



**EVALUATION OF FUNGICIDE EFFICACY FOR THE CONTROL OF
LEAF SPOT DISEASE ON OKRA (*ABELMOSCHUS ESCULENTA L.*)
CAUSED BY *CURVALARIA LUNATA* (WAKKER) BOEDIJN IN PORT
HARCOURT, NIGERIA**

¹Okoi Arikpo Ikpi, ¹Okon Essien A. and ²Ataga A. E.

¹Department of Biological Sciences, Cross River University of Technology, PMB 1123,
Calabar Nigeria.

²Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria.

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***Correspondence for
Author**

Dr. Okon Essien A.

Department of Biological
Sciences, Cross River
University of Technology,
PMB 1123, Calabar
Nigeria.

ABSTRACT

The efficacy of fungi toxicity of five fungicides was tested for the control of leaf spots disease of Okra, caused by *Curvularia lunata* in the Green house. The concentrations of the fungicides used for the inhibition of the fungus were 50, 100, 150, 200 and 250 $\mu\text{g}/\text{cm}^3$ on potato dextrose agar medium (PDA) for the vegetative growth and on leaf spots disease of Okra, in vivo. All the five fungicides; Kototine, Apron plus, Benlate, Captan and Dithane M-45, inhibited the

vegetative growth of *C. lunata* at all concentrations. The fungitoxic efficacy increased with increase in concentration of the fungicides. At 150 to 250 $\mu\text{g}/\text{cm}^3$ Kototine and Apron plus completely (100%) inhibited the mycelia growth of the fungus. The remaining fungicides in order of their decreasing fungitoxicity against the pathogenic fungus were Benlate, Captan and Dithane M-45. Apron plus and kototine proved to be more fungitoxic than the rest of the fungicides by recording the least number of leaf spot disease of okra, and size of leaf spots per leaf of okra plants

KEY WORDS: Evaluation, fungicide efficacy, control, leaf spots, okra, *Curvularia lunata*.

INTRODUCTION

Okra (*Abelmoschus esculentus L.*), (Moench) is a widely cultivated vegetable crop in Nigeria and other West African countries primarily for its mucilaginous fruits. It is a member of the

family malvacea and classified as an annual, herbaceous dicotyledon. The unripe, green fruit (a capsule) can be sliced and used in thickening and flavouring soups and sauces. The fruit is rich in vitamin C (Akinsoyoye, 1979; Phillips, 1974) and contains water (86%), protein (22.2%), fat (0.2%), carbohydrate (9.7%) and fibre (1.0%) (Purseglove, 1969). The leaves and stems are sources of fodder for goats and sheep. In traditional medicine, the mucilage in the fresh fruits is used in the treatment of ulcers, and for the relief of hemorrhoids.

Leaf spot disease caused by *Curvularia* species is an important pathogenic fungus that disturbs and causes a major problem in okra production in Nigeria. Several workers have reported a number of *Curvularia* species as causal agent of leaf spot diseases in the following crops; *Curvularia clavata* from maize (Arum *et al.*, 1980); *C. borreria* from pine (Kore and Bhide, 1981); and *C. abelmosci* from okra (Kenneth, 1979). The pathogen has been reported to cause leaf blight, seedling blight and grain lesions in rice (Benoit and Mathur, 1970). In Nigeria *Curvularia lunata* (Wakker) Boedijn has been reported to cause leaf spot disease of cow pea (Esuruoso, 1974), and pumpkin (Eboh and Okoh, 1980).

The fungus *C. lunata* is seed borne; Ataga and Akueshi (1980) reported that *C. lunata* is seed-borne in sunflower plant. Recent studies in our laboratory also revealed that *C. lunata* is a common seed-borne pathogen of several vegetables and food crops like okra (*Abelmoschus esculenta*), groundnut (*Arachis hypogea*), Ogbono (*Irvingia gabonensis*) and African yam bean (*Sphenostylis stenocarpa*) (Singh, 2003).

