asia and Latin America (Tamooka *et al.*, 1992). Annual world mung bean production is estimated at 3 million metric tons harvested from about 5.5 million hectares (Weinberger, 2003; Samson *et al.*, 2004), of which 90% is in South Asia (Shanmugasundaram, 2004). In sub-Saharan Africa, mung bean production still depends on the small-seeded traditional varieties, which reach maturity in 90 -110 days and are low yielding, producing only about 200 - 500 kg ha⁻¹ (Agugo and Chukwu, 2011). The desired characteristics and performance of the ideal variety needed to transform mung bean from a marginal to a major crop in sub-Saharan Africa include synchronous maturity, larger seeds, higher seed quality and yield, and resistant to diseases and insect pests.

Mung bean can provide significant amounts of protein (240 g kg⁻¹), carbohydrate (6g kg⁻¹) and a range of micronutrients in diets (Anwar *et al.*, 2007). Mung bean protein and carbohydrates are easily digested and create less flatulence than those derived from other legumes (Fleming, 1981). The mung bean lysine content of 504 mg g⁻¹ (Saini *et al.*, 2010) makes it a good supplement for most cereal based diets which lack this essential amino acid (Baskaran *et al.*, 2009). In addition, mung bean is lower in phytic acid, which is commonly high in cereal and other legume crops, and has a negative impact on iron and zinc bioavailability in plant-based diets. Thus, mung bean is a major protein supplement in cereal-based diets. It is eaten as boiled beans soup or mung bean pancake (Gwag *et al.*, 2006). Mung bean has been used as an iron-rich whole food source for baby food (Giridher and Reddy, 1997). Due to its high protein content and digestibility, consumption of mung bean seeds in combination with cereals has been recommended to significantly increase the

quality of protein intake as part of a vegetarian diet. of isolates from mung bean seeds (Kudre et al., 2013b). It can fix atmospheric nitrogen and improve the soil fertility.

Despite all these qualities; there are many constraints responsible for the lower yield of mung bean, however, diseases are considered dominant constraints. Amongst the production constraints, pest and diseases are the major ones. In Nigeria, sixteen diseases affecting both the foliage and seeds of mung bean have been recorded. Seeds that developed on severely infected plants are small and immature (Shevkani *et al.*, 2015).

Extract of different plant such as garlic, bitter gourd and neem were effective against fungi associated with many crop seeds. Seed health is an important factor in the control of diseases, since an infected seed is less viable, has low germination, reduced vigour and reduced yield (Shevkani *et al.*, 2015). The control of seedborne pathogens is the first step in any agricultural crop production and protection programme. Attempts have been made to reduce s seed-borne infection by chemical treatment of the seeds and some successes have been reported.

Seed-borne fungi are of considerable importance due to their influence on the over-all health, germination and final crop stand in the field. Farmers have to deal with significant losses due to infections by serious seedborne pathogens on their plants, which may start from germinating seed, seedling in the nursery, matured plants in the field and proceed till the products are harvested and seeds stored. Significant crop losses due to seedborne pathogens have been recorded. Damages due to seed-borne pathogens include seed abortion, reduced seed size, shrunken seeds, seed rot, seed necrosis and seed discolouration, all considerably affect seed quality (Muthii *et al.*, 2014).

Accurate diagnosis of seed borne diseases and their severity is a crucial step towards assessing probable crop losses and taking appropriate management measures. This necessitates the need for proper disease diagnosis that will enhance proper management which will ensue clean seeds are

used for planting. It is therefore, necessary to search for seed quality control measures that are cost effective, ecologically sound and environmentally safe to eliminate or reduce incidence of pathogens of economic importance to increase both seed germination, establishment and yield of plant crops.

Therefore, the findings of this study will pinpoint the prospects and problems of seedborne pathogens of mung bean and its effect on seed germinability. The objectives of this study therefore are: to identify fungal pathogens associated with mung bean seeds, determine the effect of the fungal pathogens on seed germinability and the effect of selected botanicals on fungi infecting mung bean seeds.

