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EFFECT OF NUTRITIONAL SOURCES ON MYCELIAL GROWTH OF *Fusarium graminearum* CAUSING HEAD BLIGHT OF WHEAT

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ABSTRACT

Laboratory experiments were carried out at Deptt. of Plant Pathology, AC&RI, Killikulum, T.N.A.U. during 2015-16 to study the effects of various carbon and nitrogen sources on mycelial growth of *Fusarium graminearum* isolated from the *Fusarium* head blight disease affected wheat plant samples collected from IARI, Regional Research Station, Wellington, Coonoor, Nilgiri district, Tamil Nadu to understand nutritional requirements and ecological survival of the isolates. Among the eight carbon sources tested, dextrose and carboxyl methyl cellulose were found to the best carbon sources in supporting the growth of *F. graminearum* on solid media exhibiting 90 mm mycelial growth and dextrose yielded the maximum dry weight of 1.25 g in liquid media. Similarly among the five nitrogen sources tested, ammonium nitrate was found to be the best nitrogen source for maximum mycelial growth both in solid media exhibiting 90 mm and in liquid media exhibiting 1.28g mycelial growth. It is inferred from this study that among the carbon sources dextrose and among the nitrogen sources ammonium nitrate were found suitable for growth *F. graminearum* in both solid and liquid media.

(Key words: Wheat, *F. graminearum.,* isolation, carbon source, nitrogen source)

INTRODUCTION

The genus *Fusarium* has been considered as one of the very interesting and important group of fungus because of its diversity and cosmopolitan distribution. They are facultative parasites and live as parasites or saprophytes depending on their host. They cause vascular wilts, crown rots, head blights, scabs, root rots and cankers in many economically important plants such as banana, cotton, legumes, maize, rice, wheat and others (Summerell *et al.,* 2003). At least 80% of all cultivated plants are associated with at least one disease caused by a *Fusarium* species (Leslie and Summerell, 2006). Thus, they are responsible for huge economic losses due to reductions in harvest yields and/or the quality of staple foods. Furthermore most *Fusarium* species could continue living in soils or being parasites to grasses if no available host around. They produce dormant structures, mostly in the form of chlamydospores to keep on living in soils for many years before these structures are stimulated to grow. *Fusarium* species produces three types of spores viz., microconidia, macroconidia and chlamydospore. Because of their diversity and cosmopolitan distribution they have attained considerable interest by the plant pathologists worldwide.

Among the fungal diseases, stripe rust /yellow rust (*Puccinia striiformis tritici*), karnal bunt (*Tilletia indica - Neovossia indica*), black point (*Alternaria alternata*), loose smut (*Ustilago nuda tritici*), powdery mildew (*Erysiphe graminis tritici*), head scab (*Fusarium graminearum*) are the most important diseases affecting wheat crop. Head blight or Head scab is a destructive disease in the humid and sub humid wheat growing areas of the world. It has been associated nearly with 17 causal organisms, of which *F. graminearum* (Schwabe) is the principal pathogen responsible for head blight in many countries including India (Saharan *et al.,* 2002). Head blight or scab of wheat caused severe epidemics in many areas worldwide (Dubin *et al*., 1997). The disease has been especially destructive in the United States since 1991 and particularly caused severe epidemic during 1993 that accounted for a crop loss of up to $1 billion (McMullen *et al.,* 1997). Totally losses due to this disease in the USA during 1990s approached $ 2.6 billion (Windels, 2000). In India, wheat grain yield losses of 15.1-29.0 per cent have been reported from Arunachal Pradesh due to head blight incidence on different wheat varieties (Chaudhary *et al.,* 1991) and also in Punjab maximum yield loss of 21.6 per cent in wheat variety PBW 222 (Kaur *et al*., 2000).

F. graminearum does not produce microconidia and produces macroconidia only which is the characteristics features of this pathogen (Leslie and Summerell, 2006). It can survive as mycelium, ascospores, macroconidia and chlamydospores (Sutton, 1982). *F. graminearum* infects wheat spike tissues primarily through anthers that protrude from the glumes, then spreads to the epidermis of glumes and to the ovaries (Xu and Hideki, 1989). Anthesis is the period of greater susceptibility, because the high levels of choline and betaine produced in the extruded anthers stimulate the growth of *F. graminearum* and promote the infection of wheat spikes by the pathogen (Strange and Smith, 1978).

A high degree of variability in physiology and

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morphological characteristic enable *Fusarium graminearum* to occupy diverse ecological niches in many geographic regions. The earlier studies on head blight caused by *Fusarium graminearum* involved only reports of field observations on disease occurrence and its symptoms. Few workers have cultured a pathogen in laboratory and observed the colony morphology. Keeping these aspects in minds present work depicts the study of the effects of different carbon and nitrogen sources on mycelial growth of *Fusarium graminearum* isolated from the *Fusarium* head blight disease affected wheat plant samples collected from IARI, Regional Research Station, Wellington, Coonoor, Nilgiri district, Tamil Nadu to understand nutritional requirements and ecological survival of the isolates which will be helpful in laboratory evaluation.

