

Response of Nitrogen and Amino Acid Sources on Development of *Alternaria Alternata* Causing Root Rot to Fenugreek

Nilkanth K. Khandare

Department of Botany, Krantisinh Nana Patil College, Walwa, 416 313, Dist- Sangli, Maharashtra, India

Abstract: During a survey in Maharashtra, it was observed that fenugreek (*Trigonella foenum graecum* L.) infected by *Alternaria alternata* (Fr.) Keissler, were found to be dominant among the diseased samples. From these samples wild sensitive (Aa-2) and highly resistant (Aa-8) isolates were identified using carbendazim. The aim of present investigation was to evaluate nitrogen, phosphate, sulphate and micronutrients sources on disease development of fenugreek caused by *Alternaria alternata*. The sensitive and resistant isolates of *Alternaria alternata*, when grown on Czapek Dox agar medium show different response to nitrogen, and amino acid sources on development of disease on fenugreek. Different nitrogen sources like peptone, ammonium nitrate, potassium nitrate and sodium nitrate were evaluated for growth response which showed variation in results. Six amino acid sources namely, Phenylalanine, Proline, Tryptophan, Leucine, Serine and Histidine were used in this study. There was variation in the growth of the sensitive and resistant isolates on different amino acids. All these amino acids show different action on the growth of sensitive and resistant isolates. There was significant variation, in the growth of development of pathogen, *Alternaria alternata*, causing disease to fenugreek, either stimulant or inhibitory, when nitrogen and amino acid sources used.

Keywords: Fenugreek, *Alternaria alternata*, roots rot, nitrogen and amino acid sources.

1. Introduction

Fenugreek is an important vegetable used for its various uses but is infected by some pathogens causing disease. A Survey of the diseases of fenugreek (*Trigonella foenum - graecum*. L.), in Maharashtra state was carried out (Khandare and Kamble, 2013). From that resistant and sensitive isolates were identified and used to study. The sensitive isolate was abbreviated as Aa-2 and resistant isolate Aa-8. The infection of *Alternaria alternata* causes severe damage in quality and yield as well as biochemical loss (Khandare, 2014). Effect of different Nitrogen (0.2%) and amino acid (0.2%) sources used to study the effect on the growth of sensitive and resistant isolates of *Alternaria alternata* amending them in the Czapek's dox agar medium and was compared with control. The aim of present study was to determine the role of nitrogen and amino acid sources in pathogenesis caused by *Alternaria alternata* causing root rot disease to fenugreek.

2. Material and Methods

2.1. Materials

Infected samples of fenugreek (*Trigonella foenum - graecum*. L), causing root rot to fenugreek by *Alternaria alternata*. Different nitrogen (peptone, ammonium nitrate, potassium nitrate and sodium nitrate) and amino acid sources (Phenylalanine, Proline, Tryptophan, Leucine, Serine and Histidine).

2.2. Methods

A Survey of the diseases of fenugreek (*Trigonella foenum - graecum*. L.), in Maharashtra state was carried out. In the survey, the samples of infected fenugreek were collected and pathogens were isolated by direct plating analysis (Pitt and

Hocking, 1997. The isolated pathogens were identified (Subramanian, 1971; Barnett and Hunter, 1972) The sensitivity of *Alternaria alternata* was identified using food poisoning method and the sensitive (Aa-2) and resistant (Aa-8) isolates were identified (Khandare and Kamble, 2013). These isolates were evaluated for assessment of growth response of Nitrogen (0.2%) and amino acid sources (0.2%), (*in vitro*) by observing linear growth of pathogen. The procedure was carried out as follows.

2.2.1. Nitrogen Nutrition

Different nitrogen sources like, peptone, ammonium nitrate, potassium nitrate and sodium nitrate were incorporated in Czapek Dox agar medium at 0.2%. The sensitive (Aa-2) and resistant (Aa-8) isolates were grown on agar medium and incubated at $28 \pm 2^\circ\text{C}$. Plates without nitrogen source served as control. The linear growth was measured at different intervals.

2.2.2. Amino Acid Nutrition:-

Effect of different amino acid like phenylalanine, proline, tryptophan, serine, leucine and histidine were selected to study growth of sensitive (Aa-2) and resistant (Aa-8) isolates by amending them Agar medium at 0.2%. Plates without amino acid source served as control. The inoculated plates were incubated at $28 \pm 2^\circ\text{C}$. The linear growth was measured at different intervals.

2.3 Statistical Calculations

Statistical calculation was calculated by using two way analysis of variance using Anova.

