International Research Journal of Public and Environmental Health Vol.1 (8),pp. 165-169, October 2014
Available online at http://www.journalissues.org/IRJPEH/
http://dx.doi.org/10.15739/irjpeh.003

ISSN 2360-8803

Original Research Article

In-Vitro antifungal efficacy evaluation of parazone, methylated spirit, and hydrogen peroxide on *Cercospora penniseti* (Chupp) the causal agent of the millet *Cercospora* leaf spot

Accepted 6 October, 2014

***[1]Kiyawa, S. A., [2]Umar, S., [3]Kutama, A.S. and [4]Datti, Y.**

[1]Department of Chemistry, Federal College of Education (Technical), Bichi, Kano, Nigeria.
[2]Department of Biological Sciences, College of Arts, Sciences and Remedial Studies, Kano,Nigeria
[3]Dept of Biological Sciences, Federal University, Dutse, Jigawa State, Nigeria
[4]Department of Chemistry, North – West University, Kano, Nigeria

*Corresponding Author
Email: ansarinagarr@gmail.com
Tel.: +2348065577180

The potency of three chemical disinfectants, namely; Parazone, Methylated spirit, and Hydrogen peroxide on *Cercospora penniseti* was evaluated in-vitro using Phenol Coefficient and Disc Diffusion techniques. Results of the experiment have shown that both techniques were effective for the evaluation of all the three disinfectants.The various disinfectants were found to inhibit the growth of the fungus with significant difference (P>0.05) between the various disinfectants. The phenol coefficients of Hydrogen peroxide, Parazone, Methylated spirit respectively, were found to be 0.9, 1.05 and 1.19. All the disinfectants were found to be active at 5000mm disc concentration against the test organism with inhibition zones ≥ 10 mm diameter indicating that the Phenol Coefficient and Disc Diffusion techniques were efficient for the evaluation of disinfectants' potency in-vitro and capable of controlling the growth of *Cercospora penniseti*.

Key words: Hydrogen peroxide, Parazone, Methylated spirit, *Cercospora penniseti,* phenol coefficient, disc diffusion techniques

INTRODUCTION

Cercospora Leaf Spot is a disease of Pearl millet caused by *Cercospora penniseti* (Chupp) with foliar lesions becoming oval, with dark brown margins and pale tan to grey or white centres, dotted with rows of black conidiophore tufts and lesions on the stems mostly found in India (Narayanaswami and Veerraju, 1970), United States, possibly Malawi (Wiehe, 1953), and possibly Tanzania (Mbwaga et al., 1993).

Cercospora leaf spot is an infectious leaf disease that affects smooth, panicle, oakleaf and bigleaf types of hydrangea in Arkansas landscapes and nurseries. This disease is caused by the fungus *Cercospora hydrangea* and is perhaps the most common disease seen on this perennial ornamental during the months of July through October. Although this disease can be widespread on hydrangeas in the landscape, it is generally an aesthetic problem for homeowners because the disease rarely kills the plant. *Cercospora* leaf spot can, if it is severe, reduce overall plant vigour by repeated defoliation. (http://www.uaex.edu)

MATERIAL AND METHODS

Study site

The Research was carried out in the Biological Sciences laboratory of the Bayero University, Kano.

Sample collection

The selected varieties of the infected millet plant leaves were collected from a farm at Bagwai town, Bagwai local

government area, Kano state. And were brought to the laboratory for series of tests, to find out whether the potentials of the three chemical disinfectants are effective on the control of *Cercospora penniseti*, that causes the *Cercospora* disease.

Collection of chemical disinfectants

The test samples were purchased from a pharmaceutical chemist in Sabon-gari market, Kano. Three (3) different brands of disinfectants namely; Hydrogen peroxide, Methylated spirit and Parazone were obtained. Samples were labelled and stored at a temperature of 25°C (British pharmacopeia, 1993).

Sample preparation

Millet infested leaves were cut into 3 mm and sterilized into 1% sodium hypochlorite for 2 minutes (Jha, 1995).