MATERIALS AND METHODS

Isolation and characterization of *Fusarium graminearum*

The *Fusarium* head blight disease affected wheat plant samples were collected from IARI, Regional Research Station, Wellington, Coonoor, Nilgiri district, Tamil Nadu. The samples were collected in clean polythene bags and the samples were brought to the laboratory at Agricultural College and Research Institute, Killikulam, Vallanad for various studies during the year 2015-16. The freshly infected wheat ear heads showing typical symptoms of *Fusarium* head blight disease collected from the field were used for isolation of the pathogen. The *Fusarium* infected ear heads were made into small pieces of three mm size cut along the edges of the lesions using a sterilized scalpel. The ear head pieces were surface sterilized with 0.1 per cent mercuric chloride solution for 30 seconds. These bits were then washed 3-5 times separately in repeated changes of sterile distilled water. The sterilized potato dextrose agar medium amended with 100 ppm of streptomycin sulphate (to avoid bacterial contamination) was prepared. The sterilized cooled (warm) medium (20 ml) was poured in to sterile petri plates (90mm) and allowed to solidify. The surface sterilized plant tissue bits were placed individually at equidistance at the rate of 3 bits plate^{-1}. All these works were carried out under aseptic conditions. The plates were incubated at room temperature (25 ± 2° C) for 5 days and observed for fungal growth. Identification of cultures was done by microscopic examination and by perithecial production. The growing fungal colony of each plant piece was sub cultured and purified by single hyphal tip method (Tutte, 1969). The pure culture of the pathogen was maintained on PDA slants for further use in this study. The stock cultures were maintained in PDA slants for long term storage under refrigerated condition at 4^0 C.

Effect of carbon sources on growth of *F. graminearum*

To study the carbon sources utilization, the complete medium containing different carbon sources *viz*., glucose, dextrose, lactose, fructose, carboxyl methyl cellulose, mannitol and starch substituting the normal carbon source (sucrose) was prepared separately and autoclaved at 1.4 kg/cm^{-2} pressure for 20 minutes and allowed to cool. The melted medium was allowed to cool (warm) and poured into sterilized petri plates (90 mm) @ 20 ml and allowed to solidify. A nine mm actively growing culture disc of *F. graminearum* cut from 5 day old complete medium culture using a sterilized cork borer was placed at the centre of the each petri plate containing the above said solid media separately and incubated for 5days at 25 ± 2ºC. Complete medium with the normal carbon source (sucrose) served as standard check and three replications were maintained for each treatment. The radial growth of the mycelium was measured when the mycelium covers the entire petri plates of the standard check.

The complete medium broth containing different carbon sources *viz*., glucose, dextrose, lactose, fructose, carboxyl methyl cellulose, mannitol and starch substituting the normal carbon source (sucrose) was prepared separately without adding agar. One hundred ml broth of each carbon source was distributed uniformly into 250 ml Erlenmeyer conical flasks separately and autoclaved at 1.4 kg/cm^{-2} pressure for 20 minutes and allowed to cool. Each flask was then inoculated with a nine mm actively growing culture disc cut from a 5 day old culture of *F. graminearum* by means of a sterilized cork borer. The flasks were incubated at 25 ± 2^{0}C for 10 days for mycelial dry weight assay. The complete medium broth with the normal carbon source (sucrose) served as standard check. Three replications were maintained for each treatment. The mycelial dry weight was assessed by subtracting the weight of the filter paper alone from the total weight.

Effect of nitrogen sources on growth of *F. graminearum*

To study the nitrogen sources utilization, complete medium containing different nitrogen sources *viz*., ammonium nitrate, urea, potassium nitrate and ammonium chloride by substituting the normal nitrogen source (sodium nitrate) was prepared separately and sterilized by autoclaving at 1.4 kg/cm^{-2} pressure for 20 minutes and allowed to cool. The sterilized melted medium was allowed to cool (warm) and was poured into sterilized petri plates (90 mm) @ 20ml and allowed to solidify. Nine mm actively growing culture disc of *F. graminearum* cut from 5 day old culture using a sterilized cork borer was placed at the centre of the each petri plate containing the above said solid media separately and incubated for at 25 ± 2° C. Complete medium with normal nitrogen source (sodium nitrate) served as standard check and three replications were maintained for each treatment. The radial growth of the mycelium was observed when the mycelial growth covered the entire petri plate in the standard check.

