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Research Article

ASSESSING THE EFFECTIVENESS OF LOCALLY AVAILABLE FUNGICIDES IN MANAGING CERCOSPORA LEAF SPOT OF SWISS CHARD UNDER FIELD CONDITIONS IN BOTSWANA

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ABSTRACT

Cercospora leaf-spot (CLS), caused by *Cercospora beticola*, is an important foliar disease of Swiss chard in Botswana. The regularity of outbreaks of the disease in Botswana is a major cause of concern and thus, have necessitated the use of fungicides under field conditions in order to control the disease and bring the crop to maturity. The objective of the study was to assess the most effective, locally available fungicides in the control of CLS of Swiss chard under field conditions. The experiment was carried out in Sebele, where a tractor-ploughed field was partitioned into 24 plots, which were split into three blocks. Seedlings were transplanted into the plots, with each plot containing a maximum of 45 plants which were left for natural infection. Disease control commenced once symptoms were observed, using Copper oxychloride; Benomyl + Mancozeb; Mancozeb + Copper oxychloride; Benomyl; Benomyl + Copper oxychloride; Mancozeb; Benomyl + Mancozeb + Copper oxychloride applied at recommended rates. Where more than one fungicide was used, the fungicides were applied at alternating intervals. Control plants were sprayed with water only. The treatments were applied for a period of twelve weeks at two week intervals. The average number of leaf spots per leaf was recorded two weeks after each fungicide application and samples were collected prior to spraying. Data were subjected to analysis of variance and means separated using LSD test at p=0.05. Cercospora leaf spot severity was significantly lower in all treatments involving Benomyl than in those treatments involving Mancozeb and Copper oxychloride used alone or in combination. This study confirms that when Benomyl is alternated with Copper oxychloride, it is much more effective in managing Cercospora Leaf spot than Mancozeb or Copper oxychloride used alone or alternated.

Keywords**:** *Cercospora Leaf Spot, Swiss Chard, Fungicides, Control, Botswana*

INTRODUCTION

Swiss chard, [*Beta vulgaris* subsp. *cicla* (L.) W.D.J. Koch] also known as spinach beet belongs to the Chenopodiaceae or goosefoot family (Tindall, 1988). A study carried out by Madisa *et al*., (2010) revealed that Swiss chard is grown in most regions of Botswana and farmers that were involved in the study within Gantsi, Southern and Kgalagadi regions produced Swiss chard. This correlated with results obtained by Obopile *et al*., (2008) which showed that the most commonly grown vegetable in Gaborone region (Kgatleng) and western region (Gantsi and Kweneng) was Swiss chard. According to farmers' ranking of the important vegetable crops in Botswana, brassicas, Swiss chard and tomato are the most popular vegetable crops (Bok *et al*., 2006; Munthali *et al*., 2004). These crops (except Swiss chard) are significantly associated with major pests of vegetable crops.

The Horticultural sector in Botswana is still at its infancy stage, with a lot of government programs mainly targeting this sector due to its potential to reduce the rates of poverty, unemployment and imports of vegetables. This therefore, necessitates information sharing with farmers who are already in production and those that are upcoming. Swiss chard being a potentially worthwhile vegetable to produce in Botswana needs not only to be promoted among farmers in Botswana, but information should be shared with farmers on its production limitations especially the disease known as Cercospora leaf spot (CLS), caused by *Cercospora beticola* Sacc. Losses caused by this disease may initially be underestimated, however intense pressure from this pathogen results in increased lesions which would ultimately lead to necrosis of whole tissue (Windels *et al.*, 1998). Most literature on fungicidal control of this disease is on sugar beet and very little studies have been carried out on Swiss chard and none in Botswana. Producers in affected areas must thoroughly apply fungicides in order to bring the crop to maturity (Windels *et al*., 1998; Meriggi *et al*., 2000). Without such measures, the leaf canopy can be destroyed by outbreaks of *C. beticola,* resulting in complete loss of the crop (Duffus and Ruppel, 1993; Rossi *et al.*, 2000). The objective of this study was to assess the most effective, locally available fungicides in the control of Cercospora Leaf Spot of Swiss chard (CLS) under field conditions through the monitoring of leaf spot numbers.

MATERIALS AND METHODS

Experimental Location

A field experiment was carried out at the Botswana University of Agriculture and Natural Resources, Sebele, Gaborone (Latitude 24 34'S, Longitude 25 57'E, Altitude 994 above sea level) from August 2013 to April 2014 in order to assess the effectiveness of three selected locally available fungicides, namely Benomyl, Mancozeb and Copper oxychloride.