Culture media

Two media were used; Potato Dextrose Agar (PDA) as general culture medium and Sabouraud Dextrose Agar (SDA). They were prepared according to the manufacturers' instructions. Cholorophenicol is also added as anti-Bacteria (Kiyawa et al., 2014).

Sterilization was done at 121°C for 15 minutes in the autoclave. Media were poured into 90mm diameter Petri dishes (Harris, England) (25ml per plate) placed on a levelled surface for uniform depth (Cheesbrough, 2000).

Isolation of the fungi

The infected samples were cut into 3mm pieces with sterile razor blade, surface sterilized in 1% sodium hypochlorite for 2 minutes, then placed on Potato Dextrose Agar (PDA) and incubated at room temperature for 5days. After incubation, colonies of different shapes and colors were observed on the plates. A pure culture of each colony type, on the plates was obtained. The maintenance was done by sub-culturing each of the different colonies on to the SDA plate an incubated at room temperature again for 5days (Jha, 1995).

Identification of the isolated fungi

The technique of James and Natalie (2001) was adopted for identification of the unknown isolated fungi using cotton blue in Lactophenol stain. The identification was achieved by placing drop of stain on clean slid with the aid of a needle. A cover slip was gently applied with little pressure to eliminate air bubbles. The slid was then mounted and observed with X10 and X40 objective lenses respectively. The species encountered were identified in accordance with Cheesbrough (2000).

Dilution techniques for evaluation of anti-fungal potency of disinfectants

Two colonies of the isolate were picked using sterile wire loop and inoculated into 10ml of nutrient broth in sterile test tube. These were then incubated overnight for 18hrs at 37°C as demonstrated by Mukhtar and Okafor (2002). The disinfectants were diluted according to the specification on the labels (the use dilution) 5ml of each disinfectant were placed in 7 sterile test tubes. 0.05ml of *Cercospora penniseti* is added to the various tubes. The tubes were labeled appropriately and then each was mixed to obtain each mixture containing fungi and disinfectant were transferred to separate test tube of sterile nutrient broth using sterile 1ml syringe at intervals of 5, 10, and 15 minutes.

All tubes were incubated for 4 to 5 days at 25-37°C for fungi. Each tube was shaken and observed for growth. Presence of growth was recovered as (+) and the absence as (-). Results were tabulated.

Preparation of sensitivity disc

Paper discs of about 6.0mm were punched from whatman No.1 filter paper, using paper puncher and sterilized by dry heating in an oven at 140°C for 60 minutes (Cheesbrough, 2000).

After cooling at room temperature, 0.1ml each disinfectant solution was dispensed into each screw capped bottle of 100 paper discs so that the discs are saturated at an equivalent potency of 100% concentration (Kiyawa et al., 2014).

Determination of phenol coefficient

A series of seven-graded concentration of the disinfectant was prepared, the 5ml volumes of the different concentrations were dispensed into sterile stopper test-tubes. 3-100ml of a 5% stock solution of "pore" phenol was prepared in sterile distilled water. From this, dilutions of 1 in 95, 1 in 100, 1 in 110 and 1 in 115 were prepared. 5ml of the different concentrations was dispensed into sterile stopper test-tubes. 0.5ml of *Cercospora penniseti* was added to each of the tubes containing 5ml volume of the solutions of the test disinfectant. The mixture was shaken well using a mixer. The tubes were kept at 18°C before and during the test. At intervals of 5, 10 and 15 minutes, a larger loopful was removed from each mixture using standard wire loop. It was then transferred to test-tubes containing 5 ml nutrient broths. The same procedure was repeated for the rest of the phenol solutions. The broth test-tubes were incubated at 37°C for 48hrs (Delatt, 1979).

