In Vitro Evaluation of Selected Fungicides Against *Curvularia geniculata* on *Digitaria iburua* Kippist Stapf in Riyom, Plateau State

Mamza, W.S[1*], A.B. Zarafi[2], B.D. Kashina[2] and S.E.L. Alao[2].

[1]National Cereals Research Institute, Badeggi, Acha Station Riyom, P. O Box 8395, Anglo Jos, Plateau State, Nigeria. [2] Crop Protection Department, Faculty of Agriculture/ Institute of Agricultural Research, Ahmadu Bello University, Zaria, Nigeria

[*]Corresponding author email: wadimamza@yahoo.comTel: +2348038388876

Summary

Five fungicides at varying concentrations (0 x; 0.5x; 1x and 1.5x where x is manufacturer's recommended rate) were evaluated *in vitro*, against *Curvularia geniculata* isolated from Fonio. Seven days old culture was placed on Petri dishes containing Potato Dextrose (PDAs) amended with fungicides at varying rates and mycelial growth measured at two days intervals starting from 2 days to 12 days after inoculation (DAI). On the 14 DAI, conidia were counted using a haemocytometer. The inhibitory effect on mycelial growth and sporulation significantly varied amongst the fungicides and rates with the highest effect recorded with Mancozeb while the least was from Thiomethoxan+Metalaxyl-M+ Difenoconazole although it was not different from Carbendazim, Benomyl and Carbendazim+ Mancozeb.

Keyword: *Curvularia geniculata*, Fonio, Fungicides, *in Vitro*, Mycelia, Conidia, Inhibition

FONIO or hungry rice (*Digitaria exilis* and *Digitaria iburua),* belongs to the family Poaceae (25; 13). Fonio is short seasoned, fast growing and thrives in marginal sandy and rocky soils of the Sahel with possibility of two or three crops per year hence serving as "gap crop" during months of low food supply (12). Fonio, locally named acha is tolerant to drought and flood; and mostly grown in the central and northern states of Nigeria such as Bauchi, Kaduna, Kebbi, Nassarawa and Plateau States (3). Among the cereals, fonio is the most nutritious with its grain

containing 7% crude protein, 9.8% leucine, 5.6% methionine and 5.8% valine more than other cereals like sorghum, rice, wheat or barley (14; 28; 29). Belton and John (4) also reported fonio to be richer in magnesium, zinc, iron, manganese, Calcium, phosphorus, thiamine, riboflavin and lower in glycemic index than other cereals. The methionine and cysteine in fonio are suppliers of sulphur, amino acid and other compounds required for normal body metabolism and growth (7; 4).The world three largest Fonio (acha) producers are Guinea, Nigeria and Mali with production of 429 000, 165 000 and 45 000 t from 200 000, 90 000 and 26 000 ha, respectively (8). Fonio which is traditionally consumed as stiff or thin porridge, couscous, or mixed with other flours to make bread also has medicinal values particularly in the management of diabetes and new lactating mothers (13; 1). Although both fonio species have shown low susceptibility to insect pests and diseases (29;16), the fungi *Phyllachorasphearosperma*, *Puccinia cahuensis* and *Helminthosporium* sphave been reported on the crop in Benin republic (11). Similarly, *Helminthosporim* sp., *Fusarium* sp. and *Curvularia* sp. have been reported on *Digitaria iburua* on NCRI Research farm Riyom (18; 20). High incidence of Brown leaf spot disease was also reported on *Digitaria iburua* and *D. exilis* at Badeggi (17). The symptoms of Brown leaf Spot caused by *Curvularia geniculata* on fonio are brown irregular lesions about 0.01mm – 0.5 mm on the leaf lamina and leaf sheath, it later coalesces to form large lesions resulting into blight on the leaf margins and wilting of leaf tips (Plate 1 and 2). However there is no documented research works conducted on the control or management of brown leaf spot of fonio in Nigeria. In view of this, the choice of synthetic chemicals over other alternatives was preferred because chemicals are effective and provides a cheaper and reliable source for control of plant pathogenic fungi (27). Many *in vitro* studies have demonstrated that some fungicides restrict or prevent the growth of fungal pathogens (15; 21). In view of the nutritional importance of the crop and the effect of fungal diseases on the crop, there is a need to identify management options for disease associated with this important and relatively new crop. The aim of the study was to determine the *in vitro* effects of selected fungicides on the mycelial growth and sporulation of *Curvularia geniculata* isolated from fonio.

