International Research Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **8(7),** 9-14, July **(2019)** *Int. Res. J. Biological Sci.*

Fungi intercepted in seeds of pigeon pea (*Cajanus cajan* (L.) grown in northern Tanzania and relation to quality attributes of the seeds

Theophili Tarmo[1], Dunstan Msuya[2] and Paul Njau[2*]
[1]Research, Community and Organizational Development Associates (RECODA), P.O. Box 10633, Arusha, Tanzania
[2]Crop Science and Horticulture Department, College of Agriculture, Sokoine University of Agriculture, P.O.Box 3005, Morogoro, Tanzania
pjnjau@yahoo.com

Available online at: www.isca.in, www.isca.me
Received 23th January 2019, revised 10th April 2019, accepted 14th June 2019

Abstract

Fungi associated with Pigeon pea seed were studied using 80 seeds samples of pigeon pea collected from Babati and Karatu districts in northern Tanzania. The standard moist blotter test was used to detect fungi on seeds. The tested pigeon pea samples yielded more than 12 different fungal species. Fusarium udum which is a pathogen of seed health certification significance was detected in 33 samples from Babati (equivalent to 82.5%) and 36 samples from Karatu districts (equivalent to 90%) of the samples. Eleven other seed infesting fungi were also intercepted, with Rhizopus spp appearing in all samples and having the highest incidence of 23.2% for Karatu and 16.1% for Babati district followed by Aspergillus flavus having the incidence of 20.3% and 15.7% for Karatu and Babati districts, respectively. The other species ranged between 1.1% and 10.1% for Babati and 0.7% and 13.7% for Karatu. Significant correlation existed between seed purity and incidence of Cladosporium spp and between seed moisture content and incidence of Fusarium moniliforme; but the correlation with purity was positive against expectation. Even though farm-saved seeds may be localized with the practicing farmer or within a restricted locality, generally it is suggested that in those areas where seed borne pathogens are endemic and farm-saved seeds is predominant farmers' awareness on Fusarium wilt disease should be created. It is also suggested that farmers should be trained on how to reduce seed transmission of the diseases at least by rouging the infected plants in the field and selective harvesting of the crop to be used as seed.

Keywords: Blotter paper method, Fungi, Pigeon pea seeds, Tanzania.

Introduction

The Pigeon pea [*Cajanus cajan* (L.) Millsp] is a grain legume of great importance in northern Tanzania and has gained considerable national economic significance in recent years. In many parts of the country, pigeon pea has been grown essentially by small scale farmers as a cash income generating crop that is mostly exported, but is also used as an occasional food legume[1]. The use of farm-saved, untreated seed in the smallholder farming sector is a common practice in Tanzania[2] . These seeds are always not protected before storage. The storage techniques used are also poor that may allow infestation of the grains by storage insect pests especially bruchids[3]. Seed borne fungi are also a major constraint faced by small scale farmers[4]. The storage fungi such as *Aspergillus*, *Penicillium* and *Rhizopus* do not only spoil seeds but they may also produce toxins and chemicals that may affect human health when the infested grains are used for food[5].

Currently, low productivity of pigeon peas has been observed in Tanzania. Production trends show that between 1999 and 2013, pigeon pea area under cultivation increased from 124,638 to 290,000 hectares (132.7%) and output from about 81,015 to 210,000 tons (159.2%). The crop's yields, however, remained not much changed, at about 0.65 and 0.72 tons/ha, respectively. This implies that the gains in production over the said period are attributable to area expansion rather than increase in productivity[6]. The yields are far below the crop′s potential of up to 4.6 tons/ha[7].

