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Antifungal Studies of Some of the Medicinal Plants Against the Growth of Fungus *Macrophomina phaseolina*

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Abstract

Three medicinal plants, *Solanum nigrum, Viola odorata* and *Mentha sylvestris*, collected for extraction means from Sudhangalli, district Bagh Azad Kashmir, Pakistan were tested against *Macrophomina phaseolina* (*in vitro*) at different concentrations of their extracts, temperature and nutrition. The linear mycelial growth, biomass and number of sclerotia were found to be maximum at 35°C in check wehereas growth decreased above and below this level. Maximum growth rate was observed when extracts were supplemented with Kno3 than when supplemented with glucose, fructose and urea. There was no linear mycelial growth when urea was mixed with extracts of *Solanum nigrum* and *Viola odorata* and it was very low in case of *Mentha sylvestris* even in check. The fungus *Macrophomina phaseolina* showed very poor growth in presence of urea, below and above 35°C and in low concentrations of the extracts of these medicinal plants.

Key words: Medicinal plants, *Macrophomina phaseolina*

Introduction

Macrophomina phaseolina is a soil born, facultative saprophyte and survive as microsclerotia in soil or in crop or weed host debris (Mikhail, 1989). This fungus has wide host ranges, widely distributed in warm climate and compete well when soil nutrient levels are low and temperature are above 30°C (Collins *et al.,* 1991). *M. phaseolina* causes serious losses to the field crops, vegetables, fruits and plants every year in Pakistan. Man is directly dependant upon plants for his survival, because plants are his prime source of food, fiber and drug. Plant diseases effect the lives and individuals. The solution of control problems is the ultimate purpose of a distinct science of plant pathology.

Nwsu *et al.,* (1995) studied antifungal activities of extracts of ten medicinal plants collected from Southeastern parts of Nigeria tested against seven pathogenic fungi using the broth dilution and agar plate methods.

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All the extracts at 1:10 dilution inhibited the growth of *Basidiobolus haptosporus* and *B. vanarum* but did not inhibit those of *Aspergillus fumigatus*, *Geotrichum cndidum* and *Candida albicans*. Whereas extracts from *Piper guineense* and *Ocimum graticimum* inhibited the growth of *Trychophyton rubrum*.

Prithiviraj et al (1996) reported a significant decrease in the mycelial dry weight and number of sclerotia of Sclerotium rolfsii at 150 ppm extract of medicinal plant Aegle marmelos. Mastura et al (1999) stutied essential oils from 11 species of Cinamomum against the microorganism activities. They found that oils inhibited the growth of microorganisms at very low concentration values ranginging from 0.47-2.52 mμg/ mμl.

In the present study three medicinal plants, *Solanum nigrum, Viola odorata* and *Mentha sylvestris*, were used for extraction to test against *Microphomina phaseolina*. It is a well known soil-borne fungus causing root rot diseases in different leguminous and cucurbitaceous plants (Nene and Reddy, 1987).

Materials and Methods

Three available wild medicinal plants, *Solanum nigrum, Viola odorata* and *Mentha sylvestris*, were collected from Sudhan galli, district Bagh Azad Kashmir, Pakistan. The whole plants were withdrawn and pressed in presser containing news papers, dried carefully for identification and extractions. The medicinal parts of the plants were dried for a month, sterilized with calcium hypochlorite solution and then washed with sterilized distilled water. Six conical flasks were washed with acetone and were kept in oven at 100°C for a week for sterilization. Dried sterilized plants were crushed to form a powdery material. Weighed powdery material was placed in the sterilized conical flask containing 100ml methanol at 100°C for ten days. This suspension was filtered in sterilized conical flask. An oily layer appeared after a few days, was used for next experiments.

The extract of medicinal plants was added to 20 ml aliquots of molten potato dextrose agar (PDA) at 40°C in 9 cm diameter Petridishes to determine the linear mycelial growth of *Macrophomina phaseolina*. The extract was added to 20 ml aliquots of 2% D-glucose solution (D-GS) in conical flasks to determine the biomass of this fungus. The final concentrations of the

extracts viz. 5, 10, 15, 20 and 25 were prepared from 100 per cent pure extracts. The above Petridishes and conical flasks were rotated to mix the extract solution with PDA and D-GS respectively. The experiments were run in triplicate except otherwise mentioned. Each Petridish containing PDA or conical flask with D-GS was inoculated in the centre with 8 mm disc cut from the margin of an actively growing culture of *Macrophomina phaseolina* on PDA or D-GS respectively. These cultures were maintained at 35°C in an incubator. The diameter of each colony in case of linear mycelial growth and dry weight of colony in case of biomass were determined after 24 hours and at 5 days intervals respectively. The effect of different nutrition viz. glucose, fructose, urea and Kno3 and temperature on the radial growth of *Macrophomina phaseolina* was determined in 5 per cent extracts.

Results and Discussion

Linear growth rate and biomass of *Macrophomina phaseolina* was maximum in 0 per cent concentration of extracts of *Solanum nigrum*. The growth is significantly decreased with increasing of every 5 per cent concentrations level of extracts of *Solanum nigrum*. The minimum growth rate was in case of 25 per cent concentration of extracts. The production of sclerotia of *Macrophamina phaseolina* also decreased with the increasing of concentration of extract (Table 1). Radial growth of the fungus growing in 5 per cent extracts of *Solanum nigrum*, *Viola odorata* and *Mentha sylvestris* is maximum at the temperature of 35 °C. Above and below of this level the radial growth rate was decreased. However, did not differ significantly at 30-40 °C in the extract of *Viola odorata*. The mycelial growth rate of fungus was higher in case of extract of *Mentha sylvestris* at all temperatures as compared to two other medicinal plants (Table 2). The result showed that the pathogen grew well in 5 per cent extracts of all three medicinal plants when mixed with KNO_3. However, the growth rate of the fungus is not significantly different when growing in the extracts of *Mentha sylvestris* mixed with glucose and fructose. The pathogen did not grow in the presence of urea when mixed with extract of *Solanum nigrum* and *Viola odorata*, whereas, it showed very low rate of growth in the extract of *Mentha sylvestris* (Table 3). Present studies indicated that chemical constituents of medicinal plants inhibited the growth of pathogen *Macrophomina phaseolina* (Table 1-3).