Bioassay and the calculation of the phenol coefficient

After incubation at 37°C for 48 hours, the presence of growth in the test-tubes was detected by the appearance of

Table 1: List of chemical disinfectants

Brand of disinfectant	Manufacturers	Recommended dilution	Composition	Batch No	NAFDAC No
Methylated Sprit	Ugolab Nig.Ltd	Undiluted	Ethanol 95%v/v Methanol 5%v/v	3050SP	4728
Hydrogen Peroxide	Ugolab Nig. Ltd	Diluted	H_2O_2 6%w/v	300HP	04-04-5745
Parazone	Nigeria-German Chemical PLC	Diluted	Nacl 3.5%	PR088	02-0012

Table 2: *In-vitro* activity of the test disinfectants against *Cercospora penniseti* (disc concentrations in 5000 micromililitres)

Brand of disinfectant	Chemical composition	Disc concentration (micomilliliters)	*In-vitro* activity
Methylated Sprit	Ethanol 95%v/v Methanol 5%v/v	5000	Resistant
Hydrogen Peroxide	H_2O_2 6%	5000	+
Parazone	NaCl 3.5%	5000	+++

KEY:
+ = inhibition zone diameter < 10mm
++ = inhibition zone diameter = 10mm
+++ = inhibition zone diameter >10mm
Cercospora penniseti showed a significant *in-vitro* activity against all the disinfectants except methylated spirit which showed a complete resistance.

turbidity. Absence of turbidity or cloudiness was an indication of no growth. The phenol coefficient was calculated by dividing the numbers indicating the dilution of the disinfectant that showed fungicidal growth after exposure for 10 minutes (but not in 5 minutes) by the greater dilution of phenol showing the same result. The number obtained was recorded as the phenol coefficient for that particular test disinfectant (Pelczer et al., 1993). However, it was assume that a dilution of 20 times that of the coefficient gave an effective dilution. (E.g. for top, 20 x 0.9 = 1:18) according to the Association of Official Agricultural Chemists (AOAC, 1955).

RESULTS

All the three (3) disinfectants tested against *Cercospora penniseti* causing *Cercospora* disease of millet were generally effective *in- vitro* against the tested isolated with only few variations.

All the disinfectant agent tested showed inhibition zones greater or equal to or less than 10mm diameter. This indicates that all the tested disinfectant were effective in-vitro against the test organism, at disc concentration of 5000micromililiter.

Phenol coefficient technique

The results obtained for the determination of phenol coefficient were showed in the Tables 1 and Table 2, for Parazone, Methylated spirit and Hydrogen peroxide respectively. Standard strain of *Cercospora penniseti* was used as the test organism for all the three (3) test disinfectant. Hydrogen peroxide had a phenol coefficient of 0.9 as shown in Table 3, parazone had 1.05 as shown in Table 4 while methylated spirit had a phenol coefficient of 1.19 as showed in Table 5.

DISCUSSION

The three (3) disinfectants differ in their spectrum of activity due to the variations in their chemical compositions and formulations. Some would destroy fungi, bacteria, spores, viruses and other infectious agents. However, most disinfectants have a limited spectrum of activity. All the three (3) disinfectants tested for their *in-vitro* activities against the isolates of *Cercospora penniseti* were found to be active against the target organism with minimal variations. This could be attributed to the differences in their chemical compositions (Kiyawa et al., 2014)

The high activity of methylated spirit of the isolates could be due to the presence of ethanol (95% v/v) and methanol (5% v/v).

The activity of hydrogen peroxide could be due to the presence of hydrogen peroxide in 6%. The activity of parazone could be due to the presence of sodium hypochlorite (3.5%).

Considering the phenol coefficient technique, the phenol coefficient of hydrogen peroxide was 0.9 and for parazone was 1.05 and methylated spirit was 1.19. This variation could be attributed to errors that might have been encountered during the course of the laboratory work as well as dilutions used by manufacturers in their test for determination of the coefficient value.

