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Research paper

Growth determination of agriculturally important fungi of Black Cotton Soil rhizosphere of Soybean in Ujjain region

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Abstract: Black cotton soil is characteristics of Malwa region (Madhya Pradesh) and covers Ujjain district. This soil type is rich in nutrients as well as diverse microbial population. This region cultivates Soybean as main *kharif* crop with different high yielding, resistant varieties suitable for climatic and edaphic conditions. Present study was undertaken to isolate and identify the rhizospheric fungi of JS–335 cultivar of soybean grown in the Black Cotton Soil. After identification selected fungi (*Trichoderma harzianum, Aspergillus niger* ANRT1 and *Aspergillus niger* ANRT2) were tested their growth response against environmental conditions such as temperature (15, 20, 25, 30, and 35°C) pH (5, 6, 7, 8, and 9) and carbon source (Glucose, Sucrose, Lactose, Maltose and Mannitol). Fungal growth was determined by radial growth on agar medium plates while biomass was determined by centrifugation dry–weight method. All data were tested statistically. Results showed that out of three studied fungi, *Trichoderma harzianum* grow best at temperature 25°C and showed maximum radial growth and biomass production, while 6 pH was suitable for all the three fungal isolates. Glucose was found best among all five, as all three fungi showed maximum growth in terms of radial growth and biomass. Present study emphasizes and proves that optimum conditions are very necessary for the growth and activity of agriculturally important fungi. Study also suggests that environmental conditions should be checked before and after crop seed sowing and cultivation so that these fungi can work efficiently and ultimately increase the yield of the crop.

Keywords: Fungi, Agriculturally Important Microorganisms (fungi).

INTRODUCTION

Growth is defined as an increase in biomass and number of an organism. It can be determined by various methods such as counting of cells, dry weight, total weight, number of spores etc. In soil, microbial growth is mainly referred to indigenous microorganisms comprising of both beneficial and harmful microorganisms

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that may influence the plant growth and development. Fungi and bacteria are main habitants of the different soils. Black cotton soil also harbours various types of beneficial microorganisms collectively known as Agriculturally Important Microorganisms (AIMs). In total soil, the rhizosphere is comparatively very rich in nutrients, due to exudates secreted by the plant roots (Bais *et al*., 2006). It is a known fact that the soil rhizosphere is simply are association of the plant root and adjacent soil which is very important for agricultural prospects because of high microbial activity. Also, it is the region from where AIMs can be easily isolated. According to Weinert *et al.*, (2011), the microbial population of rhizosphere is further influenced by the genetic make-up of a plant species. There is a direct impact and correlation of soil type, carbon and nitrogen presence, temperature, pH, moisture and plant factors (e.g. plant species and age) on microbial activities in the soil. If these external factors fluctuate or are inappropriate, fungi and other AIMs may show poor performance in the soil that may directly affect the crop growth.

Malwa region of Madhya Pradesh covers 6 district viz., Ujjain, Indore, Ratlam, Shajapur, Dewas and Mandsaur. These districts primarily have Black Cotton Soil considered as best for the agriculture purpose. Soybean (*Glycine max*) is the main *kharif* crop (June to October) of this region with various cultivars having different characteristic features such as resistance to pathogens, seed size, maturation time etc. JS–335 is a leading cultivar in the area, covers almost 20% of all soybean cultivars. Present study was undertaken to study different environmental conditions viz., temperature, pH and carbon source on the three indigenous fungi (AIMs) isolated from the rhizosphere of the JS–335 cultivar of the soybean.

MATERIALS AND METHODS

This study can be broadly divided into following subheading:

Isolation of fungi:

For the isolation of fungi following method was followed:

2.1.1 Serial dilution and plate count for fungi:

Serial dilution and plate count method was used for isolation of fungi following Johnson (1957). Ten grams of soil sample was taken in the conical flask containing 95 ml distilled water and was shaken vigorously to obtain a suspension for preparing the dilutions. Two dilutions viz., 10^{-3} and 10^{-4} were used for fungal isolation. Of each, 1 ml of sample was transferred into the sterilized Petri-plates containing the media. Media used for experiment were potato dextrose agar medium and potato dextrose broth medium.