Fungicides have played a vital role in the control of economic crop plants and several vegetable diseases, especially when disease resistant varieties are not in vogue. A good fungicide should be toxic only to the pathogen but not to the host plant (Pandey, 2011). Chemical compounds called fungicides were often applied to seeds, soil and foliage to form local barriers against fungal invasion in plants. Such treatments were most successful when used before the onset of infection as they achieved full disease control of host plants (Singh, 2006).

Apron plus is a good example of fungicide with a broad spectrum activity. Tunwari *et al* (2014) reported that Apron plus 50DS significantly inhibited and reduced the severity of gray leaf spot disease of sorghum cultivars from 40 to 25% over four other fungicides. Apron plus was also reported to have significantly prolonged the storage life of three varieties of soybean seeds stored under ambient conditions for 6 months from 2 to 3 months before deterioration

than the untreated seeds (Adebisi *et al.*, 2004). Also, Dithane M-45, another fungicide at higher concentration completely inhibited the growth of *Alternaria alternata*, the causal agent of leaf spot disease of chilli fruit (*Capsicum annum*) in vitro (Manoj *et al.*, 2013 and Ratan *et al.*, 2003). Low concentrations of Benlate, another systemic fungicide was reported to have prevented the vegetative growth of powdery mildew of kidney bean cotyledons caused by *Erysiphe graminis* (Natti and Crosier, 1971). Bambridge *et al.*, (1985) also reported that low concentrations of Benonyl (Benlate) and chlorothalonil inhibited the germination and growth of *Septoria apicola*, the causal agent of leaf spot disease of Celery plant (*Apium graveolus* L.).

Fungicides, as shown above therefore, plays a vital role in the control of diseases of vegetable crops, (Singh and Rai, 2003), especially in the absence of disease resistant varieties as is the case with most okra varieties found within Port Harcourt and environs. This study therefore, evaluates the efficacy of five fungicides for the control of leaf spot disease of okra (*Abelmoschus esculenta*) caused by *Curvularia lunata* (fungus) in Port Harcourt, Nigeria.

MATERIALS AND METHODS

Isolation of *C. lunata* from okra seeds

Seeds of a locally important okra variety (cv. Velvet-35) were obtained from the Cross River State Agricultural Development Project (ADP) Calabar, Nigeria. Isolation of *C. lunata* associated with okra seeds was carried out in accordance with the recommendation of International seed Health Testing Association (ISTA, 1966), using the blotter method. Okra seeds (500g) were surface sterilized by immersing in a 100% mercuric chloride solution for 1 minute and transferred into 95% ethanol for 10 seconds. The seeds were immediately removed and rinsed in four changes of sterile distilled water. The seeds were then dried between layers of sterile filter papers. Ten seeds of the sterilized okra were plated on a three-layer sterile Whatman filter paper, moistened with sterile distilled water in a 9cm sterile Petri dish. The Petri dish was incubated at a temperature of 27°C for 7 days. The incubated seeds were later examined under a stereobinocular microscope for identification of *C. lunata* fungus. Mycelia from okra seeds were picked with a sterile inoculating loop, sub-cultured on sterile PDA medium until pure cultures were obtained. The pure cultures of *C. lunata* were stored in a refrigerator at a temperature of 4°C until needed.

Preparation of spore suspension of *C. lunata*

Spore suspension of *C. lunata* was prepared by pouring 10ml of sterile distilled water into a pure culture of the fungus. The culture was stirred gently with a sterile glass rod to dislodge the propagules. The conidial suspension was filtered through a single layer of filter paper and adjusted to a concentration of 5×10^4 spores/cm³ with sterile distilled water, using a haemocytometer.

Preparation of fungicide concentrations

A stock solution of 1000µg/cm³ of each of the five fungicides (Benlate, Dithane M-45, Captan, Apron plus and Kokotine) was prepared in sterile water and serial dilutions made with sterile distilled water to give five concentrations of 50,100, 150, 200 and 250µg/cm³. Spore suspension of *C. lunata* (5×10^4 spores/cm³) was used for each investigation.