MATERIALS AND METHODS

The experiment was conducted at the Mycopathology laboratory of Crop Protection Department Ahmadu Bello University, Zaria in 2015. Diseased fonio leaves were brought to the laboratory washed and sterilized using 5% sodium hypochlorite and rinsed three times with sterile distilled water. Leave samples were placed on Petri dishes with Potato Dextrose Agar amended with Streptomycin. Petri dishes were placed in an incubator at 28^0 C and observed for mycelia growth. Five fungicides (Table 1) were evaluated at four concentrations (1.5 x, 1.0 x, 0.5 x, and 0x where x is manufacturer's recommended formulation rate). The required quantities of fungicides were weighed separately, dissolved in 5 ml of ethanol, made up to 100 ml with freshly prepared Potato Dextrose Agar with Streptomycin (PDAs) cooled to 45^0 C and dispensed into 9 cm diameter Petri dishes at 20ml/plate rate. Upon solidification (24 hours after pouring), using a 0.5 cm cork borer, 7 day old *Curvularia geniculata* pure culture was inoculated centrally in the labeled Petri dishes laid in completely randomized design (CRD) with each fungicide making a treatment replicated five times (a Petri dish representing a replicate) and incubated at 28^0 C± 2^0C. Radial mycelial growth was measured at two days interval (2, 4, 6, 8, 10 and 12 days after inoculation (DAI)) along two perpendicular lines on each petri dish underside. At 14 DAI, for each treatment, a stock conidial suspension was prepared by bulking its 5 petri dishes from which 10 ml was pipette into 90 ml. With the aid of a haemocytometer, conidia were counted four times for each treatment and using the formula of Booth (5) below, total conidia/ ml was obtained.

$$\text{Conidia/ ml } \frac{n}{256} x4x10^6$$

Where: n=number of conidia counted in the chambers, 256= constant volume obtained from 16x16, $4x10^6$= constant. The Experiment was repeated twice.

Data collected were subjected to statistical analysis using (25) and means that were significant were compared using the Student Newman Keuls (SNK) test at 5 % level of significance.

RESULTS

The effect of varying rates of fungicides on the mycelial growth and sporulation were highly significant ($P \leq 0.01$) (Table 2, Plates 1, 2 and 3). Thiomethoxan + Metalaxyl-M + Difenoconazole (Apron plus) treated plates recorded significantly the highest mycelial growth (2, 4, 6, 8, 10 & 12 DAI), and there were no

significant differences among the rates as the days of inoculation increases. Followed by Carbendazim (Forcelet), Benomyl (Benomy), Carbendazim + Mancozeb (Funguforce) and mancozeb (Z Force), respectively except at 2 and 4 DAI, Funguforce and Z Force did not differ significantly (Table 2). Mancozeb which had the highest inhibitory effect on mycelial growth also induced significant inhibitory effect on sporulation in all the DAI compared to all other tested fungicides which did not differ from each other (Table 2). The mycelial growth and sporulation were significantly decreasing with increase in fungicide rate, which was highest at 0 x and least at 1.5x concentration (Table 2).

Fungicides x rates (F x R) interaction was highly significant for mycelial growth at all the DAI and sporulation (Table 3). The inhibitory effect of & 1.5 x).

each fungicide was significantly increased with increase in the concentration/ rate with 1.5x recording the least conidia count and mycelial growth all through although by 8DAI, the 0 x treated plates and those treated with Thiomethoxan + Metalaxyl-M + Difenoconazole (Apron plus) at all tested rates were statistically not different from each other as well as at 10 and 12 DAI (Table 3). Similarly Carbendazim at 0 x and 0.5 x was not significantly different from Thiomethoxan + Metalaxyl-M + Difenoconazole. Mancozeb (Z Force) which induced highest inhibitory effect on this pathogen recorded least mycelia growth at all tested rate although it was statistically similar with Carbendazim + Mancozeb (Funguforce) at all rates at 2 and 4 DAI. Z Force also completely inhibited sporulation at all rates (0.5x, 1.0 x

