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Effect of Carbon, Nitrogen Sources and Ascorbic Acid on the Colony Growth and Acervulus Production of *Pestalotia psydii*

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ABSTRACT

Out of three carbon sources viz., fructose, maltose and sucrose, the colony growth of the fungus was medium (54.75 mm) in maltose, 82.25 mm in fructose and 79.25 mm in sucrose as compared to control (89.50 mm). Comparison of carbon sources indicated that sucrose and fructose were better utilized by the fungus for its growth as compared to maltose. Similar response was observed in acervulus production. Among the four nitrogen sources potassium nitrate maximally supported the growth of fungal colonies (70.50 mm) followed by sodium nitrate (63.88 mm), urea (51.38 mm) and ammonium phosphate (34.13 mm). Minimum size (2.5 mm) of acervuli was observed when ammonium phosphate was added to the medium. Among the four nitrogen sources, potassium nitrate favoured acervuli production to its maximum (9.0 mm) followed by sodium nitrate, urea and ammonium phosphate. The effect of four different concentrations indicated that with the increase in ascorbic acid concentrations a retardative effect was observed and growth was decreased (9.2 mm) at 240 mg L^{-1} concentration while acervuli production was totally checked at this concentration.

Key Words: Carbon; Nitrogen; Guava; Die-back; *Pestalotia psydii*

INTRODUCTION

Guava (*Psydium guajava*) is very important fruit having 82% water, 0.7% protein, 11% carbohydrates and enough amount of vitamins A, B, B_2 and C plus some minerals (Ardi, 1975). In Punjab province of Pakistan, it is cultivated on 62.7 thousand hactares with an annual production of 531.6 thousand tonnes with average yield 8478 kg ha^{-1} (Anonymous, (2004), which is very low as compared to other guava producing countries. Among the other yield limiting factors, diseases play a crucial role. Wilt of guava and anthracnose of guava are very destructive diseases which lead to very heavy yield losses.

Anthracnose of guava is caused by *Gloeosporium psydii* which attacks all the above ground parts of the plant resulting in the death of the branches. Spots on unripe fruits develop especially during the rainy season. Wilt of guava is another important disease which is characterized by yellowing and browning of leaves and the tips of twigs. The most characteristic symptoms include appearance of small pin head sized spots on the fruits. Affected fruits later on drop off. In moist weather, acervuli are produced in abundance on dead twigs. Disease is favoured by comparatively higher temperature i.e. $30\text{-}37^0$ and relatively high humidity (Tandon & Singh, 1969). This disease has recently been reported in Pakistan (Shakir *et al*, 1991). Taxonomically, the Deuteromycetous genera of fungi viz. *Gloeosporium*, *Colletotrichum* and *Pestalotia* cause plant diseases like anthracnose, withertip and die back, etc. belong to the order Melanconiales (*Sensu saccardo*).

Die back is a common disease of guava which is caused by *Pestalotia psyddii*. No research work on the physiology of *Pestalotia psydii*, has been carried out. Studies on the different physiological aspects like, ascorbic acid concentrations, carbon and nitrogen sources was carried out in order to have a better understanding of the physiology of this fungus. The main object of this study was to determine the suitable carbon, nitrogen sources, and ascorbic acid concentration on colony growth and acervulus production by this fungus for the proper management of disease.

MATERIALS AND METHODS

The diseased specimens of guava were collected from district Sheikhupura and pathogen isolations from the diseased parts were made by following usual isolation technique (Riker & Riker, 1936). Diseased specimens were cut into small bits and immersed in 1% sodium hypochlorite solution for two minutes and then rinsed in sterilized water in each petri plate. After treatment, the bits were put on filter papers in sterilized petri plates in order to absorb excess of water present on the treated bits. These bits were then transferred to solidified potato-dextrose agar (PDA) plates. To avoid bacterial contamination, streptomycin sulphate (1: 10,000) and rose Bengal (1: 30,000) was added to the medium after sterilization and before pouring. These plates were incubated at 30 ± 2^0C. On sporulation of the fungus temporary mounts (glycerin water) were made and isolate was identified (Pathak, 1980).

Table I. Effect of Carbon and Nitrogen sources on the colony growth of (*Pestalotia psydii*), the cause of die-back disease of guava

Observations	Colony growth (mm) with different carbon sources				Colony growth (mm) with different nitrogen sources				
	Fructose	Maltose	Suerose	Control	KNO_3	$NaNo_3$	$(NH_4)_2Po_4$	Urea	Control
1	84	56	80	90	70	64.5	35	50	90
2	83	56	78	90	71	63	33	52	89
3	81	53	79	89.5	72	63	34	52	90
4	81	54	80	89.5	69	65	34.5	51.5	90
Mean	82.25b	54.75 d	79.25 c	89.50 a	70.50 b	63.88 c	34.13 e	51.38 d	89.75 a

F. value for carbon sources = 633.203; **Highly significant (P<0.01); F. value for nitrogen sources = 184.93

Table II. Effect of carbon and nitrogen sources on the acervulus production of (*Pestalotia psydii*) the cause of die-back disease of guava