Land Preparation and Cultural Practices

A field where Swiss chard was previously grown was ploughed by a tractor and partitioned into 24 plots with an area of 10 m^2 (5mx2m) each and a spacing of 1m between the plots. The plots were split into 3 blocks each with eight plots. Seedlings were raised in a net shade in three seedling trays, each with a capacity of 200 plants per tray. The trays were watered twice daily using a watering can with a nozzle. Two weeks after emergence (WAE) the plants were given Starke Ayres plant food 3:1:6(46). One gram was dissolved in 1litre of water and each tray was watered with 1litre of the solution.

The seedlings were transplanted into the field 6 weeks after emergence at a spacing of 40 cm between the rows and 30 cm between the plants. Each plot had a population of 45 plants. The plots were weeded as the weeds emerged. Watering was carried out twice a day in the morning and afternoon during the 1st month after transplanting, thereafter watering was done once in the afternoon. Lime ammonium nitrate (LAN) fertilizer was added at a rate of 140 g per plot at 21 day intervals.

Treatments and Experimental Design

Control of Cercospora leaf spot of Swiss chard using fungicides commenced once the first symptoms were observed within the plots. The treatments were as follows: 1. No fungicide application (Control); 2. Copper oxychloride; 3. Benomyl alternating with Mancozeb; 4. Mancozeb alternating with Copper oxychloride; 5. Benomyl; 6. Benomyl alternating with Copper oxychloride; 7. Mancozeb; 8. Benomyl alternating with Mancozeb and Copper oxychloride. Fungicides were applied at 14 day intervals using a 5-litre Knapsack sprayer. A plastic cage was made around each plot during spraying in order to avoid drift of the fungicide to other plots using plastic and wooden stakes. Benomyl was applied at a rate of 50g/100l of water, copper oxychloride at 40g/ 100l of water and mancozeb at 200g/100l of water as per manufacturer's instructions.

The experimental design was a randomized complete block design (RCBD) with fungicide treatments as the main factor (Factor A) and sampling period as a subplot (Factor B).

Data Collection

Data collected were the average number of leaf spots per leaf. For each plot a total of 21 experimental plants were numbered for use after excluding the border plants. At each sampling time ten plants were randomly chosen by placing numbers 1 to 21 in a bag and picking 10 number for sampling. The first sample was collected 2 weeks after the first fungicide spray and subsequently every 2 weeks just before fungicidal application for the weeks that followed. One leaf with most spots was taken per plant. Spot count was done under a magnifier using an inoculating needle. Temperature and rainfall data for the duration of the study were collected from the Department of Agricultural Research Meteorological Station.

Data Analysis

Data were subjected to one-way analysis of variance (ANOVA-1) and if the f-value was significant (p=0.05), means were separated using Least Standard Deviation (LSD) test. The MSTATC statistical package (Michigan State University) was used to analyze the data.

RESULTS AND DISCUSSION

Meteorological Data

Total monthly rainfall received during the study period (August 2013 to April 2014) ranged from 0 mm in August to 137.7 mm in December 2013 with a cumulative total of 427.7 mm (Figure 1). Rainfall distribution was good from mid-November to the end of January with rain showers almost every week creating ideal conditions for development of CLS.

The season was also characterized by relatively high temperature condition with the average maximum temperatures ranging from 25.5°C in April 2014 to 32°C in November 2013 and minimums ranging from 6.2°C in September 2013 to 19.4°C in March 2014.