Table 3. Phenol coefficient for the *in-vitro* activity of Hydrogen peroxide against (*Cercospora penniseti*)

Brand of disinfectant	Dilution	Growth after 5 minutes	Growth after 10 minutes	Growth after 15 minutes
Hydrogen Peroxide	1:100	+	-	-
	1:125	+	-	-
	1:150	+	+	+
	1:175	+	+	+
	1:200	+	+	+
Phenol	1:195	+	+	-
	1:100	+	+	+
	1:105	+	+	-
	1:110	+	+	-
	1:115	+	+	+

Phenol coefficient =100/110=0.9
KEY:
- = No growth
+ = Growth

Table 4. Phenol Coefficient for the *In-Vitro* activity of Parazone against (*Cercospora penniseti*)

Brand of disinfectant	Dilution	Growth after 5 minutes	Growth after 10 minutes	Growth after 15 minutes
Hydrogen Peroxide	1:100	-	-	-
	1:125	-	-	+
	1:150	+	+	-
	1:175	+	+	+
	1:200	+	+	+
Phenol	1:195	+	-	-
	1:100	+	+	+
	1:105	+	+	+
	1:110	+	+	+
	1:115	+	+	+

Phenol coefficient =100/95=1.05
KEY:
- = No growth
+ = Growth

Table 5. Phenol coefficient for the *in-vitro* activity of Methylated spirit against (*Cercospora penniseti*)

Brand of disinfectant	Dilution	Growth after 5 minutes	Growth after 10 minutes	Growth after 15 minutes
Hydrogen Peroxide	1:100	-	-	-
	1:125	+	-	-
	1:150	+	+	+
	1:175	+	+	+
	1:200	+	+	+
Phenol	1:195	+	-	+
	1:100	+	-	+
	1:105	+	-	+
	1:110	+	+	+
	1:115	+	+	+

Phenol coefficient =125/105=1.19
KEY:
- = No growth
+ = Growth

Conclusion:

The disinfectants tested showed inhibition zone greater than, equal to or less than 10mm diameter. This indicates that all the tested disinfectants were effective *in-vitro* activity against the test organisms at disc concentration of

5000 micromililiters.

All the three (3) disinfectants tested against the isolate were found to be active. The phenol coefficients of parazone, methylated spirit and hydrogen peroxide were found to be 1.05, 1.19 and 0.9, respectively.

Recommendation

1. Based on the study performed, the best chemical disinfectant is methylated spirit, followed by parazone and hydrogen peroxide according to their phenol coefficients of 1.19, 1.05 and 0.9 respectively.

2. Other disinfectants available in the market could also be studied as to their action against this and other fungi of economic importance.

REFERENCES

Association of official Agricultural Chemists (AOAC, 1955). Official Methods of Analysis of the AOAC. 9th edition. Washington, D.C.

British Pharmacopeia (1993). Cheesbrough, M. (2006). District laboratory practices in tropical countries part 2 microbiology. The ford Press Ltd, England.

Cheesbrough M (2000). District laboratory practice in tropical countries. E.C.B.S.Cambridge University Press edition 2:256-267.

Delatt PN (1979). Instructions for Testing Chemical Disinfectants. Fischer Stuttgart. Pp223-231

James GC, Natalie S (2001). Microbiology. A laboratory Manual (ed.). pp. 211-223.

Jha DK (1995). Laboratory Manual on seed Pathology. Vikas Publishing House (PVT) Ltd. P. 13-30.

Kiyawa SA, Kutama AS, Umar S (2014). In Vitro Evaluation of Antifungal Potency of Some Disinfectants on *Colletotrichum Graminicola*, (*Cesati*) Wilson the Causal Agent of Sorghum Leaf Anthracnose. Int. J. Anal. Bioanal. Chem.; 4(2):53-57

Mbwaga AM, Pande S, de Milliano WAJ, Karunakar RI (1993). Diseases and parasitic weeds of pearl millet in Tanzania: occurrence and incidence from 1986 to 1990. Phytophylactica 25: 271-273.

Mukhtar MD, Okafor AC (2002). Bioactive Evaluation of Ethanol Extracts of Leaf and Stem – Bark of *Guiera Senegalensis* . Nigeria J. Res. Prod. 1 (1):114–121.

Narayanaswami R, Veerraju V (1970). A new *Cercospora* leaf spot of pearl millet. Indian Phytopathology 23:135-136.

Pelczer MJ, Chan ECS, Krieg NR (1993). Microbiology concepts and applications. Mc Graw Hill inc. New York pp.231-233.

Wiehe PO (1953). The plant diseases of Nyasaland. Kew, UK: Mycology, Paper 50, CMI.