The use of medicinal plants to avoid infection by different disease causing microorganisms has been observing for a long time. The medicinal plants possess certain antifungal and antibacterial properties. These Plants contain strong chemical compounds such as alkloids, terpenoids and phenolic compounds, which inhibit the growth of microorganisms. A higher level of phenolic compounds in resistant cultivars than in susceptible to various pathogens is well known (Bhatia (*et al.*, 1972 b).

Table 1: Effect of different extract concentrations (%) of *Solanum nigrum* (*in vitro*) on radial growth rate (mm/day), biomass (mg/5days) and production of sclerotia of *Macrphomina phaseolina* (mean values ± SEM, n = 3)

Extract Concentration	Radial growth	Biomass	No. of sclerotia
0	18.02 ± 0.19^{a}	51.17 ± 0.07^{a}	43.33+0.15
5	14.12 ± 0.10^{b}	35.60 ± 0.08^{b}	36.45+0.18
10	10.68 ± 0.12^{c}	30.07 ± 0.07^{c}	28.55+0.18
15	08.25 ± 0.20^{d}	25.23 ± 0.05^{d}	23.11+0.18
20	07.58 ± 0.18^{d}	21.97 ± 0.05^{e}	14.44+0.88
25	01.58 ± 0.06^{e}	16.30 ± 0.04^{f}	9.66+0.27

Values in the same column followed by the same letter are not significantly different ($p \leq 0.01$)

Table 2: Effect of temperatures(°C) on the radial growth of *Macrophomina phaseolina* at 5 per cent extract concentration (mean values ± SEM, n = 3)

Temp. °C	Radial growth in the extract of			Control
	Solonum nigrum	Viola odorata	Mentha sylvestris	
25	1.88±0.10c	1.33±0.06b	03.00 ± 0.23^{c}	08.60 ± 0.24^{c}
30	6.38±0.06b	3.68±0.18a	08.06 ± 0.31^{b}	12.10 ± 0.16^{b}
35	8.50± 0.01a	4.21±0.12a	10.60 ± 0.13^{a}	18.58 ± 0.23^{a}
40	6.50±0.58b	3.49±0.60a	07.21 ± 0.50^{b}	13.20 ± 0.24^{b}

Values in the same column followed by the same letter are not significantly different ($p \leq 0.01$)

Present studies indicated that the extracts of the medicinal plants inhibited the growth and reproduction of the fungus *Macrophomina phaseolina* even at very low concentrations. Whereas, at 25 per cent the radial growth and biomass is 2mm and 16 mg/day respectively (Table 1). It is too much clear from this data that growth rate of the fungus decreased with the increase in the concentration of these extract of these medicinal plants. Nwosu *et al.*, (1995) reported similar results of 10 medicinal plants against the fungus *Basidiobolus haptosporous* and *B. ranarum*. Damayanti *et al.*, (1996) studied certain medicinal plants to find out the antifungal and antibacterial activities against some *Xanthium strumarium* and *Allium stivum*

pathogens and found that extracts of medicinal plants inhibited the growth of these pathogens. However, the growth rate of the fungus was 4.21mm/day in the extract of *Viola odorata* and 10.16 mm/day in the extract of *Mentha sylvestris* indicating that fungus grew well at 35 °C in the extracts of *Mentha sylvestris* as compared to the other two medicinal plants and above and below of this level the growth was decreased. However, at 30 and 40°C this did not differ from each other (Table 2). Collins *et al.,* (1991) reported the similar results. The growth rate of *Macrophomina phaseolina* was better in the extract of all the medicinal plants when mixed with KNo3, but was the best in the extract of *Mentha sylvestris* as compared to the other medicinal plants (Table 3).

Table 3: Effect of different carbon and nitrogen sourced nutrition on radial growth rate (mm/day) of *Macrphomina phaseolina* at 5 per cent extract concentration (mean values ± SEM, n = 3)

	Radial growth in the extract of			Control
	Solanum nigrum	Viola odorata	Mentha sylvestris	
Fructose	11.04±0.20^{b}	13.10±0.31^{a}	13.18±0.30^{b}	18.58±0.24^{a}
Glucose	09.00±0.23^{c}	09.63±0.15^{b}	13.00±0.23^{b}	15.53±0.24^{b}
Urea	00	00	02.58±0.18^{c}	04.86±0.05^{c}
KNo3	14.54±0.21^{a}	13.39±0.04^{a}	19.60±0.30^{a}	18.58±0.24^{a}

Values in the same column followed by the same letter are not significantly different ($p \leq 0.01$)

Present studies showed that the constituents of the extracts inhibited the growth of *Macrophomina phaseolina* and can be used as a control measure to control the disease of plants caused by *Macrophomina phaseolina*. Although these studies are *in vitro* but the extracts of these medicinal plants can be tried in the pots or even in the field as control measure of the disease caused by soil-born fungi in the future research studies.

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