Effect of fungicides on the mycelial growth of *C. lunata*

The relative efficacy of five fungicides was tested against *Curvularia lunata* in vitro using potato dextrose agar (PDA) medium. The names of the fungicides, their chemical composition and doses are given in Table 1. Exactly 18ml of freshly prepared sterile PDA medium was poured into each of the sterilized 90mm Petri dishes. Also, 2ml of each of the fungicide solution was added into each Petri dish and agitated slightly to give a thorough mixing of the contents, and left to solidify. The media plates were inoculated at the centres with a 4mm inoculum disc from a freshly prepared culture of *C. lunata* using a sterile inoculating needle. PDA plates inoculated with the test fungus but without the fungicides served as control. All the plates were incubated at $27 \pm 1^\circ\text{C}$, after which the zones of inhibition on colony diameter were measured for 7 days and compared with the controls. The experiment was repeated thrice in a randomized block design with 5 treatments and three replicates.

Table 1: Fungicides used for in vitro evaluation against *Curvularia lunata*

Fungicide (Trade name)	Chemical name	Dose
Benlate (Benomyl)	Methyl 1- (butyl carbamoyl) -2- benzimidazole carbamate	5g/kg seed
Dithane M-45	Manganese, zinc, ethylene bisdithio carbamate ion	5g/kg seed
Captan	N-(trichloromethyl thio) – 4 – cyclohexene – 1, 2- dicarboximide	5%/kg seed
Apron plus	Metalaxyl, Carboxin, Furathiocarb.	5g/kg seed
Kokotine (Lindane)	a-Hexachlorocyclohexane	5%/kg seed

The efficiency of various fungicides was assessed by measuring the radial growth of the fungal colony in millimeters (mm). Growth inhibition percentage of colony was calculated by the formula:

$$I = \frac{C - T}{C} \times \frac{100}{1}$$

Where I = inhibition percentage of colony
C = average diameter of colony in control
T = average diameter of colony in treatment.

Evaluation of fungicides for the control of leaf spot disease of okra in the field (in vivo)

Okra seeds were pre-treated with five fungicides and sown in perforated plastic buckets (22 x 22cm in size) filled with sterilized loamy soil and watered daily where necessary in the green house. The fungicides used and their concentrations were: Benlate (5.0g/kg seed, 5 mins dust); Captan (5%/kg seed, 5 mins. dip); Apron plus (5.0g/kg seed, 5 mins dust); Dithane M-45 (5.0g/kg seed, 5 mins. dust) and Kokotine (5%/kg seed, 5 mins. dip). Each bucket was sown with 3 okra seeds and later thinned down to 2 plants per bucket after seven days of germination. After attaining 3 to 4 leaf stage (14 days after planting), all the leaves of the test plants were sprayed to run-off with a freshly prepared spore suspension of *C. lunata* using a spray atomizer (Sterling products, Manchester). Each inoculated plant was enclosed in a transparent water proof bag and tied at the base using a thread to create a humid environment for the plants for 24 hours. After seven days, the number of leaf spots per leaf and size of leaf spots were recorded in comparison with the type and concentration of fungicide applied, and the controls.

RESULTS

Results of the effect of fungicides on the mycelia growth of the fungus are shown in Table 2. All the five fungicides tested under laboratory conditions significantly inhibited the vegetative growth of *C. lunata* at all concentrations. The result showed that there was a general reduction in the vegetative growth of the fungus with increase in concentration of the fungicides. The highest percentage inhibition of mycelia growth of the fungus was recorded at 150 to 250 $\mu\text{g}/\text{cm}^3$ fungicides concentrations where Apron plus and Kokotine completely inhibited the mycelia growth of the fungus, each at 100% inhibition. At 50 $\mu\text{g}/\text{cm}^3$ concentration, the lowest percentage inhibition of the fungus was recorded in Dithane M-45 (20.0%) closely followed by Captan (33.3%) as compared to 30mm in control with no (0%) inhibition (Table 2). The remaining fungicides in order of their decreasing fungitoxic efficacy

against the pathogen were Benlate, Captan and Dithane M-45. The fungicides significantly ($P < 0.05$) differed from one another in checking the growth of the fungus at different concentrations used.