Use of improved quality seeds could solve some of the yield problems. Contrarily, however, about 96% of pigeon pea farmers use their own saved seeds whose quality status is not known[8]. The seed supply is dominated by informal system such as borrowing from neighbours/relatives, exchanging or purchasing stored food grains to be used as seeds. Such un-improved quality seeds could have high genetic variability and incidences of severe diseases and insect pest susceptibility. High genetic variability leads to indeterminate maturity dates of pigeon pea crop which leads to improper management hence affecting yield[9]. Also, differential disease and insect pest susceptibility and non-uniform harvested grains greatly reduce yield[10]. In addition, majority of the farmers store their grains/ seeds in bags/jute sacks and the seeds are not previously treated hence easily infested with storage pests which can cause a lot of physical damage to the grains or seeds rendering them un-germinable or germination with very low vigour. Therefore, there is a concern about seed health and quality of farmers own saved seeds. To ascertain the health of these seeds, seed health tests are required. This study aims at providing clear vision on

the health statuses of the farmers own pigeon pea seeds with the view of recommending appropriate management practices to produce good quality seeds and improve pigeon pea productivity in the study area.

Materials and methods

Location of the study: Laboratory seed tests were performed in the plant pathology laboratory of the African Seed Health Centre (ASHC) at Sokoine University of Agriculture (SUA), Morogoro, Tanzania in 2016-17. SUA is located at 525meter above sea level and lies at 6.8527°S latitude and longitude 37.6564°E, in eastern Tanzania. Average annual rainfall at the experimental site is 900mm, with mean maximum and minimum temperature ranging from 28.1 to 31.6^{o}C and 17.5 to 22.7^{0}C, respectively.

Seed samples collection: Samples of pigeon pea seeds for laboratory examination to isolate and identify seed-borne fungi were collected from ten villages (Orngadida, Qash, Endanoga, Gedamar, Galapo, Endagile, Mamire, Endakiso, Sangara and Riroda) in Babati district and also from ten villages (Kambi ya Faru, Getamock, Basodawish, Endamarariek, Rhotia, Qaru, Kansay, Endabash, Endanyawet and Ayalaliyo) in Karatu District. The villages were selected based on significant producers and accessibility or closeness to roads and input shops for easy sampling. Four farmers were randomly selected in each village for samples collection. One kilogram of seeds was collected from each farmer in sterilized paper bags, brought to the African Seed Health Center (ASHC) seed testing laboratories in Morogoro and stored at 4-5^{o}C until testing.

Isolation and identification of seedborne fungi: Seed samples were studied for the presence or absence of fungi. Isolation of different seed infesting fungi was performed using the standard blotter method according to ISTA[11]. The white, sterile blotter papers of 8.5cm diameter were soaked in pairs in sterile distilled water and placed in pre-sterilized 90mm diameter Petri dishes. The test sample seeds were then placed on top of the moist blotter paper in Petri dishes, ten seeds per Petri dish at approximately equal distance. For every sample 20 Petri dishes were incubated, therefore two hundred seeds per sample. The Petri dishes were incubated at 28ºC±2ºC under diurnal conditions. After the period of incubation individual seeds were thoroughly examined at varying magnifications of compound and stereo-microscopes for observation and identification of fungal contaminants. Morphological characteristics of the fungi were the bases for identification. These included characteristics of the fungal growth colony, morphology of conidiophores, shape and septation of conidia and other structures, alongside comparison with appropriate literature. Number of infected seeds by individual fungal species was converted into percentages for each sample, according to procedures of ISTA[12].

Relation of Intercepted Seed Borne Fungi with Physical Attributes of the Collected Seeds: The study included finding the correlation between the intercepted fungal pathogens with quality attributes (Purity, Germination and Moisture content) of collected farm saved pigeon pea seeds in Babati and Karatu Districts.

Data analysis: Descriptive statistical methods were used to compute means and percentages. Pearson's correlation analysis was also performed to establish significant relationship between physical quality attributes and incidences of mycoflora infesting the seeds.