MATERIALS AND METHODS

Source of Mung bean seeds and botanicals

Mung bean seeds were collected from Agronomy Department, College of Crop and Soil Sciences, Michael Okpara University of Agriculture, Umudike. Two varieties of mung bean namely: MN 92 and MN 94 were obtained. Uziza (*Piper guineense*) and Ehuru (*Monodora myristica*) seeds were purchased from the Orié Ugba market in Umuahia dried properly and then ground to fine powder for use in this study.

Isolation of Fungi from Mung bean and identification of isolated fungi

Fungal contaminants were isolated and detected by agar plate method. Ten seeds were plated on each 9 cm Petri dish in triplicates. Mung bean seeds were plated on prepared and solidified Potato Dextrose Agar (PDA) in Petri dishes. The seeds were incubated for 5-7 days, and each fungi colony was transferred to fresh PDA in Petri dishes to obtain pure cultures of fungi. Different fungal colonies that emerged were counted and identified by making slides from fungal colonies; mounting on the Olympus reflected light optical microscope and using mycological reference books and research articles (Poehman, 1991; Barnett and Hunter, 1999; Alexopoulos *et al.*, 2002). Different fungal colonies were counted and recorded.

The total number of each isolate in all samples was obtained against the total number of all the isolates in all the samples screened. Frequency of occurrence was determined using the method described by (Giridher and Reddy, 1997).

$$\text{Percentage incidence} = \frac{\text{No of observations in which a species appeared}}{\text{Total number of observation}} \times 100$$

Pathogenicity test

Healthy mung bean seeds were surface sterilized using 10% sodium hypochlorite and washed in three changes of sterile distilled water before ten seeds were inoculated with spore suspension of identified fungi. The inoculated seeds were placed on two layers of a moistened filter paper and incubated for 7 days. The control was inoculated with sterile water. Observation of visible growth of fungi on seeds and microscopic visualization was to confirm that fungi growing on seeds were same as the fungi used for inoculation.

Effect of isolated fungi on Mung bean seed germinability

Mung bean seeds were surface sterilized, washed and then inoculated with each fungus and plated on moistened filter paper as described above. The percentage germination (i.e. sprouted seeds to form seedling) of the inoculated seeds was recorded. The control was inoculated with sterile water. The experiment was laid in CRD with three replicates.

Colonization percentage was determined by visual assessment of seed colonization of the scales of 1-10 as follows: 1= 10% of seeds covered by fungal mycelium, 2= 20% of seeds covered by fungal mycelium, 3= 30% of seeds covered by fungal mycelium, 4= 40% of seeds covered by fungal mycelium, 5= 50% of seeds covered by fungal mycelium, 6= 60% of seeds covered by fungal mycelium, 7= 70% of seeds covered by fungal mycelium, 8= 80% of seeds covered by fungal mycelium, 9= 90% of seeds covered by fungal mycelium and 10= 100% of seeds covered by fungal mycelium

Effect of botanicals on fungi Infecting Mung bean seeds

Piper guineense and *M. myristica* seeds purchased and processed as described earlier were used in this study. Using the modifications of Obani and Ikotun (2014), 20g of *Piper guineense* and *M. myristica* seeds powder was separately suspended in 100ml of sterile water for 6 hours, filtered with cheese cloth into clean bottles separately; then 20 ml of each botanical extract was used to treat healthy seeds (i.e. seeds from pathogen free seed lot as determined by blotter test) of mung bean for 3 hours and then air dried for six hours before inoculation with different fungi and then plated out on PDA in 9cm Petri dishes. This was incubated for 6 days. The controls were inoculated with sterile distill water. Percentage fungal growth were recorded after the incubation period. The percentage fungi colonization on treated seeds were recorded and percentage fungal growth reduction was calculated using the formula below as reported by Obani and Ikotun (2014):

$$\text{Percentage reduction} = \frac{\text{Control} - \text{treated}}{\text{Control}} \times 100$$

Statistical Analysis

Data were analyzed using analysis of variance of the SAS (version 9.4, SAS Institute, Cary, NC). Means were compared using least significant difference test (LSD) at P = 0.05 procedure in SAS (SAS Institute Inc., Cary, NC, USA) to compare the differences among the means of samples.