3. Results

3.1. Nitrogen Nutrition

In nitrogen sources, Peptone, ammonium nitrate, potassium nitrate and sodium nitrate were incorporated in medium at 0.2%. It appeared that nitrogen sources are essential for growth of both the isolates of *Alternaria alternata*. There was slight variation in the growth of both sensitive and resistant isolates, between nitrogen sources. Growth rate of resistant strain was found to be always higher than the sensitive one. Growth rate of both the resistant and sensitive isolates were found to be higher on ammonium nitrate in all nitrogen sources used as well as control. The three nitrogen sources Potassium nitrate, sodium nitrate and peptone stimulate the growth of *Alternaria alternata* (Table.1).

3.2. Amino Acid Nutrition

For the study six amino acids (Phenylalanine, Proline, Tryptophan, Leucine, Serine and Histidine) were used in this study. There was variation in the growth of the sensitive and resistant isolates on different amino acids. All these amino acids show different action on the growth of sensitive and resistant isolates. Leucine show higher growth response to pathogen among all amino acids applied, where as tryptophan and histidine stimulated the growth of both the pathogens. The amino acids, Phenylalanine, Proline and Serine show inhibition in growth of both resistant and sensitive isolates of *Alternaria alternata* (Table.2).

Table 1: Response of different Nitrogen sources on the linear growth (mm) of *Alternaria alternata* isolates Sensitive and Resistant to carbendazim on Czapek Dox agar medium

Nitrogen sources 0.2%	Sensitive isolate						Resistant isolate					
	Days						Days					
	2	4	6	8	10	12	2	4	6	8	10	12
Ammonium nitrate	13.33	18.00	26.33	37.66	49.00	64.33	15.00	22.33	32.66	42.33	55.66	68.33
Potassium nitrate	12.00	17.00	25.33	35.66	48.00	63.33	13.33	19.00	27.66	36.66	50.33	65.66
Sodium nitrate	13.33	18.33	26.00	37.66	52.00	64.33	14.33	20.33	28.66	38.33	51.33	66.66
Peptone	11.33	17.33	25.00	35.33	48.33	62.00	13.00	19.33	27.33	37.33	50.66	65.33
Control	10.00	16.33	24.00	34.66	47.33	61.66	12.33	18.00	26.33	36.00	49.33	64.33

Sensitive isolate- $P = 0.0271$, Resistant isolate- $P = 0.0065$

Table 2: Response of different Amino acid sources on the linear growth (mm) of *Alternaria alternata* isolates sensitive and resistant to carbendazim on Czapek Dox agar medium

Amino acid source 0.2%	Sensitive isolate						Resistant isolate					
	Days						Days					
	2	4	6	8	10	12	2	4	6	8	10	12
Phenylalanine	10.66	14.00	20.66	33.66	45.33	51.66	11.66	15.33	25.66	30.33	42.00	54.33
Proline	11.66	14.33	21.66	34.66	46.33	56.66	12.00	16.33	26.66	32.33	46.00	56.33
Tryptophan	12.00	18.00	26.00	38.33	53.00	66.66	14.33	22.33	29.33	40.33	56.33	68.66
Leucine	12.66	19.66	28.33	41.33	55.66	69.33	14.66	24.33	32.66	43.33	59.66	71.00
Serine	11.33	15.33	22.33	32.66	47.33	59.00	12.33	17.66	24.33	34.66	48.33	61.66
Histidine	11.66	17.00	25.66	36.00	52.66	64.00	13.66	20.33	28.66	38.33	54.66	66.00
Control	11.66	16.66	24.33	34.66	50.00	62.66	13.33	18.66	26.33	36.33	52.33	64.66

Sensitive isolate- $P < 0.0001$, Resistant isolate $P < 0.0001$

4. Discussion

Alternaria alternata is a pathogen which causes root rot disease to Fenugreek. The requirement of nutrients in the development of disease is variable in pathogens. The present study was an attempt to investigate the requirement of the nitrogen and amino acids as nutrient source (*in vitro*) for development of growth of pathogen so that to overcome disease development in the host. The study will enable the cultivar to know the possible sources of development of pathogen so that those can be avoided during cultural practices. There are many workers who studied physiology of fungi. Reddy *et.al* (1965) did physiological studies of *Alternaria ricini*, causative agent of *Alternaria* blight of castor bean. Lilly and Barnett, (1951); Hasija (1970), as well as Cochrane (1951), studied physiology of the fungi. Taber *et.al* (1968), made a comparative nutritional study of *Alternaria raphani*, *A. brassicae*, and *A. brassicicola* with special reference to *A. raphani*. Natrajan and Govindrajan (1974), studied on the physiology and control of *Alternaria*