2.1.2 Identification and screening of fungi:

Total 15 fungi were isolated from the rhizosphere of JS–335 cultivar of soybean. These fungi were tested on different plant growth parameters (not mentioned here) and only three were found suitable and considered as AIMs. These three fungi were identified as *Trichoderma harzianum*, *Aspergillus niger* ANRT1 and *Aspergillus niger* ANRT2 on the basis of colony morphology, spore (size, shape), mycelium structure, arrangement, color etc.

Effect of physical conditions on mycelium dry weight and radial growth:

Effect of temperature, pH and carbon source on radial growth and mycelial dry weight of these three fungi was studied using standard methods.

Effect of temperature:

From each of the growing culture, piece of 5 mm diameter was taken from periphery of the colony with the help of a sterilized cork borer. Potato dextrose agar (PDA)

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plates were inoculated with the pieces and incubated at different temperature viz., 15, 20, 25, 30 and 35°C, and observed on 7th day of incubation. Growth was measured in milli–meter (mm). For dry weight of mycelial content, fungi were grown in 50 ml potato dextrose broth (PDB) in a 100 ml conical flask. After 7 days of incubation at their respective temperature the content was centrifuged at 5000 rpm (rotation per minute) for 15 minutes and then filtered through sterilized filter paper and filtrate was kept for drying at 72°C for 24 h. After drying mycelium weight was taken.

Effect of pH:

The method was same as described in the previous section but different pH were set: 5, 6, 7, 8, and 9. PDA plates were incubated at 25°C for 7 days. Radial growth was measured in mm, and for determination of mycelial dry weight similar method was followed as mentioned earlier.

Effect of carbon source:

Glucose, sucrose, lactose, maltose and mannitol (5%) were used in the potato infusion medium. These carbon sources were replaced manually with dextrose (glucose) in the medium. The method was same as described in the previous section. Potato infusion agar plates were incubated at 27°C for 7 days. Radial growth and mycelial dry weight were determined.

Statistical analysis:

All data collected from the present study was tabulated and tested for their significance using MS–Excel and *Biostat* Software.

RESULTS

All the results of the present experiment were statistically tested and presented in the form of tables and graphs. Table 1, 2 and 3 represent the effect of temperature, pH and carbon sources on radial growth of fungi respectively. While Figure 1, 2, and 3 showing dry weight of biomass. The best suitable temperature for radial growth as observed was 25°C. *Trichoderma harzianum* showed maximum growth (45.8 mm) among all three fungi and minimum radial growth was noticed in *Aspergillus niger* ANRT2 (6.9 mm) at 20°C (Table 1). There was no radial growth showed by any fungus at 15°C. Same pattern was observed in the dry weight biomass in which maximum growth was observed at same temperature i.e., 25°C (Fgure–1).

Table 1. Effect of temperature on the radial growth (in mm) of fungi

S.No.	Parameters	Range	*Trichoderma harzianum*	*Aspergillus niger* ANRT1	*Aspergillus niger* ANRT2
1	Temperature (°C)	**15**	00.0	0.0	0.0
		20	28.1±2.1	31.2±1.3	6.9±1.4
		25	45.8±1.8	38.0±1.4	34.7±0.8
		30	22.6±1.1	37.5±1.1	12.8±0.9
		35	10.4±0.9	11.0±1.4	10.8±1.2

Each value is average value of three (± indicates standard deviation) (P value= 0.004559)