RESULTS

Percentage incidence of fungal isolates

Seven fungal genera belonging to *Aspergillus* (*A. tamarii*, *A. ochraceus*, *A. niger*, *A. flavus*), *Rhizopus* sp, *Fusarium* sp, *Phomopsis* sp, *Paecilomyces* sp, *Botryodiplodia theobromae* and *Penicillium* species (*P. oxalicum*, *P. montanense* and *P. chrysogenum*) were encountered in the two mung bean varieties studied. *Aspergillus tamarii* (40.0%, 60.0%), *A. Ochraceus* (100.0%, 0.0%), *A. niger* (40.0%, 60.0%), *A flavus* (71.4%, 28.6%), *Rhizopus* sp (100.0%, 0.0%), *Fusarium* sp (100.0%, 0.0%), *Paecilomyces* sp (100.0%, 0.0%), *Botryodiplodia theobromae* (66.7%, 33.3%),

P. oxalicum (71.4%, 28.6%), *P. montanense* (75.00, 25.0%), *P. chrysogenum* (100.0%, 0.0%) and *Phomopsis* sp (100.0%, 0.0%) were encountered in MN 92 and MN 94 respectively ((Fig 1). In MN 92 *Penicillium chrysogenum*, *Rhizopus* sp, *Aspergillus ochraceous*, *Fusarium* sp and *Paecilomyces* sp (100.0%) were the most prevalent species followed by *Penicillium montanense* (75.0%), while *Aspergillus tamarii* and *A. niger* (40.0%) were the least. In MN 94 *Phomopsis* sp (100.0%) was the most prevalent fungus, followed by *Aspergillus tamarii* and *A. niger* (60.0%), while *Penicillium montanense* (25.0%) was the least (Figure 1).

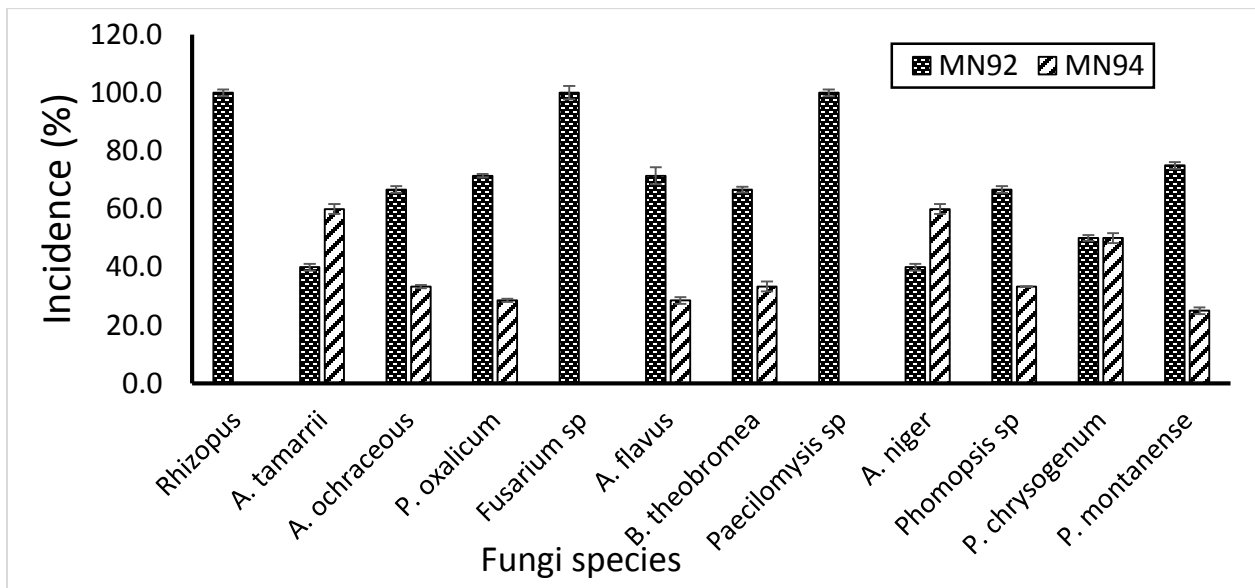


Figure 1: Percentage incidence of fungal species isolated from Mung bean seeds

Table 1 shows the colonization and germination percentages of mung bean inoculated with various isolated fungi. For colonization percentage, *Phomopsis* did not colonize the mung bean seeds. The seeds inoculated with *A. ochraceous* had the highest colonization percentage of 55.00%, followed by *Rhizopus* (47.5%) while the control (0.0%) and *Phomopsis* (0.0%) had no growth.