Table 1: Fungicides evaluated *in vitro* against *Curvularia geniculata*

Trade name	Active ingredient	Formulation	Manufacturer	Recommended rate
Apron Plus	20%Thiomethoxan + 20% Metalyxl-M + 2% Difenoconazole	42% SD	Sygenta	10g /4kg seed
Forcelet	50% Carbendazim	50% WP	Jubaili	1.5 Kg/ 200L / ha
Benomy	50% Benomyl	50% WP	Shanghai Chem Ltd	250g /100L/ ha
Z Force	80%Mancozeb	80% WP	Jubaili	2kg/ 400L / ha
Funguforce	12 %Carbendazim + 53%Mancozeb	65% WP	Jubaili	2.5 kg /400L / ha

Table 2: Effects of varying fungicides rates on mycelial growth and conidia sporulation of *Curvularia geniculata*

	Mycelial growth at days after inoculation (DAI)/ cm						Conidia count ($4x10^6$)/mls
reatments	2	4	6	8	10	12	
ungicide (F)							
hiomethoxan + Metalaxyl-M + ifenoconazole	2.26a	4.35a	7.50a	9.00a	9.00a	9.00a	17.25a
arbendazim	1.92b	3.72b	5.06b	5.54b	8.02b	8.08b	16.64a
enomyl	1.24c	2.22c	3.42c	4.47c	5.63c	5.77c	16.19a
lancozeb	1.00d	1.68d	2.35e	2.62e	2.62e	2.62e	6.21b
arbendazim+ Mancozeb	1.06d	1.68d	2.60d	3.38d	3.98d	4.15d	16.78a
E±	0.030	0.118	0.032	0.040	0.024	0.023	0.675
NK	*	*	*	*	*	**	*
ate (R)							
	2.80a	5.26a	5.95a	9.00a	9.00a	9.00a	24.48a
.5	1.30b	2.06b	3.63b	4.52b	5.76b	5.75b	15.79b
.0	1.06c	1.78b	2.75c	3.53c	4.58c	4.90c	10.83c
.5	0.92d	1.82b	2.49d	3.21d	3.93d	4.05d	7.36d
E±	0.027	0.106	0.029	0.036	0.022	0.020	0.604
NK	**	**	**	**	**	**	**
nteraction(F x R)	**	**	**	**	**	**	**

Table 3: Fungicide x Rate interaction effect on mycelial growth of *Curvularia geniculata*

Fungicides	Rates	2	4	6	8	10	12	
Thiomethoxan + Metalaxyl-M + Difenoconazole	0	2.83ab	5.36a	8.05a	9.00a	9.00a	9.00a	23.80a
	0.5	2.11de	3.49de	7.34b	9.00a	9.00a	9.00a	19.46b
	1.0	2.14cd	3.96de	7.28b	9.00a	9.00a	9.00a	15.28d
	1.5	1.95e	4.57bc	7.32b	9.00a	9.00a	9.00a	10.49ef
Benomyl	0	2.69b	5.25a	7.97a	9.00a	9.00a	9.00a	25.36a
	0.5	1.11f	1.74g	2.97e	4.38c	6.20c	6.38c	17.14b
	1.0	0.67g	1.41g	1.65f	2.55e	4.11e	4.25e	15.12cd
	1.5	0.50g	0.50h	1.10g	1.94f	3.21g	3.45g	7.15f
Carbendazim	0	2.80ab	5.23a	7.98a	9.00a	9.00a	9.00a	23.93a
	0.5	2.29cd	4.08cd	6.05c	6.78b	9.00a	9.00a	18.90b
	1.0	1.48e	2.52fg	3.58d	4.46c	8.50b	8.60b	12.21de
	1.5	1.14f	3.06ef	3.02e	3.52d	5.58d	5.75d	11.54de
Mancozeb	0	2.74abc	5.25a	7.92a	9.00a	9.00a	9.00a	24.84a
	0.5	0.50g	0.50h	0.50i	0.50h	0.50j	0.50j	0.00g
	1.0	0.50g	0.50h	0.50i	0.50h	0.50j	0.50j	0.00g
	1.5	0.50g	0.50h	0.50i	0.50h	0.50j	0.50j	0.00g
Carbendazim + Mancozeb	0	2.93a	5.24a	7.87a	9.00a	9.00a	9.00a	24.45a
	0.5	0.50g	0.50h	1.27g	1.95f	3.67f	3.87f	23.49a
	1.0	0.50g	0.50h	0.72h	1.15g	1.90h	2.17h	11.54de
	1.5	0.50g	0.50h	0.54i	1.10g	1.36i	1.58i	7.66f
SE±		0.061	0.237	0.065	0.084	0.049	0.045	1.350
SNK		**	**	**	**	**	**	**