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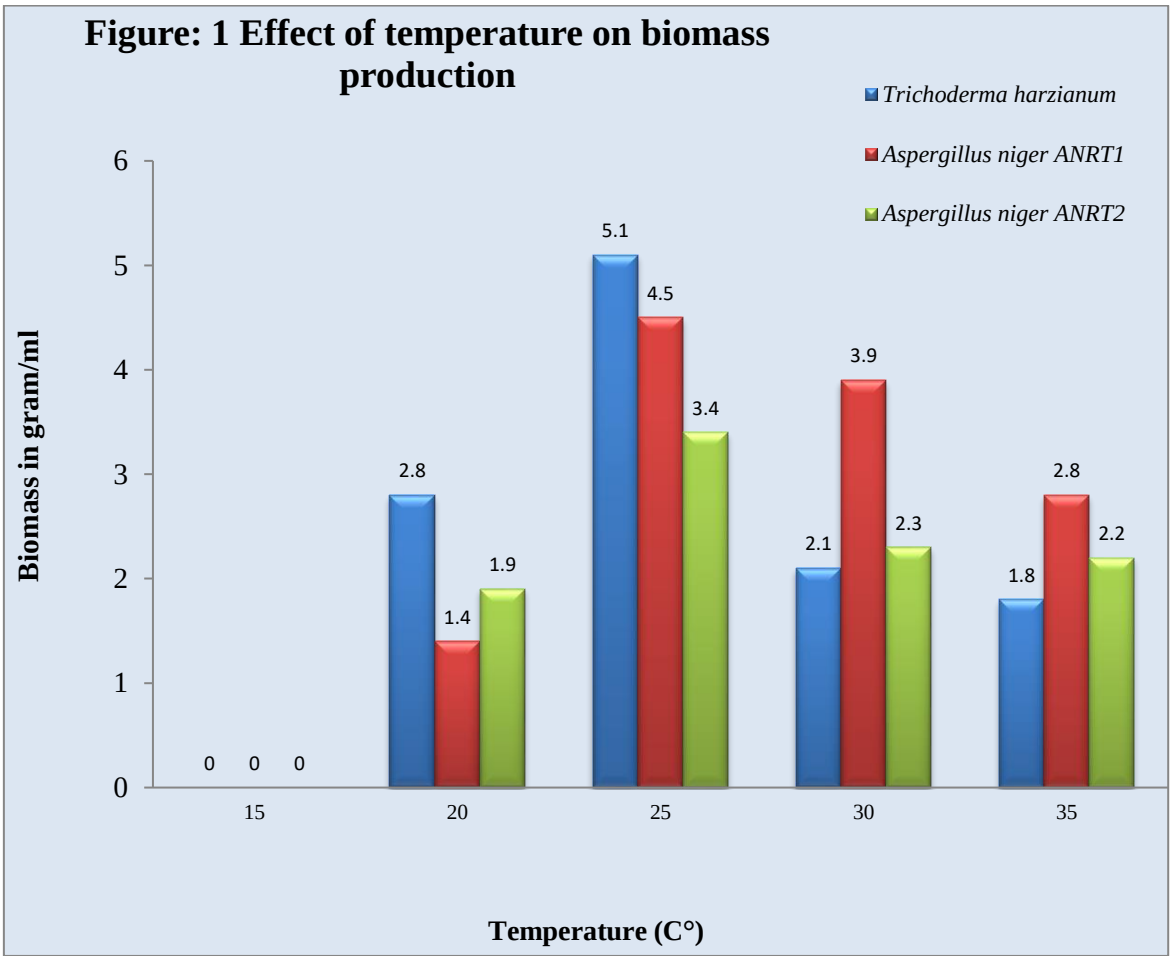

Table 2 and Figure 2 represent radial growth and biomass respectively of fungi at different pH (5, 6, 7, 8, and 9). There was variation in the optimum pH of each fungus. As mentioned in the table 2, *T.harzianum* and *A.niger* ANRT1 showed maximum radial growth (45 mm) and (37 mm) at 6 pH respectively, while *A.niger* ANRT2 showed maximum growth (26.5 mm) at pH 7. All three fungi showed very little growth at pH 5.0 while there was no growth at pH 9.

Table 2. Effect of different pH on the radial growth (in mm) of fungi

S.No.	Parameters	Range	*Trichoderma harzianum*	*Aspergillus niger* ANRT1	*Aspergillus niger* ANRT2
1	pH	**5**	12.5±1.1	10.5±1.0	8.9±2.1
		6	45±2.0	37±0.7	25±1.7
		7	39.5±1.8	32.6±1.3	26.5±1.4
		8	12.1±1.4	10.5±1.4	10.9±0.9
		9	0	0	0

Each value is average value of three (± indicates standard deviation) P value: 0.083175

