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Research Article

UTILIZATION OF SOME CARBON AND NITROGEN SOURCES FOR GROWTH OF *CERCOSPORA BETICOLA* SACC., THE CAUSAL AGENT OF LEAF SPOT DISEASE OF SPINACH BEET

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ABSTRACT
The present study evaluates the effect of some carbon and nitrogen sources on growth of two isolates of *Cercospora beticola in vitro*. *C. beticola* isolates (A & B), were obtained from infected spinach leaves collected from two farms at Glen valley and Otse respectively in Gaborone, Botswana. Six carbon sources, fructose, cellulose, sucrose, lactose, glucose and starch, and six nitrogen sources, ammonium chloride, ammonium sulphate, aspergine, ammonium orthophosphate, sodium nitrate and urea were incorporated into carrot decoction and inoculated with the pathogen to evaluate their effect on the growth of *C. beticola* after 20 days of incubation at 25^{o} C.Glucose supported maximum growth of the pathogen (Isolate A: 1.9565 g, Isolate B: 1.8495 g) followed by sucrose, fructose and lactose. Minimum growth of the pathogen was observed in case of cellulose (Isolate A: 0.4813g, Isolate B: 0.4903g) and starch (Isolate A: 0.2174g, Isolate B: 0.3381g).*C. beticola* isolates grew best when sodium nitrate was used as a source of nitrogen (Isolate A: 1.3317g, Isolate B: 1.4735g) followed by L-Lysine, L-Argenine and ammonium sulphate. Least mean dry mycelia weight was obtained in case of urea (Isolate A: 0.8377g, Isolate B: 0.9432g) compared to control for both isolates, A and B.

Keywords: *Cercospora beticola, Spinach Beet, Growth, Carbon Sources, Nitrogen Sources*

INTRODUCTION
Cercospora leaf spot of spinach beet caused by *Cercospora beticola* Sacc., is considered to be the most economically important foliar disease of spinach beet in Botswana, reducing quality, quantity and marketability of spinach leaves. Spinach beet (*Beta vulgaris var.cicla*(L.) W.D.J. Koch, also known as Swiss chard is a leafy green vegetable and grown for its dark green leaves. The most common varieties of spinach beet are Ford hook Giant, Rhubarb Chard and Lucullus.

Ford hook Giant and Lucullus varieties of spinach beet are usually grown in Botswana and heavily infected with *Cercospora beticola*. Other major foliar diseases of spinach beet include blue mold (*Peronospora effusa*), white rust (*Albugo occidentalis*), anthracnose (*Colletotrichum spinacicola* and *C. spinaciae*) and Cladosporium leaf spot (*Cladosporium macrocarpum*) which have been reported elsewhere but not of significantly important in Botswana (Correll *et al*., 1994).

The ability of C. *beticola* to infect wide range of plants shows that it is an aggressive pathogen which can destroy many different crops growing within the same area. Recent study revealed that *C. beticola* could infect sun flower during artificial inoculation experiments (Weiland and Koch, 2004). During periods of warm temperatures and high humidity or leaf wetness, *C. beticola* on spinach beet form tan necrotic spots on lower leaves which will turn gray and blighted all over the leaf and thus lowering quality or making the spinach leaves unmarketable.

To authors knowledge there is no information available on the pathogen and the disease in Botswana. Therefore, a research project was undertaken to study the different aspects of this important disease and its causal agent with ultimate goal to manage the disease. This paper however, reports the effect of some carbon and nitrogen sources on growth of two isolates of *Cercospora beticolain vitro*.

MATERIALS AND METHODS

Isolation and Identification of Cercospora beticola

Fresh infected spinach beet leaves collected from randomly selected farmers fields at two different locations, Glen valley and Otse in Botswana were observed through transmitted light under the stereo microscope and preserved at 4^0C for subsequent use. Some of leaves with a lot of lesions were kept in a moist chamber to induce sporulation for isolating and characterizing the pathogen. The pathogen was then isolated from infected leaves pieces and cultured on the Potato dextrose agar. The plates containing infected tissue were incubated at 25^oC. These were examined daily to check growth of fungus around the tissue pieces. Once the growth of the fungus has occurred it was sub-cultured on other sterile PDA plates and incubated at 25^oC. The symptoms of the disease and the characteristics of conidia both from the infected leaves and the cultures were examined. After consulting references Chupp (1953), Groenewald *et al*., (2006) and Crous *et al*., (2006) the pathogen isolated was identified to be *Cercospora beticola*. The colony that was identified as *C.beticola* was sub cultured into other prepared sterile PDA plates and cultures thus obtained were kept at 4^o C for later use in the experiment.

Preparation of Media for the Experiment

Carrot decoction liquid medium was prepared by boiling 200 g of graded carrot in 500 ml bottle containing 500 ml of distilled water for 15 minutes, and then the extract was filtered through a muslin cloth into 1000 ml bottle. The filtrate was then mixed with twenty gram dextrose and fifteen gram powder agar and the mixture was then made to 1000 ml. The mixture was dissolved in hot plate using magnetic stir and dispensed into 100 ml bottles which were autoclaved at 121^oC for 15 minutes.