Table 2: Radial growth of *C. lunata* (mm) and percentage (%) inhibition PDA treated with fungicides, 7 days after inoculation, incubated at 27⁰C

Conc. ($\mu\text{g}/\text{cm}^3$)	Captan		Dithane M-45		Benlate		Apron Plus		Kokotine	
	Growth (mm)	Inhibit. (%)	Growth (mm)	Inhibit. (%)	Growth (mm)	Inhibit. (%)	Growth (mm)	Inhibit. (%)	Growth (mm)	Inhibit. (%)
50	20	33.3	24	20	15	50	12	60	6	80
100	15	50	18	40	12	60	5	83.3	2	93.3
150	9	70	13	56.7	6	80	0	100	0	100
200	5	83.3	7	76.7	3	90	0	100	0	100
250	2	93.3	3	90	1	96.7	0	100	0	100

Control = 30mm, 0% inhibition

Results of the average number of leaf spots per leaf and size of leaf spots of okra are presented in Table 3. The size of leaf spots of the treated plants significantly reduced from Dithane M-45 (3.3mm) to kokotine (1.5mm). Okra plants pretreated with kokotine gave the smallest average leaf spot size of 1.5mm, closely followed by Apron plus (1.7mm). Benlate and Captan had a common leaf spot size of 2.4mm each, while Dithane M-45 showed the largest leaf spot size of 3.3mm. However, the average leaf spot size in the control plants was 4.3mm (Table 3).

Okra plants pretreated with Kokotine also gave the least average number of leaf spots per leaf (3.3), closely followed by Apron plus (4.7), while Dithane M-45 gave the highest number of 7.8 (Table 3). However, the control plants recorded a maximum average number of 12 leaf spots per leaf.

Table 3: Average number and size of leaf spots (mm) of okra seven days after inoculation with *C. lunata* pretreated with fungicides in the Green house

Fungicide	Dose	Number of leaf spots per leaf	Size of leaf spots per leaf (mm)
Control	Water spray	12	4.3
Benlate	5g/kg seed	4.8	2.4
Dithane M-45	5g/kg seed	7.8	3.3
Captan	5g/kg seed	6.7	2.4
Apron plus	5g/kg seed	4.7	1.7
Kokotine	5g/kg seed	3.3	1.5

LSD ($P = 0.05$)

0.9

DISCUSSION

The present study showed that all the five fungicides inhibited the vegetative growth of *Curvularia lunata* at all concentrations in vitro. Out of the five fungicides, Kokotone and Apron plus appeared to be more effective fungicides than others as they completely inhibited the vegetative growth of the pathogen at 150 to 250 $\mu\text{g}/\text{cm}^3$ concentrations used. Benlate, Captan and Dithane M-45 were also found to be significantly superior when compared to control in inhibiting the mycelial growth of the fungus. These observations are similar to the findings of previous researchers in the control of fungi diseases on similar vegetable crops (Maude and Kyle, 1970); Maude *et al.*, (1969); and recently, Tunwari *et al.* (2014).

The results of the efficacy of the fungicides in the field (in vivo) also showed that Kokotone and Apron plus were the most effective fungicides by reducing significantly the number and size of leaf spots on okra plants. This observation is similar to the report of Sharma and Sohi (1981) in India, on the effectiveness of Bavistin another systemic fungicide in the control of *Cercospora* leaf spot diseases of chilli (*Capsicum annum*). According to their report, 1.1% leaf area of chilli got infected as compared to 11.3 in control. In comparison to the effectiveness of Kokotone in this study, only 3.3 of the total number of leaf spots per leaf were formed by plants pre-treated with Kokotone as compared to 12 in control. Similar results were also reported by Tunwari *et al.*, (2014) that Apron plus 50DS significantly inhibited and reduced the severity of gray leaf spot disease of sorghum cultivars from 40 to 25% over other four fungicides in vivo.