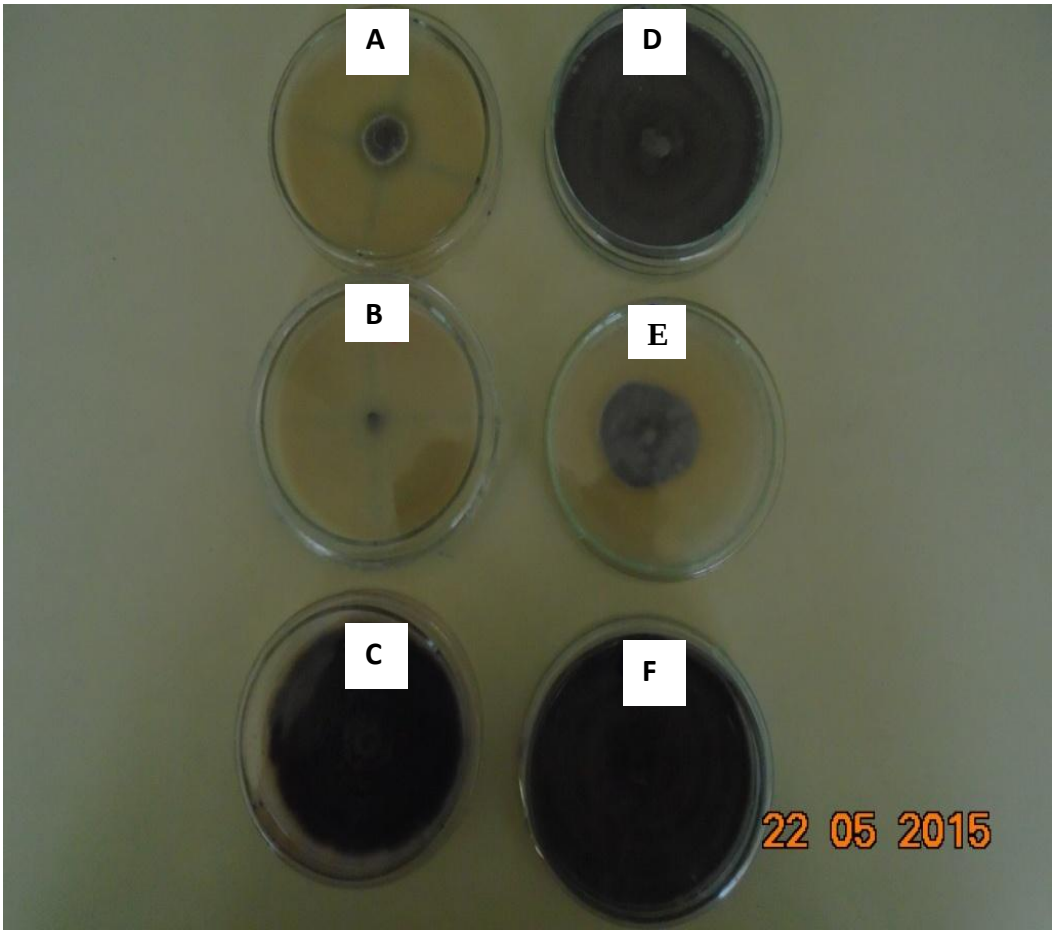

Plate 1: Mycelial growth of C*urvularia geniculata* on different fungicides at 0.5 x mg a.i/ mls at 7 days after inoculation (DAI)

KEY:

x- Manufacturers Recommended Rate
A- Carbendazim + Mancozeb
B- Mancozeb
C- Carbendazim
D- Thiomethoxan + Metalyxl-M + Difenoconozole
E- Benomyl
F- Control