Fusarium sp, *Paecilomyces* sp, *A. tamarii*, *A. ochraceous*, *Penicillium oxalicum*, *P. montanense*, control and *Phomopsis* had 100.0% germination, followed by *Rhizopus* sp 92.5% while *A. flavus*

(50.0%) had the least germination which was significantly lower than that recorded in others. It is worthy of mention that after germination the seedling rotted as a result of the colonization of the fungi.

Table 2 shows the effects of *Piper guineense* and *Monodora myristica* extracts on the percentage growth and reduction of different fungi isolated from mung bean seeds. In *Piper guineense* treated seeds the control had higher percentage seed colonization (95.00%) while the treated seed had (80.00%) for *Rhizopus*; and there was

Table 1: Effects of Isolated Fungi on mung bean seeds and it's Germinability

	Colonization (%)	Germination (%)
<i>Rhizopus</i> sp	45.50	92.50
<i>Aspergillus flavus</i>	37.50	50.00
<i>Aspergillus tamaraii</i>	30.00	1000.0
<i>Aspergillus niger</i>	17.50	100.00
<i>Aspergillus ochraceous</i>	55.00	100.00
<i>Penicillium montanense</i>	2.50	75.00
<i>Paecilomyces</i> sp	15.00	100.00
<i>Phomopsis</i> sp	0.00	100.00
<i>Botryodiplodia theobromae</i>	35.00	75.00
<i>Penicillium chrysogenum</i>	7.50	75.00
<i>Penicillium oxalicum</i>	15.00	70.0
Control	0.0	1000.0
LSD (p=0.05)	28.96	23.90

significant difference (P = 0.05) in the mean percentage colonization. Similar trends were obtained in seeds inoculated with *Aspergillus niger*, control had 80.0%, treated 43.0%, *A. tamaraii* (control 74.0%, treated 25.0%), *A. flavus* (Control 68.0%, treated 17.0%, *A. ochraceous* (control 56.00%. treated 40.0%) except for *Penicillium* where control had 45.0% and treated had 22.0%, although

control had higher percentage seed colonization of 45% compared to treated seeds which had 22.0%. (Table 2). The percentage reduction of the growth of the various fungi on *Piper guineense* treated mung bean seeds ranged from 10.5 – 90.0% (Table 2).

For *Monodora myristica* treated seeds, *Rhizopus* inoculated seeds had higher percentage colonization of 95.0% while the control (treated seeds) had 85.0% and there was significant difference in their mean percentage colonization. For seeds inoculated with *Aspergillus niger*, *A. tamarii*, *Botryodiplodia* and *A. ochraceus* and *Penicillium* the control also had higher percentage seed colonization than the treated mung bean seeds and there were significant differences between the treated and the control. Although the control had higher percentage seed colonization of 68.00% compared to treated seeds which had 41.00% for *A. flavus* inoculated seeds, there was no significant difference in their mean colonization percentages. The percentage reduction of the growth of the various fungi on *Monodora myristica* treated mung bean seeds ranged from 15.7–66.2% (Table 2).

Table 2: Effects of *Monodora myristica* and *Piper guineense* on Fungi associated with Mung bean seeds

	Percentage fungal growth and reduction (%)						
	<i>Aspergillus tamarii</i>	<i>Penicillium oxalicum</i>	<i>Rhizopus</i> sp	<i>Aspergillus ochraceus</i>	<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>	<i>Botryodiplodia</i>
<i>M. myristica</i>	7.00	22.00	85.00	26.00	36.00	41.00	28.00
Control	74.00	45.00	95.00	56.00	80.00	68.00	57.00
Growth							
reduction	90.54	51.11	10.53	53.57	55.00	39.71	50.88
LSD (P =0.05)	5.65	0.00	0.00	3.26	2.31	21.07	5.65
<i>P. guineense</i>	25.00	22.00	80.00	40.00	43.00	17.00	23.00
Control	74.00	45.00	95.00	56.00	80.00	68.00	57.00
Growth							
reduction	66.22	51.11	15.79	28.57	46.25	75.00	59.65
LSD (0.05)	0.00	2.80	0.00	2.31	4.61	14.22	5.65