Results and discussion

Seed borne fungi isolated: Results of micro-organisms specifically fungi infestation isolated from the seeds used by farmers in the two districts where study was conducted are presented in Tables-1 and 2 for Babati and Karatu Districts respectively and the incidence of each species detected from the samples presented in Table-3. Of greatest interest in seed health was *Fusarium udum* which is a serious pathogen of the crop and seed-borne. Four other *Fusarium* species (or belonging to *Fusarium* group) were also intercepted in the seeds. These were *F. moniliforme*, *F. pallidoroseum*, *F. equiseti* and *F. poae*. Other species of essentially saprophytic fungi detected were *Rhizopus* spp, *Penicillium* spp, *Cladosporium* spp, *Botrytis cinerea*, *Aspergillus niger, Aspergillus flavus*, and *Curvularia lunata*. Among the *Fusarium* spp, *F. moniliforme* appeared in greatest frequency (100% of samples in Babati and 97.5% of samples in Karatu Districts) followed by *F. pallidoroseum, F. equiseti,* and *F.udum* whose frequencies were respectively > 80%. In Karatu District, *F. moniliforme* was missing in one of the samples. Each sample in both Districts was infested with at least 7 of the fungal species. Least infested was sample number 14 from Gedamar and No. 27 from Mamire villages (Data not shown) in Babati District. Nevertheless, sample No. 14 was still not free from *F. udum* (Data not shown). Seven samples in Babati District and four samples in Karatu District were free from *F. udum* (Data not shown).

None of the detected fungi other than *F. udum* is of any serious seed health significance. Apart from *F. udum*, other fungi reported to be pathogenic in pigeon pea include *Colletotrichum cajanae*[13], *Diplodia cajani*[13], *Macrophomina phaseoli*[13], *Phoma cajani*[13], *Phaseolus manihotis*[13], *Phyllostica cajani*[13], *Phytophthora* spp[13], *Cercospora* spp[13], *Corticium solani*[13], *Leveilula taurica*[13], *Rhizoctonia bataticola*[13], *Rosellinia* spp[13], *Sclerotium rolfsii*[13] and *Uredo cajani*[13]; economic damages of which is reported to be negligible (Ibid). Furthermore, there is one bacterial species, *Xanthomonas cajani*, reported to cause disease in pigeon pea; and two viral infections: Sterility mosaic virus and Yellow mosaic virus[14]. Several species or groups of species detected during this study have been detected in pigeon peas previously. Thirty two different species of fungi of 15 different genera, from 10 samples of pigeon pea seed collected from seed market have been reported[14]. Among those are some species which have also been currently detected during this study. These are: *F. moniliforme*, *F. equiseti*, *Penicillium spp*, *Cladosporium spp*, *A. flavus*, *A. niger* and *Rhizopus spp*.

Table-1: Microbial contamination of seeds of pigeon pea collected from farmers in various villages of Babati District.

Fungi	*Villages											
	1	2	3	4	5	6	7	8	9	10	Total	%
Rhizopus spp	4	4	4	4	4	4	4	4	4	4	40	100
Fusarium moniliforme	4	4	4	4	4	4	4	4	4	4	40	100
F. pallidoroseum	2	3	4	3	1	4	4	4	4	4	32	82.5
F.udum	4	3	3	4	4	2	3	3	4	3	33	82.5
F.equiseti	3	3	3	2	4	4	4	3	3	4	33	82.5
F.poae	1	3	2	-	1	2	2	2	2	1	16	40
Penicillium spp	3	3	4	3	4	4	4	4	4	4	37	92.5
Aspergillus niger	3	4	4	4	4	4	4	4	3	4	38	95
A. flavus	2	3	4	4	4	4	4	4	3	4	36	90
Cladosporium spp	2	3	2	-	2	4	3	3	4	4	27	67.5
Curvularia lunata	1	4	1	3	2	3	3	3	3	3	26	65
Botrytis cinerea	1	2	1	1	3	4	3	2	3	4	24	60

*1= Orngadida; 2= Qash; 3= Endanoga; 4= Gedamar; 5= Galapo; 6= Endagile; 7= Mamire; 8= Endakiso; 9= Sangara; 10= Riroda.

Table-2: Microbial contamination of seeds of pigeon pea collected from farmers in various villages of Karatu District.