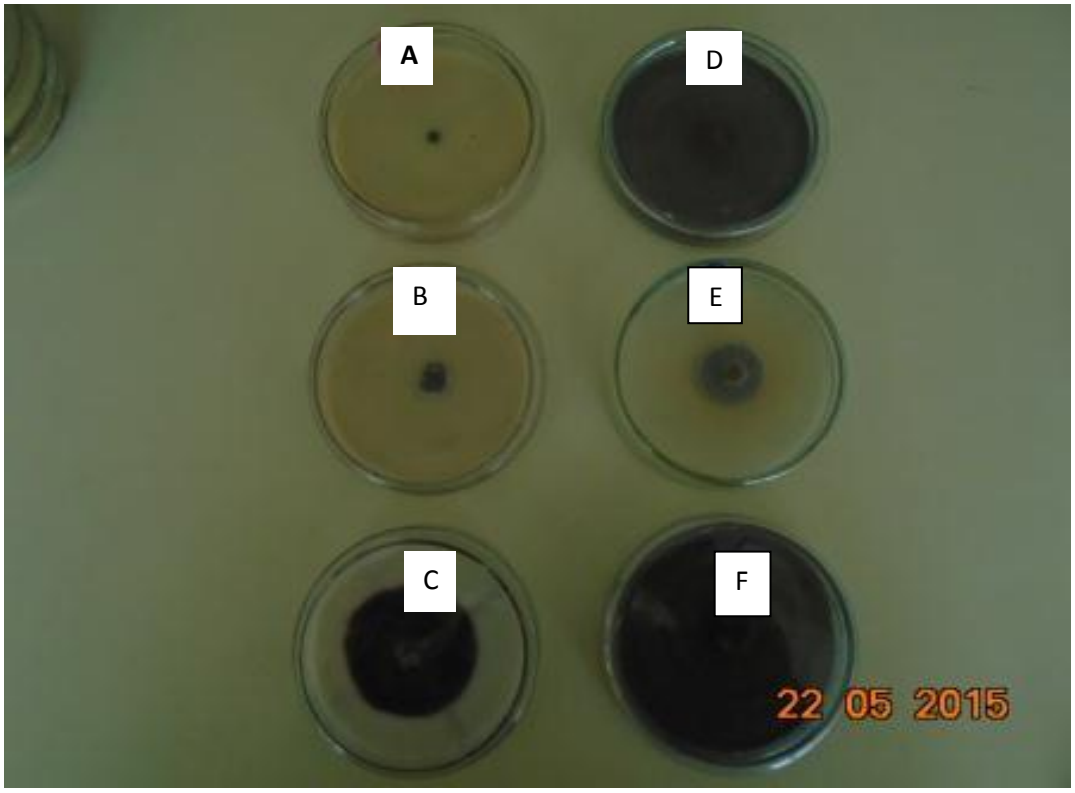

Plate 2: Mycelial growth of *Curvularia geniculata* at 1.0x mg a.i / mls fungicide at 7 days after inoculation (DAI)

KEY:

x- Manufacturers Recommended Rate
A - Carbendazim + Mancozeb
B - Mancozeb
C- Carbendazim
D- Thiomethoxan + Metalyxl-M + Difenoconozole
E- Benomyl
F- Control