DISCUSSION

Mung bean seeds were found to be highly infested with different fungi. A total of ten fungal species were isolated using the agar plate method which in most of cases was found to be superior to blotter for the isolation of seed mycoflora (Tripti *et al.*, 2012). Seven (7) out of the ten fungal species (*Aspergillus tamarii*, *A. ochraceous*, *A. niger*, *A. flavus*), *Rhizopus* sp, *Botryodiplodia theobromae* and *Penicillium. oxalicum*) caused deterioration of mung bean varieties studied.

Aspergillus niger, *A. flavus*, *A. ochraceous* and *A. tamarii*, *Botryodiplodia theobromae*, *Penicillium oxalicum*, and *P. montanense*) were the dominant group of fungi in the two mung bean varieties. *Fusarium* sp, *Penicillium chrysogenum*, *Paecilomyces* sp, *Phomopsis* sp. and *Rhizopus* sp were isolated from one of the mung bean varieties. The occurrence of these fungi in mung bean seeds have been reported by several other authors (Bakir and Rahman, 2001; Fakir, 2001; Tripti *et al.*, 2012); as well as Al-Yahaya (1999) and Krasauskas *et al.* (2005) in Arab and Nepal, respectively. The result indicated the high and low incidences of various fungal species. This high incidence of saprophytes may be due to ability to colonize, rapid germination of spore, fast hyphal invasion, high competitive nature and their ability to utilize a wide variety of substrate and their nutrient composition (Novak *et al.*, 2001). On the contrary, low incidence of species might be due to antagonistic effect of *Aspergillus* species and *Rhizopus* sp, which were dominant. This agrees with the report that *Aspergillus niger*, *Penicillium* sp., *Rhizopus arrhizus* suppressed the growth of other fungi (Tripti *et al.*, 2012).

For seed treatment with botanicals, results showed that treated seeds yielded less growth of seed-borne fungi than the untreated seeds that is in close conformity with the findings of Shafun-Nahar *et al.* (2005) and other numerous authors. Similar results have also been reported from seeds other than mung bean, e.g. sunflower, egusi and groundnut seeds (Embaby and Abdel-Galil, 2006; Obani and Ikotun, 2014). Both plants' extracts considerably inhibited the growth of these organisms on inoculated seeds, however, *Piper guineense* inhibited the fungi growth more than *Monodora myristica*. The difference in efficiency of the extract of the plants may be due to the presence of

different active ingredients (Obani and Ikotun, 2014). Various authors have reported anti-fungal activities of *P. guineense* (Obani and Ikotun, 2014; Amadioha and Nwazuo, 2019). Both plants are environmental-friendly and have less financial implications to obtain. Which implies that they can serve as a good alternative to chemical fungus considering their safety and degradable qualities (Amadioha and Nwazuo, 2019).

CONCLUSION

This study showed that mung bean seeds are infected with pathogenic fungi which could reduce seed viability and may result in seed establishment failure even after they have successfully germinated. The botanicals tested were effective in reducing fungal colonization of the seeds at varying levels and have no harmful effect on germination of treated seeds, the seeds germinated after treatment with extracts showing that the extracts are not phytotoxic. Hence, the seed borne pathogens of mung bean seeds can be managed using the extracts of the plant tested in this study.

Results obtained show that fungi associated with mung bean seeds when treated with Ehuru (*Monodora myristica* and Uziza (*Piper guineense*) extracts and were considerably controlled. Therefore, it can be recommended that mung bean seeds should be treated with *Monodora myristica* and (*Piper guineense*) seed extracts to control seed borne fungal that usually hinder seedling establishment when planted. Further, research should be carried out to ascertain the effect of tested botanicals on seedling establishment in the fields to further authenticate the findings of this study.

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