Fungi	*Villages											
	1	2	3	4	5	6	7	8	9	10	Total	%
Rhizopus spp	4	4	4	4	4	4	4	4	4	4	40	100
Fusarium moniliforme	4	4	4	4	4	4	4	4	3	4	39	97.5
F. pallidoroseum	3	4	4	4	4	4	4	4	3	4	38	95.0
F.udum	4	4	4	4	4	4	2	4	3	3	36	90.0
F.equiseti	2	3	4	3	3	3	3	3	3	2	29	72.5
F. poae	2	2	4	3	2	2	-	1	-	-	16	40.0
Penicillium spp	4	4	4	4	4	4	3	4	4	4	39	97.5
Aspergillus niger	4	4	4	4	4	1	4	4	4	4	37	92.5
A. flavus	3	4	4	4	4	4	3	4	4	4	38	95
Cladosporium spp	3	2	3	3	3	2	4	3	4	2	29	72.5
Curvularia lunata	3	2	4	4	2	3	4	1	2	4	29	72.5
Botrytis cinerea	2	3	4	4	4	2	4	3	3	1	30	75

*1= Rhotia; 2= Qaru; 3= Kansay; 4= Endabash; 5= Endanyawet; 6= Ayalaliyo; 7= Kambi ya Faru; 8= Basodawish; 9= Getamock; 10= Endamarariek.

Table-3: Fungal species intercepted in samples of pigeon pea seeds collected from farmers in Babati and Karatu Districts and their incidences.

Fungal species intercepted	Incidence		
	Babati District	Karatu District	Mean
Fusarium udum	2.0	3.1	2.55
F. moniliforme	8.5	9.6	9.05
F. poae	1.1	0.7	0.9
F. pallidoroseum	6.0	6.7	6.35
F. equiseti	4.5	3.5	4.0
Rhizopus spp	16.1	23.2	19.7
Aspergillus flavus	15.7	20.3	18.0
Penicillium spp	10.1	12.3	11.2
Aspergillus niger	10.1	13.0	11.55
Cladosporium spp	7.6	13.7	10.65
Curvularia lunata	3.5	2.3	2.9
Botrytis cinerea	3.2	3.7	3.45

Incidence of isolated fungi: Table-3 shows extensiveness of the fungal infestation in the samples tested in terms of incidence equated with number of seeds infested out of 200 seeds tested in each sample. From the Table we see that percent incidence for *F. udum* was 2% in Babati District and 3.1% in Karatu District. This was generally low even though frequencies of interception of the pathogen in the tested samples were high. Fungi whose observed incidences were highest were *Rhizopus spp* and *Aspergillus flavus*. Mean incidence averaged over the two Districts was about 19.7% for *Rhizopus* and 18% for *A. flavus*. Species with lowest incidence was *F. poae* (0.9%) followed by *F. udum* (2.5%).

The observed incidences of the various fungi in the tested samples must naturally have meaning and implications. Most often, however, meaning and implications can only be predictable (predicted syntheses). Actual implication may be a matter of more research. Comparatively very high incidence of highly saprophytic fungi like *Rhizopus spp*; for example, may simply mean that the seeds were having high quantities of plant debris particles some of them decaying or decayed. This simply means purity was low and inert matter present was mostly plant debris. Indeed purity was low and sub-standard for the great majority of the samples[15]. Considerably also high incidences of the storage fungi *A. flavus, A. niger* and *Penicillium spp*; may imply that the seeds reached the stores in considerably dry condition, drier than conditions that would support field inhabiting saprophytes which need wetter conditions. Under dry store conditions, field fungi like *Fusarium spp*; *Cladosporium, Curvularia* and *Botrytis spp* would not thrive competitively very well with the more dry condition-adapted storage fungi. In another prediction, presence of *Botrytis cinerea* in some of the samples may suggest that among intercropping practices[15] there was intercropping with sunflower (*Helianthus annuus*). *B. cinerea* is an important pathogen of *H. annuus* and in presence of infection of the crop with *B. cinerea*, intercropping may be a source of plant debris particles with *B. cinerea* accompanying the pigeon pea seeds.

Relation of intercepted seed borne fungi with germination and other physical attributes of the collected seeds: Table-4 relates fungal incidences of seed infestation with physical quality attributes of seeds tested during this study. No very conclusive observations, however, have been realized on expected relationship between infestation and quality. Established correlation coefficients showed in-existence of any significant ($P \leq 0.05$) relationship between germination capacity of the seeds and microbial infestation (incidence).