cymopsidis, the incitant of blight disease of guar. Kumara and Rawal (2008), studied influence of carbon, nitrogen, temperature and pH on the growth and sporulation of some Indian isolates of *Colletotrichum gloeosporioides* causing anthracnose disease of papaya (*Carrica papaya* L). Naim, and Sharoubeem (1963), described Carbon and nitrogen requirements of *Fusarium oxysporum* causing cotton wilt. Vakeeshan (2014), studied some carbon and nitrogen sources for growth of *Cercospora beticola*. The pathogen studied here made excellent or good growth on nitrates of potassium and sodium, peptone and ammonium nitrate. A majority of fungi are known to utilize nitrate and ammonium nitrogen. A great majority of fungi are unable to utilize nitrite as nitrogen source at their optimum pH value which is generally below 7.0. Nitrites have been reported to be toxic on the pH range (Cochrane, 1958; Lilly and Barnett, 1951). Bais *et. al* (1970), studied effect of different carbon and nitrogen sources on the growth and sporulation of *Curvularia pallescens*. B.P. Singh (1976), made nutritional study, on two species of *Curvularia* causing leaf spot disease IV. Nitrogen nutrition while Thind

and Madan (1969), observed effect of various nitrogen sources on the growth and sporulation of *Cephaloecium roseum* causing pink rot of apple. Growth and nutrition study of pathogenic fungi was also supported by Rajdekar (1966), Sami Saad (1970) and Khilare (2011).

An attempt was made in the present study to find out amino acid requirement of *Alternaria alternata* in the cultural conditions. Tryptophan, Leucine and Histidine were stimulant to *Alternaria alternata* while Phenylalanine, Proline and Serine were inhibitory amino acids in present studies. Lewis (1957), studied amino acid nutrition of *Alternaria solani*. According to some workers, Lysine supported fair growth of *C. capsici* (Thind and Randhwa, 1957) and *P. indica* (Mandahar, 1965). Niharika Yadav and Supriya Sarkar (2014), studied production of L-Asparaginase by *Fusarium oxysporum* using submerged fermentation. Radhika Tippani and Girisham Sivadevuni (2012), worked on nutritional factors effecting the production of L-asparaginase by the *Fusarium* sp. Aspartic acid is a poor growth of *Zygorhynchus* spp., (Sarbhoy, 1965).

A. Imada (1973), studied Asparaginase and glutaminase activities of microorganisms. However glutamic acid, arginine and threonine have been reported to show excellent or good growth of many fungi such as *C. hibiscina* (Thind and Mandahar, 1965).

5. Conclusion

From above results it is quite clear that all nitrogen source used were stimulant while ammonium nitrate was most stimulant for the pathogen *Alternaria alternata*. The source selected among amino acids, leucine was the most stimulant of pathogen while phenylalanine, proline and serine are inhibitory. This observation was useful for cultivar while managing the disease.