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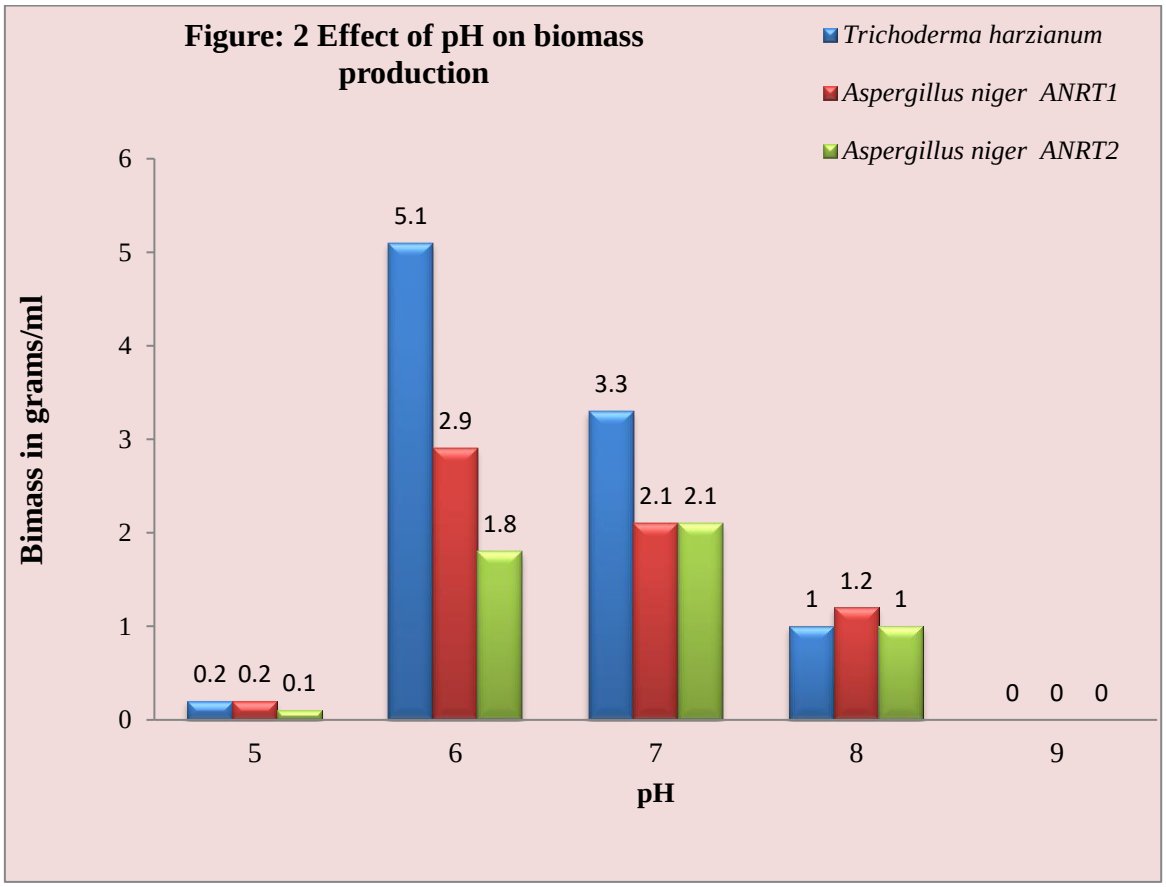

Glucose containing medium was best for all three fungi for radial growth and biomass (Table 3 and Figure 3). *A. niger* ANRT2 showed maximum radial growth (53.8 mm) and minimum (11.6 mm) with mannitol supplemented potato infusion medium (Table 3). Similarly, 6.9 g of biomass was obtained by *A. niger* ANRT2 in potato dextrose medium (Figure 3).

Table 3. Effect of Carbon source on the radial growth of fungi

S.No.	Parameters	Range	*Trichoderma harzianum*	*Aspergillus niger* ANRT1	*Aspergillus niger* ANRT2
3	Carbon Source	Glucose	47.8±1.3	45.9±1.4	53.8±0.8
		Sucrose	33.6±1.1	37.4±1.8	41.5±1.1
		Lactose	42.5±1.2	39.6±2.2	36.5±1.7
		Maltose	32.1±0.9	40.5±1.2	40.9±0.9
		Mannitol	12.0±0.4	15.7±1.1	11.6±1.2

Each value is average value of three (± indicates standard deviation) P-value: 0.421758