Among the physical quality attributes significant correlations existed only between Purity and incidence of *Cladosporium spp* (r= 0.274*) and between Moisture content and *F. pallidoroseum* (r=0.249*). The rest of significant correlations were between the fungal species amongst themselves. All of these correlations were positive. Correlations between *A. flavus* and *Penicillium spp*; between *F. equiseti* and *F. moniliforme*; between *B. cinerea* and *A. flavus*; *B. cinerea* and *F. equiseti*; *C. lunata* and *F. equiseti*; *C. lunata* and *B. cinerea*; and between *F. poae* and *C. lunata* were highly significant ($P < 0.01$). *F. udum* did not have significant correlation with any of the physical quality attributes nor with any of the other fungal species.

Correlation analysis, nevertheless, did not show very pronounced association between incidence of the seed infesting mycoflora and quality attributes of the seeds. Seemingly the mycoflora did not influence germination; only significant correlations existed between *Cladosporium spp* and purity; and between *F. pallidoroseum* and moisture content. Ideally high purity would discourage incidences of the debris-loving fungi, since there would be less inert matter. Consequently therefore predictable significant correlation would be negative. Contrarily the correlation was positive, meaning that increasing cleanness of seeds increased incidence of the fungus *F. pallidoroseum*. Ironically here it may be suggested that it is seed purity that is dependent variable, that the purity is increased by increasing incidence of the fungus. This may be sensible if we say that the microbe upon its saprophytic activity on decaying plant inert matter increases the pure seed fraction of seed purity.

Predictions may also be said about observation that all observed significant correlations among incidences of the different fungi

were positive. This eliminates any existence of competitive growth or infestation of the seed by the fungi and contrarily suggests synergistic action. It may be indeed assumed that the fungi exerted combined damage or stress on the seed and each microbe's weakening ability of the seed tissues to resist colonization by the fungus increased opportunity for the other microbe to establish.

Table-4: Correlation between physical quality attributes and fungal species incidences, and amongst the fungal species infesting tested pigeon pea seeds.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	1.00														
2	0.26*	1.00													
3	-0.08	0.05	-1.00												
4	-0.09	-0.11	-0.01	1.00											
5	0.13	0.17	-0.03	0.04	1.00										
6	0.11	-0.04	-0.01	0.06	0.22	1.00									
7	-0.01	-0.25*	0.02	-0.07	0.22	0.25*	1.00								
8	0.28*	-0.02	-0.05	0.14	0.19	0.04	0.15	1.00							
9	-0.01	0.16	0.01	0.15	0.11	0.25*	0.08	0.08	1.00						
10	0.04	-0.14	-0.08	0.21	0.05	0.30**	0.27*	0.13	0.19	1.00					
11	0.09	-0.11	-0.10	0.01	0.34**	0.13	0.20	0.01	0.11	0.12	1.00				
12	0.09	-0.14	-0.11	0.11	0.13	0.20	0.27*	0.25*	0.29*	0.36**	0.31**	1.00			
13	0.14	-0.12	0.13	0.07	0.18	0.26*	0.07	0.15	0.20	0.09	0.30**	0.31**	1.00		
14	0.04	-0.13	0.05	0.06	0.21	0.21	0.16	-0.08	0.05	0.09	0.25*	0.18	0.40**	1.00	
15	0.20	-0.06	-0.08	0.03	-0.07	0.05	-0.08	0.02	0.11	0.18	-0.19	0.11	-0.10	-0.01	1.00

Number of observations: 80 *Significant at 0.05 level, **Significant at 0.01 level, 1= Purity, 2= Moisture content, 3= Germination, 4= *Rhizopus*, 5= *Fusarium moniliforme*, 6= *Penicillium*, 7= *Fusarium pallidoroseum*, 8= *Cladosporium*, 9= *Aspergillus niger,* 10= *Aspergillus flavus,* 11= *Fusarium equiseti,* 12= *Botrytis cinerea,* 13= *Curvularia lunata,* 14= *Fusarium poae,* 15= *Fusarium udum*

Conclusion

This study has shown that majority of the seed samples collected and hence being used by farmers posed a hazard of transmitting *Fusarium udum.* Only few samples from both Districts were free from the infectious pathogen; or in other words majority of the farmers' farms saved seeds were potentially hazardous.

