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Effect of Carbon, Nitrogen Sources and Carbon to Nitrogen Ratios on *Sclerotinia sclerotiorum*

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***Sclerotinia sclerotiorum* is a soil borne plant pathogenic fungus that causative white rot disease in most of vegetable crops, including bean (*Phaseolus vulgaris* L.). In this study, the effect of different carbon, nitrogen sources and C/N ratios on the growth of *S. sclerotiorum* were examined. Sclerotia development and sclerotia dry weight were also assayed. The mycelial growth of the fungus was increased with most of carbon sources tested, but the highest levels were found when D-glucose and saccharose were added that produced 1.34 and 1.32 mg/ml mycelial dry weight. Whereas, L-alanine and L-arginin were the best nitrogen sources for the growth of *S. sclerotiorum* with 0.95 and 0.66 mg/ml mycelial dry weight, respectively. However, ammonium chloride gave moderate growth of the fungus that produced 0.49 mg/ml mycelial dry weight. The best C/N ratios were 9:1 and 20:1 with mycelium dry weight of 1.40 mg/ml in to ratios and 28.75 and 16.25 sclerotium/flask, respectively.**

Kew words: White rot, Nutritional factors, C/N ratio.

Sclerotinia sclerotiorum (Lib.) de Bary[1] is a destructive phytopathogenic fungus capable of infecting a broad range of plant hosts, including vegetables, ornamentals, and field crops causing economic yield losses. This fungus able to infecting about 75 families, 278 genera, and 408 species[2]. Sclerotia of *S. sclerotiorum* serve as the survival structure that is resistant to environmental factors, allowing the fungus to persist viable in the soil for up to many years reach to eight years[3]. Under favorable climate conditions, sclerotia germinate carpogenically producing apothecia that can last for several weeks. Apothecia produce ascospores that become air-borne and infect host plants during the susceptible stage[4].

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The common failure of economically important crops to improve germplasm resistant to this fungus has focused interest on the need for a more detailed understanding of the factors affecting the pathogen, which contribute to the progression of the disease[5,6]. Fungal pathogenicity is dependent on a coordinated of interaction between many different pathological determinants. Nutrients are substances that interfere in the biosynthesis of proteins and enzymes, which is the impetus for the growth of the object and causing injury[7]. Several carbon and nitrogen sources can support the growth and oxalic acid accumulation[8]. Similar complex and simple carbohydrates have been revealed to support growth and oxalic acid which have an important role in the pathogenesis of *S. sclerotiorum*[9].

The objective of this study was to deterimed the ability of *S. sclerotiorum* to use a

variety of carbon, nitrogen sources for growth and sclerotia development as well as C/N radios.

MATERIALS AND METHODS

Effect of carbon on the growth, sclerotia formation and sclerotia dry weight of *Sclerotinia sclerotiorum*

A defined glucose–alanine medium based on Czepek mineral salts medium[6] was used routinely and contained (1 liter distilled water; 1 g KH_2PO_4, 0.5 g $MgSO_4$.7H2O; 0.5g KCl; 0.01g $ZnSO_4.7H_2O$; 0.005g $CuSO_4.5H_2O$; 40 g D-glucose; 2 g L-alanine. Batches of based medium (without D-glucose and L-alanine) were prepared by combining appropriate amounts of individual chemical solution after autoclaving. The pH of each medium was adjusted at pH 5.5 prior to autoclaving. The effect of different carbon sources (D-glucose, D- dextrose, maltose, saccharose and glycerol) was tested in basal medium with L-alanine (2 gm/l) as the nitrogen source. Data were recorded on dry weight and average number of sclerotia formed in flask as well as dry weight of sclerotia.

Effect of nitrogen source on the growth, sclerotia formation and sclerotia dry weight of *Sclerotinia sclerotiorum*

The effect of different nitrogen sources (Ammonium chloride, L-alanine, L-agrinine and Glutamic acid) were tested in basal medium with D-glucose 40 g/L as carbon source. Data were recorded on dry weight and average number of sclerotia formed in flask as well as dry weight of sclerotia.

Effect of C/N ratios on the growth, sclerotia formation and sclerotia dry weight of *Sclerotinia sclerotiorum*

The effect of carbon/nitrogen (C/N ratio) was studied.The basal medium was supplement with amount of D-glucose and L- alanine. The C/N ratio in medium was adjusted between 9:1and 200:1 by varying the concentration of D-glucose with L-alanin fixed at 2 gm/l. Each flask was inoculated separately with uniform quantity of homogenous culture suspension (1 ml) prepared by triturating mycelial mat of one flask grown on Potato dextrose broth, and inoculated at 20±2 ºC. Data were recorded on dry weight and average number of sclerotia formed in flask as well as dry weight of sclerotia.

RESULTS AND DISCUSSION

Data collected from all experiments were statistically analyzed using the Statistic Analysis System Package (SAS institute, Cary, NC, USA). Differences between treatments were studied using Fisher's Least Significant Difference (LSD) test and Duncan's Multiple Range Least[11]. All analysis were performed at P 5 % level.

Effect of carbon on the growth, sclerotia formation and sclerotia dry weight of *Sclerotinia sclerotiorum*

Data in Table 1 showed that there were significant differences between all carbon sources affecting growth of user. D-glucose was the best carbon source for mycelial dry weight with 1.34 mg/ml followed by saccharose and D-dextrose and with 1.32and 1.29 mg/ml mycelial dry weight, respectively. Conversely, glycerol gave the least affecting the mycelial growth of *S. sclerotiorum* that produced 0.37 mg/ml of the mycelial dry weight. Whereas, Maltose was not suitable for the growth of fungus.