Carbon Utilization

Ten gram of carbon from each carbon source was added to carrot decoction medium. The quantity of carbon compounds added to the medium in order to provide10g of carbon was determined on their molecular weights. Fructose, cellulose, sucrose, lactose, glucose and starch were used in the study and one set was maintained as control without adding carbon source. All the compounds were dissolved properly and the prepared carbon solutions were filter- sterilized through a membrane filter into 100ml sterile conical flasks. The liquid medium in flasks were inoculated with 5mm mycelia discs which were cut from the margins of 14 day old cultures of *C.beticola*, and incubated at 25^0C for 20 days. Each treatment was replicated thrice. Mycelia growth at the end of 20 days of incubation were harvested and filtered through Whatman No. 42 filter paper, which were previously dried to a constant weight in hot air oven at 60^0C. The mycelia mat on the filter paper was thoroughly washed with distilled water to leach out any salts associated with the mycelium. Subsequently, the filter papers along with the mycelia mat were dried to a constant weight, cooled in desiccator and weighed on an electronic balance. Weight of *C.beticola* mycelium mat for each carbon source was calculated as mentioned before. The data were analyzed statistically.

Nitrogen Utilization

Ten gram of nitrogen from each nitrogen source was added to carrot decoction medium. The quantity of nitrogen compounds added to the medium in order to provide10g of nitrogen which was determined on their molecular weights. Ammonium chloride, ammonium sulphate, aspergine, ammonium orthophosphate, sodium nitrate and urea were used as nitrogen sources. One set was maintained as control without adding any nitrogen source to the broth. All the nitrogen sources were dissolved properly and, prepared solutions were filter-sterilized through a membrane filter into 100ml sterile conical flasks. Each treatment was replicated thrice. The flasks were inoculated as described earlier and incubated at 25^0C for 20 days. After 20 days the mycelia growth was harvested and dried and, the weights of *C.beticola* mycelium mat were calculated as mentioned before. The data were analyzed statistically.

RESULTS AND DISCUSSION

The results on the effect of different carbon sources on the growth of *Cercospora beticola* presented in Table 1 revealed that glucose supported maximum growth of the pathogen (Isolate A: 1.9565 g, Isolate B:

1.8495 g) followed by sucrose, fructose and lactose. Minimum growth of the pathogen was observed in case of cellulose (Isolate A: 0.4813g, Isolate B: 0.4903g) and starch (Isolate A: 0.2174g, Isolate B: 0.3381g).

This is in agreement with Rangaswamy and Chandreasekharan (1962), Dange and Patel (1968) and Khandar *et al.*, (1985) who also reported glucose as best source of carbon for other species of *Cercospora*, while Verma and Agnihotri (1972), Lakshminarayana (1981) and Dinesha (1984) reported that sucrose was the best carbon source for *C. cruenta, Cercospora solani-melongenae and Cercospora sorghi* for growth and sporulation. This indicates that different *Cercospora* species have different priority for their carbon source. Mono-saccharides and di-saccharides supported good growth of *Cercospora* isolates.

Table 1: Effect of different carbon sources on the vegetative growth of two isolates (A & B) of*Cercospora beticola* Sacc., in carrot decoction after 20days of incubation at 25° C

S.No	Carbon source	Mean dry mycelium weight in g	
		A	**B**
1	**Glucose**	1.9565bcde	1.8495bcde
2	**Fructose**	1.4702bcd	1.4581bcd
3	**Sucrose**	1.8633bcde	1.7091bcde
4	**Lactose**	1.0745 bc	1.0891bc
5	**Cellulose**	0.4813 b	0.4903 b
6	**Starch**	0.2174 a	0.3381 a
7	**Control**	1.0073bc	1.0189bc

Means in a column followed by the common letters are not significantly different, p=0.05, LSD test

Table 2: Effect of different nitrogen sources on the vegetative growth of two isolates (A & B) of *Cercosporabeticola*Sacc.in carrot decoction after 20 days of incubation at 25° C

S. No	Nitrogen source	Mean dry mycelium weight in g	
		A	**B**
1	**NaNO3**	1.3317bcd	1.4735bcd
2	**$(NH_4)_2SO4$**	1.0510bc	1.0312bc
3	**(NH_4) Cl**	0.9633bc	1.0190bc
4	**L-Lysine**	1.1749bcd	1.2018bcd
5	**L-Arginine**	1.0632bcd	1.1134 bcd
6	**Urea**	0.8377 ac	0.9432 ac
7	**Control**	0.8361 a	0.8865 a

Means in a column followed by the common letters are not significantly different, p=0.05, LSD test

The isolates of *Cercospora beticola* Sacc. were not significantly different in the mycelia weight in different carbon sources compared to each other but the growth of mycelia in different carbon sources

were significantly different from each other according to two-way ANOVA at, F=179.34, p=0.005 (Table 1).

Results on the effect of nitrogen on growth of isolates of *C. beticola* presented in Table 2. revealed that maximum dry mycelia weight of *C. beticola* isolates were obtained when sodium nitrate was used as a source of nitrogen (Isolate A: 1.3317g, Isolate B: 1.4735g) followed by L-Lysine, L-Argenine and ammonium sulphate. Least mean dry mycelia weight was observed in case of urea (Isolate A: 0.8377g, Isolate B: 0.9432g) compared to control for both isolates A and B. Nitrogen utilization studies revealed that *C. beticola* was capable of utilizing a wide variety of nitrogen sources. The most suitable inorganic nitrogen sources in order were sodium nitrate and ammonium sulphate whereas ammonium chloride supported only slight growth (Table 2). Amino acids L-lysine and L-arginine were also well utilized by the isolates of *C. beticola*. In general, most amino acids were good nitrogen sources which supported the fungal growth (Manjunath *et al*., 2010).The isolates of *Cercospora beticola* Sacc., were not significantly different in the mycelia weight in different nitrogen sources compared to each other but the growth of mycelia in different nitrogen sources were significantly different except ammonium sulphate and ammonium chloride, and L – Lycine and L - Arginine according to two-way ANOVA at, F=50.61, p=0.005.

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