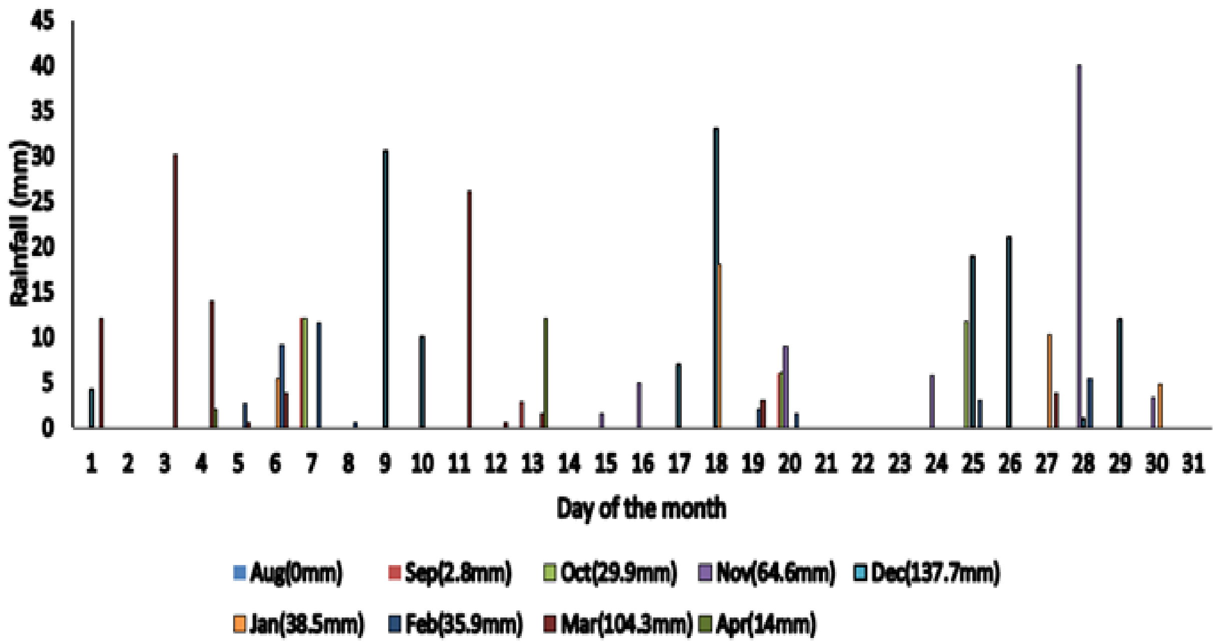

Figure 1: Daily Total Rainfall Recorded at the Department of Agricultural Research Station from August 2013 to April 2014 (Figures in Parenthesis in the Legends are Monthly Total Rainfall)

Effects of Fungicide Application on CLS Disease Development

Fungicide application significantly reduced disease development compared to the non-treated control but among the fungicides benomyl application alone or alternated with mancozeb, and copper oxychloride was the most effective resulting in 83-88% disease reduction compared to the control (Figure 2, Table 1). Copper oxychloride and mancozeb alone achieved 79% and 72%, respectively while their combination achieved 71% disease reduction compared to the control.

From the time of initiation of spraying after disease was first noticed, disease slightly increased in plants treated with copper oxychloride, mancozeb or their combinations before leveling off and eventually decline while benomyl alone or in combination with the other two caused immediate disease decline. There were no significant differences in disease intensity among the fungicide-treated plants 84 days after disease onset while the disease in untreated plants was significantly higher than in the treated plants (Table 1).