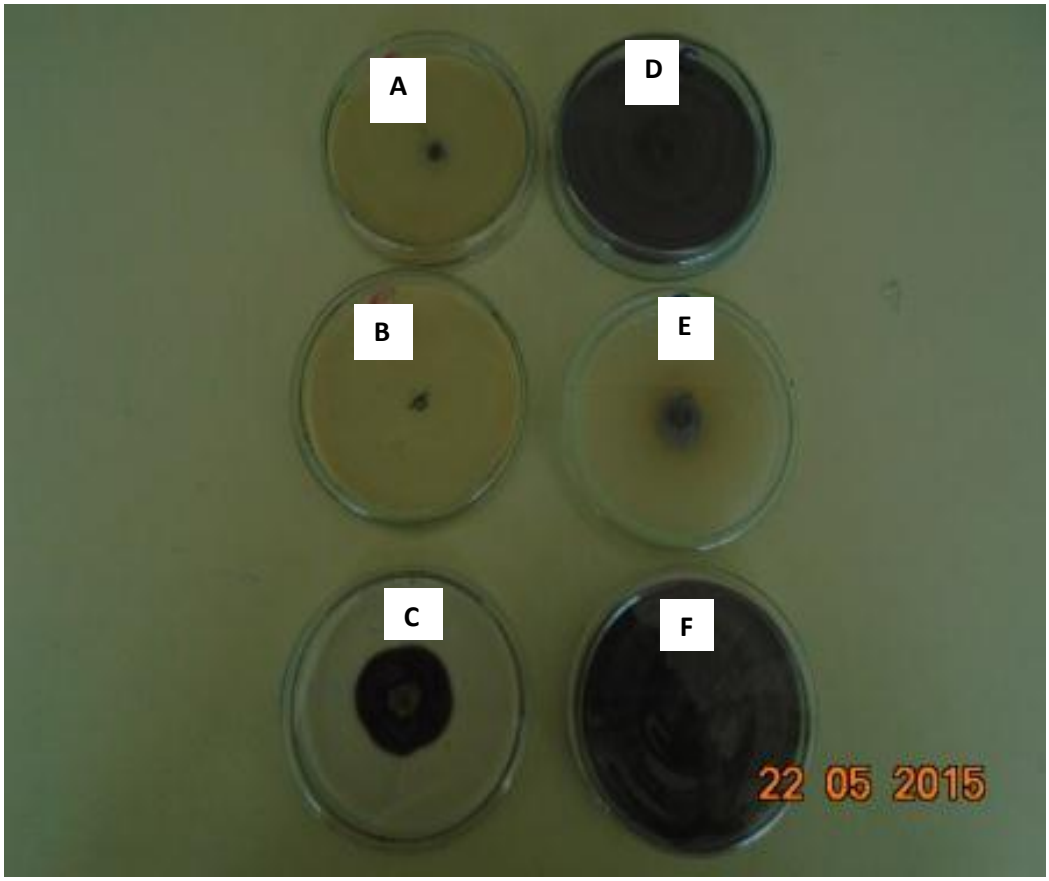

Plate 3: Mycelial growth of *Curvularia geniculata* on different fungicides at 1.5 x mg a.i/ mls at 7 days after inoculation

KEY:

x- Manufacturers Recommended Rate
A- Carbendazim + Mancozeb
B- Mancozeb
C- Carbendazim
D- Thiomethoxan + Metalyxl-M + Difenoconozole
E- Benomyl
F- Control

DISCUSSION

In this study, all tested fungicides significantly reduced pathogen development except Thiomethoxan + Metalyxl-M + Difenoconozole with Mancozeb being the best. The high efficacy of Mancozeb, Carbendazim and Benomyl, was reported on *Physoderma maydis*, causal organism ofmaize brown spot where sporangia germination was completly inhibited(23). Similarly, Benomyl at 5, 10 and 20 g was effective in controlling *Stenocarpella maydis* on maize *in vitro* (21). Also Gholve *et al.* (10) reported Mancozeb at 500, 1000 and 1500ppm was effective in controlling the mycelia growth of *Alternaria macrospora* on cotton.In a similar report, Mancozeb at 1000, 1500, 2000, 2500, 3000 and 3500 ppm completely inhibited the growth of *Alternaria solanii* irrespective of the concentration (10). Mancozeb also gave a significant reduction in sporulation of *Alternaria solanii* on Apple irrespective of concentration (9). Mancozeb also gave a significant reduction in sporulation (27; 30; 31). Obagwu (23) reported Mancozeb to be effective in the *in vitro* control of brown blotch of Bambaranut caused by *Colletotrichum capsici.* In this study the poor inhibitory effect of Thiomethoxan + Metalyxl-M + Difenoconozole (Apron Plus) on mycelial growth and sporulation was observed. Report has shown Metalaxylcould not inhibit the mycelial growth of some isolates of *Phytophthora infestans* of Potato in Serbia (7). Also Metalaxyl could not inhibit the mycelial growth of *Bremia lactucea* downy mildew on Lettuce cv Salinas (31). In this experiment Carbendazim + Mancozeb gave better inhibitory effect on mycelial growth than Carbendazim alone. This is in line with Mamza *et al*. (18; 19; 20) who reported the inhibitory effect of Carbendazim and Mancozeb on mycelial growth and sporulation of *Fusarium pallidoroseum* on Castor. Similarly, Alberto, *et al*. (2) conducted an experiment on *Collectotrichum gleosporiodes* (Penz.) where Carbendazim gave a significant reduction in mycelial growth. Meanwhile, in another experiment Carbendazim + Mancozeb, Mancozeb, Carbendazim, Ziram, Metalaxyl + Mancozeb on *Alternaria* leaf blight of sunflower, reduced mycelial growth of *A. helianth* at higher concentrations (500 and 1000 ppm) as compared to untreated however, Carbendazim was found to be more effective (22).