Observations	No. of acervuli produced with different carbon sources				No. of acervuli produced with different nitrogen sources				
	Fructose	Maltose	Suerose	Control	KNO_3	$NaNo_3$	$(NH_4)_2Po_4$	Urea	Control
1	13	8	11	17	10	6	3	5	17
2	14	9	13	18	9	8	2	6	16
3	13	8	11	16	8	8	2	5	15
4	15	10	10	16	9	7	3	6	15
Mean	13.75 b	8.75 d	11.25 c	16.75 a	9.0 b	7.25 c	2.50 e	5.5 d	15.75 a

F. value for carbon sources = 43.308; **Highly significant (P<0.01); F. values for Nitrogen sources = 154.934; **Highly significant (P<0.01)

For different physiological studies, the best medium was amended with different carbon sources (maltose fructose and sucrose), nitrogen sources (Sodium nitrate, ammonium sulphate, potassium nitrate and urea) and ascorbic acid. To observe their affect on colony growth and acervulus production, 25 mL of medium was poured in each petri plate. After solidification of the medium, 6 mm diameter agar plugs were cut from one week actively growing culture of the fungus with the help of sterilized cork borer, placed in the center of each petri plate and incubated at 30 ± 2^0C (Tutite, 1969). Hydrogen-ion concentration (pH) of medium was adjusted by Beckman pH meter. Observations on colony growth of the fungus were recorded in quadruplicate after 7 days and data on acervulus production was recorded after 15 days.

For calculating the number of acervuli culture discs from center of each petri plate were cut with the help of a 5 mm diameter flame sterilized cork borer. Data were analyzed statistically for interpretation of the results.

RESULTS AND DISCUSSION

Out of three carbon sources viz., fructose, maltose and source, the colony growth was medium i.e. (54.75 mm) in maltose. When glucose was replaced with fructose and sucrose, the growth was comparatively better (28.25 and 79.25 mm respectively), and out of the remaining two, sucrose and fructose favoured growth better than maltose (Table I). According to statistical analysis the F values were highly significant, indicating that Sucrose and Fructose were better utilized by this fungus for its growth compared to Maltose. As regards the effect of these sugars on number of acervulus production, a response similar to colony growth (13.75, 11.25 and 8.75 in Fructose Sucrose and maltose, respectively) was observed (Table I), and a similar pattern of acervulus production was noted (Table II)

Living organisms are known to utilize about forty different elements, among which carbon plays the key role. Carbon compounds serve two essential functions in the metabolism of the fungi. In our studies, among the carbon, sources, fructose gave the maximum effect on mycelial growth and acervulus production. These results agree to the findings of (Midha & Chohan, 1968), who observed that the growth of *Gloesporium psydii* was the best on Fructose.

Among the four nitrogen sources, potassium nitrate supported the growth of the fungus to the maximum (70.5 mm) followed by sodium nitrate (63.88 mm), urea (51.38 mm) and ammonium phosphate (34.13 mm) (Table I) The colony growth was lowest in case of ammonium phosphate as compared to other nitrogen sources. Least colony number (34.13) was observed when ammonium phosphate was added to the medium. Among the other nitrogen sources, potassium nitrate maximally favoured (9.0) acervulus production (Table II). Among different nitrogen sources, ammonium phosphate produced more inhibitory effect.

Like carbon sources, nitrogen is also used both for functional as well as structural purposes by different fungi. The form of nitrogen has a profound effect on metabolism of microorganism. Among organic sources of nitrogen L-glutamic acid and L-proline were found to support growth of all the fungi belonging to genus *Pestalotia*. Relatively poor growth of these fungi was observed on ammonium nitrate showing that these fungi are capable of utilizing nitrate as well as organic nitrogen (Mitra & Tasndon, 1970).

Table III. Effect of different ascorbic acid concentrations on the colony growth of (*Pestalotia psydii*) the cause of die-back of guava

Observations	Colony growth (mm) at different concentrations (mg L^{-1})				
	180	200	220	240	Control
1	25.0	20.0	15.0	10.0	90.0
2	27.0	18.0	14.0	9.0	89.0
3	26.0	17.0	15.0	10.0	90.0
4	25.0	17.0	15.0	8.0	90.0
Mean	25.75b	18.0c	14.00d	9.2e	89.75 a

F. value = 5058.462**; Highly significant (P<0.01)**; The value sharing the same letter do not differ significantly

Table IV. Effect of different ascorbic acid connections on the acervulus production of (*Pestalotia psydii*) the cause of die-back disease of guava

Observations	No. of acervulus at different concentrations (mg L^{-1})				
	180	200	220	240	Control
1	3.0	2	1	0	17
2	4.0	2	0	0	16
3	5.0	2	1	0	15
4	5.0	2	1	0	15
Mean	4.25 b	1.75c	0.75d	0e	15.75a

F. value = 361.071**; Highly significant (P<0.01)**; The value sharing the same letter do not differ significantly

The effect of four different concentrations indicated that with an increase in ascorbic acid concentration a retarding effect on colony production was observed (Table III). The effect was greatest when medium was amended with 240 mg L^{-1} ascorbic acid and the acervulus production was totally checked (Table IV). It indicates that well suited concentration of ascorbic acid is between 220-240 mg L^{-1}. This may be helpful for the researchers for the proper understanding of the physiological behavior of the fungus.

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