Even though farm-saved seeds may be localized with the practicing farmer or within a restricted locality, generally it is suggested that in those areas where seed borne pathogens are endemic and farm-saved seeds is predominant, farmers' awareness on Fusarium wilt disease should be created. It is also suggested that farmers should be trained how to reduce seed transmission of the diseases at least by rouging the infected plants in the field and selective harvesting of the crop to be used as seed.

Acknowledgement

Authors of this paper are grateful to the Head of the Department of Crop Science and Horticulture of Sokoine University of Agriculture, Morogoro, Tanzania for providing necessary laboratory space and facilities to conduct the study. The financial assistance received from the "Sustainable Intensification of Maize-Legume Cropping Systems for Food Security in Eastern and Southern Africa" (SIMLESA), which enabled conducting this study, is gratefully acknowledged.

References

1. Monyo E.C.L. and Laxmipathi G. (2014). Grain Legumes Strategies and Seed Roadmaps for Select Countries in Sub-Saharan Africa and South Asia, Tropical Legumes II Project. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India, 286.

2. Amare M., Asfaw S. and Shiferaw B. (2012). Welfare impacts of maize-pigeon pea intensification in Tanzania. *Agricultural Economics,* 43(1), 27-43.

3. Dasbak M.A., Echezona B.C. and Asiegbu J.E. (2009). Post-Harvest Bruchid Richness and Residual Activity of Pirimiphos-Methyl on Callosobruchus Maculatus F. Infested Pigeon Pea (CajanusCajan L. Millsp.) in Storage. *African Journal of Biotechnology,* 8(2), 311-315.

4. Patil D.P., Pawar P.V. and Muley S.M. (2012). Mycoflora associated with pigeon pea and chickpea. *International Multidisciplinary Research Journal,* 2(6), 10-12.

5. Bankole S.A., Eseigbe D.A. and Enikuomehin O.A. (1995). Mycoflora and Aflatoxin Production in Pigeon Pea Stored in Jute Sacks and Iron Bins. *Mycopathologia,* 132(3), 155-160.

6. FAOSTAT (2013). Pigeon pea. [http://faostat.fao.org/default.aspx] site visited on 15/10/2014.

7. Odeny D.A. (2007). The potential of pigeon pea (*Cajanus cajan* (L.) Millsp.). Africa. *Journal of Natural Resources Forum,* 31(4), 297-305.

8. Shiferaw B., Silim S., Muricho G., Audi P., Mligo J., Lyimo S., You L. and Christiansen J.L. (2007). Assessment of the adoption and impact of improved pigeon pea varieties in Tanzania. *Journal of SAT Agriculture Research,* 5, 1-27.

9. Deshmukh D.V. and Mate S.N. (2013). Evaluation of pigeon pea genotypes for morpho-physiological traits related to drought tolerance. *World Journal of Agricultural Sciences,* 9(1), 17-23.

10. Sharma O.P., Gopali J.B., Yelshetty S., Bambawale O.M., Garg D.K. and Bhosle B.B. (2010). Pests of Pigeon Pea and Their Management. National Centre for Integrated Pest Management, New Delhi, India. 99.

11. International Seed Testing Association (2005). International Rules for Seed Testing. Budapest, Hungary. [hp://www.seedtest.og] site visited on 20/8/2017.

12. ISTA (2014). International Rule for Seed Testing, Quality Standards for Supporting Advanced Technologies in Other Seed Determination. International Seed Testing Association, Switzerland. 13.

13. Shinde S. (2016). Isolation of seed borne Fungi associated with pigeon pea (*Cajanus cajan,* Linn.) seeds. *International Journal of Science and Research,* 5(7), 1766- 1769.

14. Duke J.A. (1981). Hand Book of Legumes of World Economic Importance. Plenum Press, New York, 345.

15. Theophili T., Msuya D., Njau P.J. and Ringo D.E. (2018). Post-harvest handling practices of pigeon pea seeds used by farmers in northern Tanzania and relation to quality of the seeds. *International Journal of Agriculture, Environment and Bioresearch,* 3(6), 65-79.