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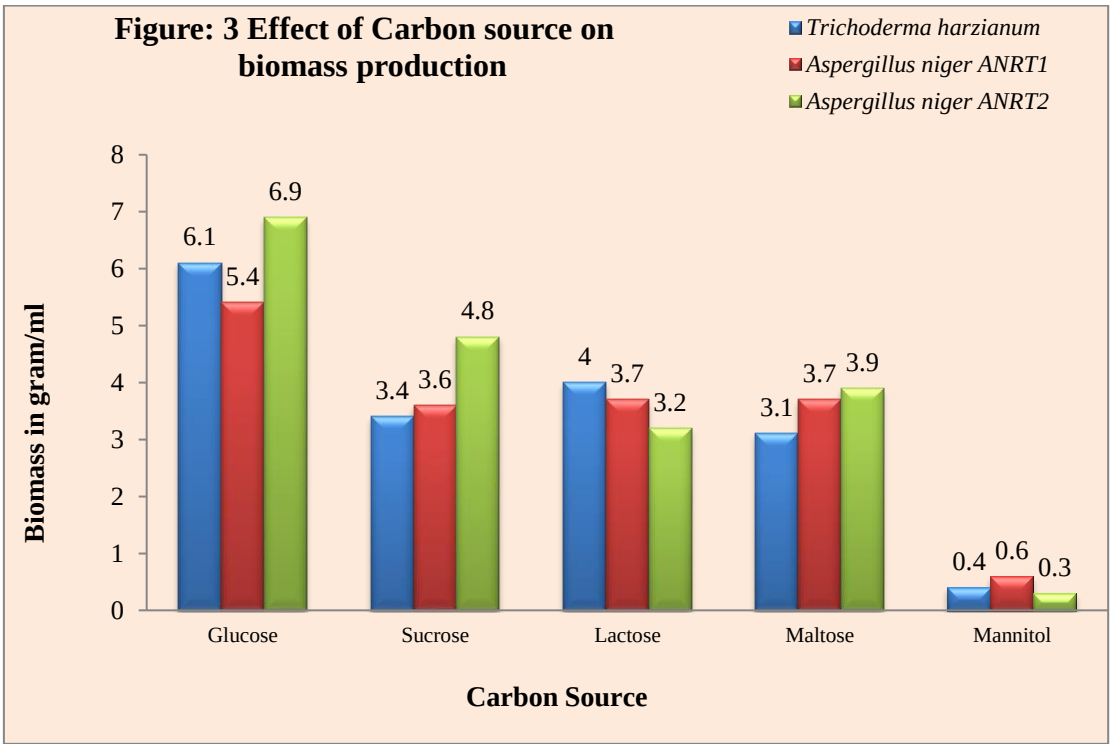

DISCUSSION

Soil health and productivity of a crop is primarily influenced by genetic and environmental factors. Genetic factors, which influence the plant growth and yield at a significant extent, can be manipulated to improve the productivity of crops. On the other hand, environmental factors such as temperature, pH of soil, nutrients level, weather, and water are difficult to control in field. According to report of FAO (1984) environmental factors such as temperature, pH etc., affect the cycling of several chemical components of the soil such as phosphorus and nitrogen. Rhizospheric microbes play a crucial role for plants during seedling stage and overall plant growth. It has been suggested that each soil harbours its own characteristic microbial diversity (Nacke *et. al.*, 2011).

According to Lupwayi *et al.*, (1998) for stability and functioning of any agro ecosystem, AIMs play an important role and for their proper functions environmental conditions should be optimum. In the present study effect of temperature was in accordance to several workers who reported 25°C as optimum for the growth of fungal isolates in terms of both mycelium dry weight and radial growth (Table 1 and Figure 1). Also, the temperature below and above 25°C caused the growth ceased. Fungi generally grow well in acidic conditions (Dix and Webster 1995), but some species favour neutral to slightly alkaline conditions (Yamanaka 2003) that is also reflected in the present study (Figure 3). Balanced ingredient in medium is very essential as nutrition for the proper and optimum growth of fungi. Carbon as a part of an ingredient in the medium is required for growth and other metabolic activities. Although, the work of El-Banna *et al.*, (2006) was mainly based on bacterial species but their results are parallel with our results (Table 3 and Figure 3) as they have studied the effect of different carbon sources on growth.

CONCLUSION

Edaphic and Environmental factors such as temperature, pH, carbon source, nitrogen

source, humidity etc., play very crucial and important role in any micro–ecosystem. The microbial population present in any soil–microorganisms–plant association depends on these factors. As it is clear in the present study that for optimum activity of AIMs (fungi) it is necessary to known the actual and suitable requirement of them. If there is any fluctuation in the environmental conditions, these AIMs are not able to work efficiently which can leads to deleterious effect on plant growth. Although, this piece of work is at laboratory level but it clearly indicates that optimization of every environmental conditions should be done before further study of AIMs.

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