Complete medium broth containing different nitrogen sources *viz*., ammonium nitrate, urea, potassium nitrate and ammonium chloride by substituting the normal nitrogen source (sodium nitrate) was prepared separately without adding agar agar. One hundred ml broth of each nitrogen source was distributed uniformly into 250 ml Erlenmeyer conical flasks separately and autoclaved at 1.4

kg/cm^{-2} pressure for 20 minutes and allowed to cool. Each flask was then inoculated with a nine mm actively growing complete medium culture disc cut from a 5day old culture of *F. graminearum* by means of a sterilized cork borer. The flasks were incubated at 25 ± 2 ^{0}C for 5days for mycelial dry weight assay. The complete medium broth with the normal nitrogen source (sodium nitrate) served as standard check and three replications were maintained for each treatment. The mycelial dry weight was assessed as described earlier.

The data were statistically analysed using the SPSS (Statistical Package for the Social Sciences) version 16.0. Prior to statistical analysis of variance (ANOVA) the percentage values were arcsine transformed. Data were subjected to analysis of variance (ANOVA) at significant levels ($P< 0.05$) and means were compared by Duncan's Multiple Range Test (DMRT). Wherever the 'F' test were found significant, critical difference (C.D.) were worked out at 5 per cent level of probability for comparison of treatment means. The treatment effects were presented by making table of means with appropriate standard error (S.E.) and C.D. value.

RESULTS AND DISCUSSION

Isolation and characterization of *F. graminearum*

The causal agent of wheat head blight disease *Fusarium* sp. was isolated from the diseased wheat plant (ear head), using PDA medium and sub cultured by the single hyphal tip method. The morphology of the mycelium initially appeared white colour. Two days later, mycelia turned pink and entire agar medium became typically reddish pink colour. Analysis of asexual spore showed that the pathogen produced only macroconidia and no microconidium was produced. This is the typical characteristics feature of *F. graminearum.* The isolated *Fusarium* sp. was identified based on morphological and cultural characters as *F. graminearum.* Similar to this result Wegulo *et al.* (2008) also showed that *F. graminearum* in agar cultures appeared white mycelial growth with grey, pink, brown and red pigments in agar. It produced asexual spores known as macroconidia in primarily fruiting structures known as sporodochia

Effect of carbon sources on growth of *F. graminearum*

Eight carbon sources were tested for growth of *F. graminearum* in solid media (Table 1 and Fig. 1) and the results showed significant variation for growth of mycelium. Among the carbon sources, CMC and dextrose amended media showed significantly the maximum growth (90.00 mm). This was followed by lactose (88.00 mm), mannitol (86.67 mm) and fructose (81.67 mm) which were all at par with one another. The minimum growth was recorded in starch (74.67 mm) and glucose (74.67mm) amended media.

Eight carbon sources were tested for growth of *F. graminearum* in liquid media and the results showed significant variation for growth of mycelium. Dry weight of *F. graminearum* was estimated in eight different carbon sources and results indicated the significant variation in biomass production (Table 1 and Fig.3). Among the carbon sources, dextrose (1.25g) and sucrose (Standard check) (1.24g) supported significantly the maximum mycelial dry weight. This was followed by glucose (1.11g), fructose (0.96 g) and mannitol (0.91g) amended media which were at par with one another. Least growth was recorded in CMC (0.29g) amended medium.

F. graminearum recorded the maximum mycelial growth in all the carbon sources indicating its wide adaptability. Among the nine carbon sources tested, dextrose and carboxylmethylcellulose were found to the best carbon sources in supporting the growth of *F. graminearum* on solid media and dextrose yielded the maximum dry weight of 1.25 g in liquid media. Selvi and Sivakumar (2013) also reported that carboxylmethylcellulose yielded maximum mycelial fresh weight of *F. graminearum.* Jiao *et al.*(2008) found that glucose, sucrose, 1-kestose, nystose, amylose, amylopectin, cellobiose, fructose, maltose, xylose and galactose supported significant mycelial growth for all strains of *F. graminearum*, although the growth varied somewhat from strain to strain. Mannitol proved to be the best suitable carbon source for the *in vitro* vegetative growth of *F. moniliforme* isolated from wilted sugar cane fields (Panchal and Rao, 2007). Naik *et al.* (2010) reported that all carbon sources used were suitable for the growth of *Fusarium oxysporum* f. sp. *vanilla* isolates. However, growth of isolates was best on sucrose followed by fructose and maltose. It was observed that maltose, starch, glucose, xylose, lactose and fructose were most favorable for the growth of *Fusarium oxysporum* f. sp. *ciceri* causing chickpea wilt (Khilare and Ahmed, (2011). Sucrose was found best carbon source for growth of all the three isolates of *Fusarium oxysporum* f. sp. *cubense* (Somu *et al.*, 2014). Shilpa *et al.* (2015) reported that the highest mycelial yield of *F. aethiopicum* was recorded in the presence of D-maltose, succinic acid D- lactose and D-fructose. L-sorbose, D-mannitol, D-lactose and D-xylose supported maximum biomass, while D-mannose, D-galactose and succinic acid was responsible for least growth of the *F. culmorum.* Among the eight carbon sources used for the physiological study of the tested soil fungi, maltose, dextrose, lactose and sucrose were best utilized by all the isolates of *Fusarium* spp. (Islam, 2015).