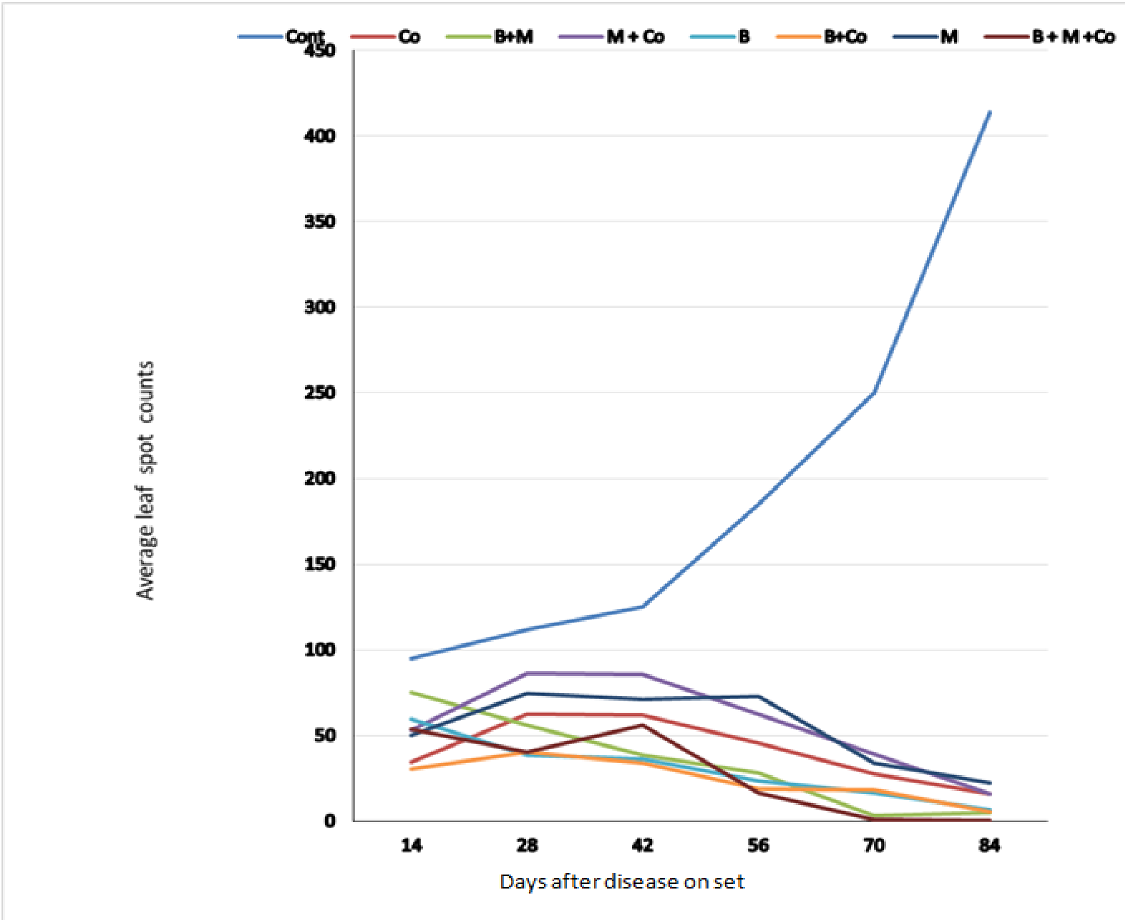

Figure 2: Effect of Fungicide Treatments on CLS Disease Progress Curve (Cont= Control, Co = Copper Oxychloride, B = Benomyl, M= Mancozeb)

Cercospora leaf spot disease outbreak from natural infection was dependent on the onset of favorable weather conditions, mainly rainfall and temperature in December 2013 and January 2014 with a cumulative total rainfall of 162.2 mm and this continued up to the end of the rain season in April 2014. Khan *et al.*, (2009) confirmed what the result showed by stating that favorable environmental conditions for the development of Cercospora leaf spot are day temperatures of 25-35ºC, night temperatures of 16ºC, and prolonged periods of relative humidity of 90-95% or free moisture on the leaves after which conidia are dispersed from their source of inoculum by wind or water splash, running water and insects onto the Swiss chard leaves. Larson (2004) correlated a higher humidity in the stomata opening with infection through the stomata, which do not need to open to facilitate fungal entry.

The most effective fungicide against *C. beticola* during the study was benomyl alternated with copper oxychloride, with a disease reduction of 88% compared to the untreated control. Copper oxychloride alone achieved 79% reduction which was significantly higher than Mancozeb (72%) or Mancozeb alternated with Copper oxychloride (71%). Benomyl is a systemic fungicide whose mode of action is inhibition of mitosis and cell division in target fungi by polymerization of tubulin into microtubules (Koo *et al.*, 2009; MacCarroll *et al.*, 2002; Gupta *et al.*, 2004). That is why the benomyl treated plants generally had declining numbers of leaf spots after the initial spray compared to the non-systemic fungicides where the disease increased. Harvesting of Swiss chard leaves commenced 3 days after spraying with all the fungicides. The 3 day waiting period rendered the leaves safe for human consumption as per manufacturer's instructions.

Table 1: The Effect of Locally Available Fungicides on the Severity of Cercospora Leaf Spot of Swiss Chard under Field Conditions in Botswana

Fungicide Treatment[1]	**Mean Number of Swiss Chard Cercospora Leaf Spots per Leaf Recorded at 14-day Interval from First Fungicide Application**						**Overall Fungicide Means**	**Percentage Disease Reduction**
	14	**28**	**42**	**56**	**70**	**84**		
Control	95.00def[2]	112.70de	125.00d	184.7c	250.00b	414.00a	196.9a[3]	0
Copper oxychloride;	34.33hijklmno	62.33fghijk	62.00fghijk	45.67ghijklmn	27.67jklmno	16.00mno	41.33bc	79
Benomyl+ Mancozeb;	75.33efgh	55.67fghijkl	38.67hijklmno	28.00jklmno	3.00no	5.00no	34.28c	83
Mancozeb + Copper oxychloride;	53.33fghijkl	86.00defg	85.33defg	62.33fghijk	39.33hijklmno	16.00 lmno	57.06b	71
Benomyl	59.67fghijkl	38.33hijklmno	36.00 hijklmno	23.67 klmno	16.33lmno	6.330 mno	30.06c	85
Benomyl + Copper oxychloride;	30.67ijklmno	40.33hijklmno	33.67hijklmno	18.67 klmno	18.33 klmno	5.330no	24.50 c	88
Mancozeb;	50.33ghijklm	74.67efghi	71.00efghij	73.00efghi	34.00hijklmno	22.00klmno	54.17b	72
Benomyl + Mancozeb + Copper oxychloride.	53.67 fghijkl	40.33hijklmno	55.67fghijkl	16.33lmno	1.00o	0.00o	27.83c	86
LSD	78.6						18.05	
Overall Sampling Means	56.542	63.792	63.417	56.542	48.708	60.583	58.264	
LSD	NS							