This study also showed that the fungicides did not vary significantly in the reduction of the size of leaf spots of okra. Except for okra plants pre-treated with Dithane M-45 (3.3mm) the rest of the test plants pretreated with the remaining fungicides showed little or no difference in the size of their leaf spots. For example, Kokotone and Apron Plus recorded 1.5mm and 1.7mm leaf spot size respectively, while Benlate and Captan recorded same leaf spot size of 2.4mm each (Table 3).

The present study also showed that low concentrations of Kokotone, Apron plus and Benlate effectively inhibited the mycelial growth and number of leaf spot disease of the fungus per leaf of okra, when compared to the control. This is in agreement with Bambridge *et al.*, (1985) who reported that low concentrations of Benomyl (Benlate) and chlorothalonil inhibited the germination and growth of *Septoria apicolo*, the causal agent of leaf spot disease of celery plant (*Apium graveolus* L.). The effectiveness of Benlate as a systemic fungicide in this study

also corroborate the findings of Natti and Crosier (1971) that low concentrations of Benlate prevented the growth of powdery mildew on kidney bean cotyledons caused by *Erysiphe grammis*. The efficacy of Apron plus in this study also agreed with the report of Adebisi *et al.*, (2004), that Apron plus, among other four fungicides significantly prolonged the storage life of three varieties of soybean seeds from 2 to 3 months before deterioration.

Dithane M-45 which showed the least fungitoxicity in this study at the lowest concentration (Table 2) was however, found to be effective in the inhibition of vegetative growth of *C. lunata* at higher concentrations. This report also agreed with Manoj *et al.*, (2013) that at higher concentrations Dithane M-45 completely inhibited the growth of *Alternaria alternata*, the causal fungus of leaf spot disease of *Capsicum annum*. However, at low concentrations the weak fungitoxicity shown by Dithane M-45 in this study is similar to the report of Singh *et al.*, (1970); Sharma and Sohi (1981) in the use of the fungicide in the control of mycelial growth of *Macrophomina phaseolina* on Indian jute (*Corchorus capsularis*).

The fungicides used in this investigation were found to be inhibitory to *C. lunata*. However, low concentrations of Kokotine, Apron plus and Benlate could be safely recommended for control of *C. lunata*, the causal agent of leaf spot disease of some of our economic crops. Based on the efficacy of the fungitoxicity of Kokotine and Apron plus over the other fungicides observed in this investigation, more research is ongoing in the field and screen house, on the possible toxicity of Kokotine and Apron plus in the control of fungal diseases of some important vegetable crops in Calabar, Nigeria.

REFERENCES

1. Adebisi, M. A., Daniel, I. O. and Ajala, M. O. Storage life of Soybean (*Glycine Max. L. Merril*) seeds after seed dressing. *Journal of Tropical Agriculture*. 2004; 42(1-2): 3-7.
2. Akinsoyoye V. O. Senior Tropical Agriculture for West Africa, Longman Nigeria Ltd. Ibadan. 1979; 75-77.
3. Arum, M. N. and K. C. B., Chaudhary. Survey and Epidemiology of *Curvularia* leaf spot of maize. *Indian phytopath.* 1980; 33(3): 337-521.
4. Ataga, A. E. and C. O. Akueshi. Changes in Protein and amino acid composition of sunflower seed inoculated with *Alternaria alternata*, *Curvularia lunata* and *Marcrophomina phaseolina* Nig. *Jour of Biotechnology* 1986; 2: 45-49.