Effect of different nitrogen sources on growth of *F. graminearum*

The influence of different nitrogen sources on growth of *F. graminearum* was studied using five nitrogen sources (Table 2 and Fig. 2). Among the nitrogen sources tested, ammonium nitrate recorded significantly the maximum growth of 90.00 mm. This was followed by potassium nitrate (80.33mm) and ammonium chloride (80.33mm) and sodium nitrate (standard check) (79.67 mm) which were at par with each other. Urea recorded least mycelial growth of 61.00 mm.

Table 1. Growth of *F. graminearum* in different carbon sources (Solid and liquid) media

S.No.	Culture media	Mycelial growth (mm) in solid media	Mycelial dry weight (g) in liquid media
1	Carboxylmethylcellulose	90.00[a]	1.25[a]
2	Dextrose	90.00[a]	1.11[ab]
3	Lactose	88.00[ab]	0.96[bc]
4	Mannitol	86.67[ab]	0.91[c]
5	Fructose	81.67[bc]	0.83[c]
6	Glucose	74.67[c]	0.66[d]
7	Starch	74.67[c]	0.29[e]
8	Sucrose (Standard check)	76.33[c]	1.24[a]
	S.E.±	2.32	0.05
	CD (P=0.05)	6.90	0.15

The treatment means were compared using Duncan's Multiple Range Test (DMRT)
In a column, means followed by a common letter (s) are not significantly different (P=0.05)

Table 2.Growth of *F.graminearum* in different nitrogen sources (Solid and liquid) media

S.No.	Culture media	Mycelial growth (mm) in solid media	Mycelial dry weight (g) in liquid media
1	Ammonium nitrate	90.00[a]	1.28[a]
2	Ammonium chloride	80.33[b]	1.19[a]
3	Potassium nitrate	80.33[b]	0.91[b]
4	Urea	61.00[c]	1.17[a]
5	Sodium nitrate (Standard check)	79.67[b]	0.82[b]
	S.E.±	1.93	0.04
	CD (P=0.05)	5.76	0.14

The treatment means were compared using Duncan's Multiple Range Test (DMRT)
In a column, means followed by a common letter (s) are not significantly different (P=0.05)

Among the five nitrogen sources tested for the biomass of *F. graminearum* in liquid media (Table 2 and Fig. 4), ammonium nitrate recorded the maximum mycelial dry weight of 1.28 g followed by potassium nitrate (1.19 g) and ammonium chloride (1.17 g) which were at par with each other. Minimum weight of 0.82 g was recorded in standard check medium (complete medium) amended with sodium nitrate.

With respect to nitrogen sources the results revealed that ammonium nitrate was found to be the best nitrogen source for maximum mycelial growth. The results obtained in the present study are in conformity with the results obtained by the earlier workers. Selvi and Sivakumar (2013) also reported that ammonium nitrate supported the maximum growth of *F. graminearum* in liquid medium. Among the inorganic nitrogen sources, sodium nitrate was found most suitable for growth all the isolates of *Fusarium* spp. (Islam, 2015). Ammonium hydrogen phosphate proved to be the best suitable carbon and nitrogen sources respectively for the in vitro vegetative growth of *F. moniliforme* isolated from wilted sugar cane fields (Panchal and Rao, 2007). Shilpa *et al*. (2015) reported that Biomass accomplished by *F. aethiopicum* was maximum in medium containing L-lysine, L-glutamine, L-tyrosine, and L-tryptophan, while it was least in medium containing L-glutamic acid, potassium nitrate, L-histidine and L-aspartic acid. Maximum biomass production of *F. culmorum* was recorded in media containing L-tyrosine, potassium nitrate, L-glycine and L-arginine.Out of ten nitrogen sources tested, *F. oxysporum* f.sp. *elaeidis* showed good growth and sporulation in sodium nitrate, ammonium nitrate, potassium nitrate, peptone and DL- leucine amended media (Oritsejafor, 1986).

Thus, it is summarized from this study that among the carbon sources, dextrose and among the nitrogen sources ammonium nitrate were found suitable for growth of *F. graminearum* in both solid and liquid media.

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