[1] Where more than one fungicide was used, the fungicides were applied alternately every two weeks and leaf samples were collected just before spraying
[2] Means for the fungicide and sampling interactions at 2 weeks interval in a column and in a row respectively followed by the same letter(s) are not significantly different, LSD test (p=0.05)
[3] The overall means in a column followed by the same letter are not significantly different, LSD test (p=0.05)

The second most effective fungicide was Copper-oxychloride which is non-systemic contact fungicide with limited therapeutic activity. Effective control of disease by copper oxychloride depends on complete coverage of plant surfaces and minimal weathering factors such as rain soon after application. When Copper oxychloride was used alone the reduction in spot counts was not satisfactory between second and fourth week, however, effective control was observed through the fifth to sixth week. This is because Copper oxychloride's action as a fungicide is due to the release of small quantities of copper (Cu^{++}) ions when in contact with water. The mode of action of Copper oxychloride is the nonspecific disruption of cellular proteins when the toxic copper ion is absorbed by germinating fungal spores, hence, for best results copper must be reapplied as plants grow to maintain coverage and prevent disease establishment (Husak, 2015). However, Copper oxychloride being an insoluble compound generally left unattractive residues on the leaves rendering them to be unmarketable as noted by Swartz and Gent (2007).

Mancozeb was the least effective compared to Benomyl and Coper oxychloride but was much better than the control (Table 1), Mancozeb is also a contact fungicide with preventive activity. Its mode of action is the inhibition of enzyme activity of fungi, by forming a complex with metal-containing enzymes including those involved in the production of adenosine triphosphates (ATP). Calviello *et al.,* (2006) suggested that Mancozeb caused post apoptotic cell membrane integrity due to its oxidative effect. Mancozeb is a typical multisite protectant fungicide and for this reason use rates and frequency of application need to be relatively high in order to counteract the effect of weathering and plant growth, which can rapidly diminish the protection afforded by the product. It must also be present on the leaf prior to the arrival of fungal spores if it is to be effective and hence, it is not as flexible as curative or eradicant fungicides. Mancozeb was first applied after the appearance of spots therefore, an increase in spots is observed on the weeks that follow however, a significant decline in the number of spots was observed on the fifth and sixth week because this is foliage that underwent preventative spraying. Although, preventive spraying can eliminate or reduce disease losses, it may result in use of more sprays than are necessary.

In Botswana the Ministry of Agricultural Development and Food Security has a number of fungicides registered for control of fungal pathogens in certain crops. These include among others Metalaxyl, Flusilazole, Terbuconazole, Benomyl, Mancozeb, Copper oxychloride and Pyraclostrobin (Plant Protection, 2016) One of the greatest challenges faced by horticultural farmers in Botswana in the control of *C. beticola* is to determine the best fungicide and time for fungicide application in order to minimize losses. Disease severity is directly related to favorable environmental conditions characterized by extended rainfall (Khan *et al.,* 2009) as was the case during the study. For effective control of the disease, monitoring of environmental factors such as rainfall, relative humidity and duration of leaf wetness and scouting for disease is critical. A number of authors have reported the development of resistance in the Benzimidazole class of fungicides, however, these provided significantly better results in the control of Cercospora leaf spot (Dexter and Luecke, 1999; Karaoglanidis *et al.,* 2000; Weiland and Halloin, 2001).

Conclusion and Recommendations

The purpose of applying fungicides against fungal pathogens at the recommended dose is to ensure production of large quantities of high quality Swiss chard yield using minimal amounts of active ingredients. It can thus, be concluded that Benomyl alternated with Copper oxychloride not only it is significantly effective but it counteracts the development of resistant strains of the *C beticola* fungus which may become problematic in controlling also noting that Copper oxychloride or Mancozeb alone or both lack the post infection activity thus, only act as protectant fungicides, killing pathogenic cells on plant surfaces. As a recommendation to farmers, in order to reduce chemical residues on produce/ Swiss chard leaves, potential environmental contamination and the occurrence of fungicide tolerant strains, biocontrol could be researched and used as an alternative.

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