References

- [1] A. Imada, S. Igarasi, K. Nakahama, M. Isona, "Asparaginase and glutaminase activities of microorganisms". *Journal of General Microbiology*, 76: 85-99, 1973.
- [2] A. K. Sarbhoy, "Nutritional studies on some member of Mucorales. VI. Utilization of different nitrogen compounds", *Path. Microbiol*, 28, 816-829, 1965
- [3] B.S. Bais, S.B. Singh and D.V. Singh, "Effect of different carbon and nitrogen sources on the growth and sporulation of *Curvularia pallescens*", *Indian Phytopathology*, 23: 511-17, 1970.
- [4] B.P. Singh, "Nutritional studies on two species of *Curvularia* causing leaf spot disease IV. Nitrogen nutrition. *Proc. national Acad: sci., India* B46, 421-424, 1976.
- [5] C.L. Mandahar, "Nutritional studies of *Pestalotia theae* and *Monochaetia indica*. I. Influence of different carbon sources on growth", *Proc. Nat. Acad. Sci.*, 36, 95-101, 1966.
- [6] C.V. Subramanian, "*Hyphomycetes*", *Indian Council of Agricultural Research*, New Delhi, India. 930pp, 1971.
- [7] H. L. Barnett and B.B. Hunter, "Illustrated Genera of Imperfect Fungi", Burgess Publication Company: Minneapolis, Minnesota, III Edition, 1972.
- [8] J. I. Pitt and A. D. Hocking, "Fungi and food spoilage", 2nd Edn. Blackie Academic and Professional, London, 1997.
- [9] J. Vakeeshan, K.B. Khare and B. A. Gashe, "Utilization of some carbon and nitrogen sources for growth of *Cercospora beticola* sacc., the causal agent of leaf spot disease of spinach beet" *International Journal of Food, Agriculture and Veterinary Sciences*, 4 (2), pp. 142-145, 2014.
- [10] Kumara K L W and Rawal R D, "Influence of carbon, nitrogen, temperature and pH on the growth and sporulation of some Indian isolates of *Colletotrichum gloeosporioides* causing anthracnose disease of papaya (*Carrica papaya* L)", *Tropical Agricultural Research and Extension* 11: 7-12, 2008.
- [11] K. S. Thind and M. Madan, "The effect of Various nitrogen sources on the growth and sporulation of *Cephaloecium roseum* Corda causing pink rot of apple (*Malus sylvestris* Mill.)", *Ibid*, 39B, 233-340, 1969.
- [12] K. S. Thind and C.L. Mandahar, "Trace element studies on some pathogenic fungi", In. *Proceedings of the Indian Academy of Sciences, Section B*, 68(1), pp 37-51, 1968.
- [13] K. S. Thind and H. S. Randhwa, "The influence of different sources of Nitrogen on the growth and sporulation on *Colletotrichum capsici*". *Proc. National. Acad. of Sci, India* B-27, 47-52, 1957.
- [14] M. N. Reddy., J. Subbaiah and A. Appa Rao, "Physiological studies of *Alternaria ricini*, causative agent of *Alternaria* blight of castor bean", *Phytopathology*, 55:1072, 1965.
- [15] M.S. Naim, and H. H. Sharoubeem, "Carbon and nitrogen requirements of *Fusarium oxysporum* causing cotton wilt", *Mycopathologia*, 22: 59-64, 1963.
- [16] Nilkanth K. Khandare, "Biochemical Changes in Carbendazim Sensitive and Resistant Isolate of *Alternaria Alternata* Causing Root Rot to Fenugreek (*Trigonella foenum graecum* l.)", *International Journal of Science and Research*, Volume 3, Issue 9, September. 1300 – 1303, 2014.
- [17] N.K. Khandare and S. S. Kamble, "Sensitivity of carbendazim against *Alternaria alternata* causing root rot of fenugreek," *Bioinfolet*, 10 (1B), pp.307 – 308, 2013.
- [18] N. K. Khandare and S.S. Kamble, "The diseases of fenugreek in Maharashtra - A survey" *Bioinfolet*, 10(1B), pp. 335 - 336, 2013.
- [19] N.R. Rajdekar, "The influence of nitrogen nutrition on growth and sporulation of *Alternaria solani*", *Mycopath. Mycol. Appl.* 29: 55-58, 1966
- [20] Niharika Yadav and Supriya Sarkar, "Production of L-Asparaginase by *Fusarium oxysporum* using submerged fermentation", *International Journal of Pharmaceutical Science Invention*, 3 (6), pp.32-40, 2014.
- [21] Radhika Tippani, Girisham Sivadevuni, "Nutritional factors effecting the production of L-asparaginase by the *Fusarium* sp", *African Journal of Biotechnology*, 11(15): 3692-3696, 2012.

- [22] R.A. Taber., T.C. Vanterpool and W.A. Taber, "A comparative nutritional study of *Alternaria raphani*, *A. brassicae*, and *A. brassicicola* with special reference to *A. raphani*. *Phytopathology*, 58: 609- 616, 1968.
- [23] R.W. Lewis, "Amino acid nutrition of *Alternaria solani*", *Phytopathology*, 47: 121-125, 1957.
- [24] S.M.Reddy, "Nitrogen requirment of five species of *Helminthosporium*", *Proc.Indian Acad.Sci.*, 74B,142-146,1971.
- [25] S. K. Hasija, "Physiological studies on *Alternaria citri* and *Alternaria tenuis*", *Mycologia*, 62: 289-295, 1970.
- [26] S. Natrajan & K. Govindrajan, "Studies on the physiology and control of *Alternaria cymopsidis* the incitant of blight disease of guar", *Indian. J. Mycol. pl. Pathol.*, 3: 33-39, 1974.
- [27] Sami Saad and D. j. Hagedorn, "Growth and nutrition of an *Alternaria* pathogenic to Snapbeans", *Phytopathology*, 60:903-906, 1970.
- [28] V.C. Khilare and Rafi Ahmed, "Effect of nutritional sources on the growth of *Fusarium oxysporum* f. sp. *ciceri* causing chickpea wilt", *International journal of Science and Nature*, 2(3) : 524 – 528, 2011.
- [29] V. G. Lilly& H.C. Barnett, "Physiology of fungi", McGraw Hill, New York 1951.
- [30] V. W. Cochrane, "Physiology of the fungi". John Wiley & Sons, N.Y. 524pp, 1958.

Author Profile



Dr. Nilkanth K. Khandare is a postgraduate in Botany and awarded Ph.D in plant pathology, from Shivaji University Kolhapur, (Maharashtra). He is assistant Professor of Botany and has 18 years teaching experience at Krantisinh Nana Patil College, Walwa,

Dist- Sangli, (Maharashtra), India.