5. Bambridge, J. M., R. B. Maude and A. Spencer. Test of fungicides as foliar sprays for the control of *Septoria apicola*, leaf spot of celery. Test of Agrochemicals and cultivars 6. *Annals of Applied Biology*. 1985; 106: 145-148.
6. Benoit, A. C. and C. O. Mathur. An account of seed-borne species of *Curvularia* on agricultural seeds in Canada. *Canadian jour of phytopath.* 1970; 3: 117-120.
7. Eboh, D. O. and C. I. Okoh. A preliminary Taxonomic Study of Fungi associated with some vegetables in Nigeria. *Nig. Jour. Agric. Sc.* 1980; 2(2): 75-81.
8. Esuruoso, O. F. Seed borne fungi of cowpea (*Vigna unguiculata*) in Western Nigeria. *Nig. Jour. Plt. Protection.* 1974; 1(1): 87-90.
9. International Seed Testing Association (ISTA),. International Rules for Seed Testing. Procedure for International Seed Testing Association 1966; 31: 1-52.
10. Kenneth, R. H. Wescott's Plant Disease Hand book. 4th ed. Van Nostrand Coy., New York 1979; 120.
11. Kore, S. S. and Bhide, V. P. A New record of *Curvularia borreriae* from India. *Indian jour of mycol. Plant pathol* 1981; 11(1): 150-151.
12. Manoj Kumar, Vijai Bhadauria, Kaushlendra Singh, Chandrajeet Sing and Anil Kumar Yadav. Evaluation of fungicide efficacy for the management of *Alternaria* leaf spot disease on chilli (*Capsicum annum*). *Indian jour. of phytopathol.* 2013; 12: 32-35.
13. Maude, R. B. and Kyle, M. Seed treatments with benomyl and other fungicides for control of *Ascochyta pisi*. *Annals of Applied Biology*. 1970a; 66: 37-40.
14. Maude, R. B.; Vizor, A. S. and Shuring, C. C. The Control of Fungal Seed-borne diseases by means of a Thiram seed soak. *Annals of Applied Biology*. 1969; 64: 245-248.
15. Natti, J. J. and Crosier, D. C. Seed and Soil Treatments for control of bean root rots. *Plt. Dis. Reprtr.* 1971; 50: 55-59.
16. Pandey, B. P. (2011). Plant Pathology, Pathogen and Plant Disease. S. Chand and Coy. Ltd., New Delhi.
17. Phillips, T. A. (1974). Notebook of Agricultural Science. Longmans Nigeria Ltd. Ibadan.
18. Purseglove, J. W. (1968). Tropical Crops, Dicotyledons Longmans, Green and Coy. Ltd. London.
19. Rata, V. H. P. Shukla and U. Narain,. Management of *Alternaria* blight of marigold. *Ann. Plant Prot. Sci.*, 2003; 11: 172-173.
20. Sharma, S. R. and Sohi, H. S. Effect of different fungicides against *Rhizoctonia* root rot of French bean (*Phaseolus vulgaris*). *Indian jour. Mycol., Plt. Pathol.* 1981; 11(2): 216-219.

21. Singh, R. S. (2003). Diseases of Vegetable Crops. Oxford and IBH, New Delhi, India.
22. Singh, K. and M. Rai, Evaluation of Chemical against *Alternaria* leaf spot of brinjal. *Ann Plant Prot. Sci.* 2003; 11: 394-395.
23. Singh, R. S. (2006). Introduction to Principles of Plant Pathology 4th edition, Oxford and IBH Publishing Coy. PVT. Ltd. New Delhi.
24. Singh, D. V., Singh, S. B. and Bains, B. S. Effect of fungicides against *Rhizoctonia bataticola* (Taub) Bult. *In vitro. Indian Journal of Microbiology* 1970; 10: 27-28.
25. Tunwari, B. A., Nahunnaro, H. and Anaso, A. B. Eco-friendly management strategies for gray leaf spot disease of sorghum using cultivar selection and seed dressing fungicides in Maiduguri, Nigeria. *Journal of Agriculture and Sustainability.* 2014; 5(1): 14-25.