CONCLUSION

This study indicated the suppressive effect of various fungicides on mycelial growth and conidia count of *Curvularia geniculata* on fonio. The inhibitory effect varies with various

fungicides. However, the inhibition in mycelial growth increased with the increase in the concentration of different fungicides. All fungicides tested effectively reduced pathogen development when compared with the control with the exception of Thiomethoxan + Metalyxl-M + Difenoconozole (Apron Plus) which could not inhibit mycelial growth and conidia sporulation of *Curvularia geniculata* at all concentrations, while Mancozeb at all rates was highly effective. Meanwhile this is the first time fungicides is being evaluated on *Curvularia geniculata* isolated from fonio in Riyom, there is a need to further evaluate the fungicides under field conditions for effective management of the pathogen.

REFRENCES

1. **Adoukonou-Sagbadja, H., Dansi, A., Vodouhè, R.and Akpagana, K. (2006)**. Indigenous Knowledge and traditional conservation of Fonio millet (*Digitaria exilis* Stapf, *Digitaria iburua* Stapf) in Togo. *Journal of Biodiversity and Conservation* 15: 2379-2395
2. **Alberto, J.O., S.A.Bello, C.P. Ruth and C.C. Reynaldo 2005**. Chemical control of *Collectotrichum gloesporiodes* (Penz)Penz and Sacc under laboratory conditions. *IDESIA* (Chile) Enero- Volume 23, No.1 pp 19-23.
3. **Aleiro, A.A. and Morakinyo, J.A. 2001**. Characterization of *Digitaria exilis* (Kippist) Stapf and *D. iburua* Stapf accessions. *Nigerian Journal of Genetics* 16:10-12
4. **Belton, P.S and John, R.N 2002**. Pseudo cereals and less common Cereals; Grains Properties and utilization potentials. Publisher Springler – Varlag, Berlin, pp201.
5. **Booth, C. (1971).** The Genus *Fusarium*. CMI, Kew, England. 237p.
6. **Emil, R., I. Potocnik, S.M. Milijasvic, M. Stepanivic, B. Todorovic and M. Mihajlovic. 2012**. Toxicity of Metalaxyl,azoxystrobin,dimethomorph,cymoxanil,zoxamide and Mancozeb to Phytophthora infestans isolates from Serbia. *Journal of Environmental Science and Health*, part B (2012) 47,403-409
7. **European Food Research and Technology (E-FRT). 2000.** Vol. 211, No. 2/July 2000. Springler Berlin Heidelberg.
8. **FAO (Food and Agricultural Organization) database 2013.** www. FAO.org / side, pp567

9. Ganie S.A., M.Y Ghani, Q. Anjum, Q. Nissar, S.U Rahman and W.A. Dar 2013. Integrated Management of early blight of potato under Kashmir valley conditions. *African Journal of Agricultural Research* Vol.8 (32) pp 4318-4325, August 2013

10.**Golve, V.M., S.M., Jogdand, G.P. Jagtap and U. Dey 2012***In Vitro* evaluation of fungicides, bioagents and aqueous leaf extracts against *Alternaria* leaf blight of cotton. *Scientific Journal of Veterinary Advances* (2012)1(1) 12-21

11. **Haq, N. and Ogbe, D. F. 1995**. Fonio (*Digitaria exilis* and *D.iburua*). **In**: Cereals and Pseudocereals (Under – Utilized crops). Williams, J.T. ed Longman, Chapman and Hall, XX 111 London, United Kingdom. pp. 225–245.

12.**Ibrahim, A. 2001**. Hungry Rice (Fonio): A neglected cereal crop.*NAQAS Newsletter* 1 For effectio (4): 4-5.

13. **Jideani, I. A. 1999.** Tradition and possible technological uses of *Digitaria exilis* (Acha) and *Digitaria Iburua*: A review. *Plant foods Human Nutrition* 54: 363-374

14. **Jideani, I. A. and Takeda, Y. 1996.** Structures and Physiochemical properties of Starches from acha (*Digitaria exilis*), iburu (*D.iburua*) and tamba (*Eleuine coracana*) *Cereal Chemistry* 73 (6): 677-685

15.Karaoglanidis, G.S., D.A Karadimos, P.M. Ioannidis and Ioannidis, P.I. 2003 Sensitivity o f *Cercosporabeticola* populations to Fentinacetate, Benomyl and Flutriatol in Greece. *Crop protection*, (2003) 22:735-740.