In contrast, D-dextrose was the best carbon source for sclerotia production with 15 sclerotium/flask, followed by D-glucose by 14 sclerotium/flask. Whereas, maltose did not have any sclerotia Table (1). This result are in good agreement with those Coman *et al*.,[12] who reported that the highest biomass of *Sclerotinia sclerotiorum* was obtained with saccharose and glucose (400-500) mg dried mycelium from 100 mL medium). Also, Galhaup and Haltrich[13] and Periasamy and Palvannan[14] confirmed that easily utilizable carbon sources such as saccharose or glucose are best substrates and well metabolized to support energy needs for the growth of the organism[7,8].

Effect of nitrogen source the growth, sclerotia formation and sclerotia dry weight of *Sclerotinia sclerotiorum*

In case of Nitrogen sources, between all nitrogen tested, it was noticed that L-alanin and L-arginin were the best nitrogen source for the growth of *S. sclerotiorum* with 0.95 and 0.66 mg/ml mycelial dry weight, respectively. Whereas, ammonium chloride and gave moderate growth of the fungus that produced 0.49 mg/ml mycelial dry weight. Conversely, methionine did not give any growth of the fungus (Table 2).

In case of sclerotia formation, L-alanin gave the maximum number of sclerotia and sclerotia dry weight with 13 sclerotium/flask and 109 mg sclerotia/flask, respectively. This was followed by L-arginine that produced 6.25 sclerotium/flask and 52 mg sclerotia/flask, respectively (Table 2). On the other hand, the Methionin did not suitable for growth and sclerotia development of *S. sclerotiorum*.

Nitrogen sources have been revealed to regulate the growth of organisms. In most cases, organic nitrogen is a better source than inorganic nitrogen[15,16]. Coman *et al*.,[12] revealed that tryptose and tryptone are best sources of nitrogen

Table 1. Effect of carbon sources on the growth, sclerotia formation and sclerotia dry weight of *Sclerotinia sclerotiorum*

Carbon source	Mycelium dry weight (mg/ml)	No. of sclerotia formed/flask (100ml)	Dry weight of sclerotia per/flask(mg)
D-Glucose	1.34[a]	14.00[a]	128.00[a]
D-Dextrose	1.29[a]	15.00[a]	123.00[a]
Maltose	0.00[c]	0.00[d]	0.00[d]
Saccharose	1.32[a]	10.25[b]	93.00[b]
Glycerol	0.37[b]	6.50[c]	55.00[c]
LSD at 5%	0.13	1.33	1.11

Values within a column followed by the same letter are not significantly different according to Duncun's multiple range test (P=0.05)

Table 2. Effect of nitrogens sources on the growth, sclerotia formation and sclerotia dry weight of *Sclerotinia sclerotiorum*

Nitrogen source	Mycelium dry weight (mg/ml)	No. of sclerotia formed/flask (100ml)	Dry weight of sclerotia per/flask(mg)
Ammonium chloride	0.49[b]	5.00[b]	37.00[b]
L-Alanin	0.95[a]	13.00[a]	109.00[a]
L-Arginin	0.66[b]	6.25[b]	52.00[b]
L-Glutamic acid	0.53[b]	5.75[b]	48.00[b]
Methionine	0.00[c]	0.00[c]	0.00[c]
LSD at 5%	0.25	2.74	1.06

Values within a column followed by the same letter are not significantly different according to Duncun's multiple range test (P=0.05)

Table 3. Effect of C/N ratios on the growth, sclerotia formation and sclerotia dry weight of *Sclerotinia sclerotiorum*

C/N ratio	Mycelium dry weight (mg/ml)	No. of sclerotia formed/flask (100ml)	Dry weight of sclerotia per/flask(mg)
9/1	1.40[a]	28.75[a]	24.00[a]
20/1	1.40[a]	16.25[b]	94.00[b]
50/1	0.71[b]	13.25[b]	106.00[b]
100/1	0.14[c]	8.75[c]	70.00[c]
200/1	0.13[c]	0.00[d]	0.00[d]
LSD at 5%	0.32	3.50	3.86

Values within a column followed by the same letter are not significantly different according to Duncun's multiple range test (P=0.05)

regarding biomass production (750-850 mg dried mycelium/100 mL medium), while nitrate and ammonium were less efficient by almost one order of magnitude.

Effect of carbon/nitrogen ratios on the growth, sclerotia formation and sclerotia dry weight of *Sclerotinia sclerotiorum*

Under C/N ratio studies, appropriate growth of *S. sclerotiorum* was obtained on Czapek salt mineral at different C/N ratio ranging from 9:1 – 200:1. Data in Table 3. indicated that the best C/N ratios were 9:1 and 20:1 with mycelium dry weight of 1.40 mg/ml in to ratios and 28.75 and 16.25 sclerotium/flask, respectively. These were followed by 50:1 that produced 0.7 mg/ml mycelium dry weight and 13.25 sclerotium/flask. Nonetheless, 100:1 and 200:1 did not suitable for growth with 0.1 mg/ml in two ratios. These results are in good agreement with those obtained on other strains[17, 18, 19]. As well the carbon and nitrogen sources, their separate concentration and the C/N ratios are known to be essential for growth in many microorganisms [20]. The influence of the high C/N ratio can be enhanced the growth of fungi by nitrogen limiting conditions[17, 21]as well as by nitrogen rich media (low C/N)[22,23] or, alternatively, can be unaffected by nitrogen concentration[24]. The activity of fungi increases significantly as C/N decreases (increase of nitrogen concentration).

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