16. **Kwon-Ndung, E.H. and Misari, S.M. 1999.** Overview of research and development of Fonio (*Digitari exilis* Kippist Stapf) and prospects for genetic improvement in Nigeria. In: Genetics and Food Security in Nigeria. *Genetics Society of NigeriaPublication*, Nigeria, p.71-76.

17. **Maji, E.A., Bright E.O., Bake, J. and Mohammed, J. 2013**. Integrated Management of acha brown leaf spot disease through the use of nitrogen fertilizer and the fungicide kitazine. Annual Research Review and Planning Workshop NCRI Badeggi, 10th – 12th June 2013 pp 101 – 105

18. Mamza, W.S., Dachi, S.N and Ukwungwu, M.N. 2009. Preliminary Studies on Insect Pests and Diseases of acha at

Riyom. National Cereals Research Institute Badeggi, 2009 Research Review and planning workshop, 12th -14th May 2009, pp 117-119

19. **Mamza, W. S., Zarafi, A.B. and Alabi. O. 2010**. *In vitro* evaluation of six fungicides on radial mycelial growth and regrowth of *Fusarium pallidoroseum* isolated from Castor (*Ricinus communis*) in Samaru, Nigeria. *Archives of phytopathology and plant protection*, vol 43 (7-9) 871-874.

20. **Mamza, W.S., Zarafi A.B., Dachi S.N., Bright E.O and Ukwungwu, M.N. 2013.** Incidence and severity of *Curvularia* leaf spot on *Digitaria iburua* (Stapf)(Fonio) in Riyom, Plateau State, Nigeria. Poster presentation during the 3rd NUS 2013 International Conference in Ghana held at Mensvic Hotel Accra, from 25th to 27th September 2013

21. **Marley, P.S. and O. Gbenga 2004.** Fungicide control of *Stenocarpella maydis* in the Nigerian Savanna. *Archives of Phytopathology and Plant Protection*. 37: 19-28.

22. **Mathivanan, N. and V.R Prabavathy 2007.** Effects of Carbendazim and Mancozeb Combination on *Alternaria* leaf blight and seed yield of sunflower *(Helianthus annus L.) Archives of Phytopathology and Plant Protection*, 40 (2):90-96

23. **Obagwu J. 1997**. Studies on the Brown blotch disease of Bambaranut (*Vigna subterranean* (L.) Verde) induced by *Colletotrichum capsici* Syd Butler and Bisby in the Zaria Area of Northern Nigeria. M.Sc. Thesis, Crop Protection Department Ahmadu Bello Bello University Zaria. p. 74.

24. **Osunlaja, S.O.and Alamutu, M.A. 1999**. Evaluation of fungicides for the control of Brown spot disease of Maize caused by *Physoderma maydis* (miyabe). *Nigerian Journal of Plant Protection.*18:84–95.

25. **Purseglove, J. W. 1975.** Tropical Crops:Monocotyledons. Longman, UK. pp. 142–144.

26. **SAS. 2013.** SAS Users guide. Statistical system Institute, Cary, NC, USA.

27. **Szkolnik, M. 1981.** Physical modes of action of sterol inhibiting fungicides against apple diseases. *Plant Disease*. 65:981-985.

28. **Temple, V.J. and Bassa, J.D. 1991.** Proximate chemical composition of Fonio (*Digitaria*

exilis) grain. *Journal of Science Food and Agriculture*. 56:561-564.

29. **Vietmeyer, N.D., Borlaugh, N.E., Axtell, J., Burton, G.W., Harlan, J.R. and Rachie, K.O. (1996).** Fonio (Acha). **In:** Lost crop in Africa. BOSTID Publication, pp 59-7

30. **Wicks, T. G., Hall, B., and Pezzaniti, P. 1994.** Fungicidal control of metalaxyl-insensitive strains of *Bremia lactuce* on lettuce. *Crop Protection*.13:617-623.

31. **Wong, F. P., and Wilcox, W. F. 2001**. Comparative physical modes of action of Azoxystrobin, Mancozeb, and Metalaxyl against *Plasmopara viticola* (grapevine downy mildew). *Plant Disease* 85:649-656