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Changes in total proteins in normal and galled stem of *Coriandrum sativum* L. caused by *Protomyces macrosporus in vivo* and *in vitro*

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Abstract
Coriandrum sativum L. (Dhania) is one of the earliest spices known to mankind. It is grown in fields for green leaves and dry fruits. Coriander suffers severely from stem gall disease caused by the fungus *Protomyces macrosporus*.
Present study deals with the changes in total protein contents of healthy and diseased tissues of coriander both *in vivo* and *in vitro*. Total protein contents were estimated following the method of Lowry *et al.* (1951). Galled tissues of coriander showed high protein contents as compared to their normal counterparts both *in vivo* and *in vitro*.

Keywords: *Coriandrum sativum, Protomyces macrosporus, in vivo, in vitro*

1. Introduction
Coriander plants suffers from severe stem gall disease caused by the fungus *Protomyces macrosporus*. The disease is reported from several parts of India.
Several aspects epidemiology, histopathology, biochemistry and control of coriander gall caused by *P.macrosporus* have been studied by many scientists *viz*. (Tayal *et al*. 1981, Goyal *et al.*1983, Sharma and Sharma 2004 and Singhania *et al.* 2006) [1-4] also worked on occurrence of the disease and its effect on seed production. Jain *et al.* (1994) [5] recorded an increase in total soluble sugars and alpha amylase activity in galled tissues of coriander both *in vivo* and *in vitro*. Mishra *et al.* (2017) [6] worked on biochemical changes in coriander due to stem gall disease.
Proteins are the principal constituents of plants and animal life. Amino acids are known to play a key role in the metabolism of plants as they are the building blocks of proteins and are involved in several metabolic activities.
Pathogen induces a spurt in cellular activity in the infected tissue. Due to host pathogen interaction metabolism is shifted in favour of the pathogen. Protein and amino acid composition of various plants under pathogenic state has been worked out by a number of workers *viz*. (Prasad *et al.* 1976, Tandon 1985, Rao and Sridevi 1987, Tavernier *et al.* 2007, Ahmed *et al*.2013.) [7-11]
Infection of plants with pathogenic fungi is found to be associated with accumulation of certain nitrogenous material. (Allen 1954) [12]. The protein content increased in the diseased plants at the early stages of infection *viz*. cabbage infected by *Plasmodiophora brassicae* (Bhattacharya and Williams 1971) [13], Soyabean infected by *Phytophthora megasperma* (Lazarovitz and Ward 1982) [14].
Chaffei *et al.* (2004) [15] studied the effect of cadmium toxicity in nitrogen management of *Lycopersicon esculentum.* Dulermo *et al.* (2009) [16] studied amino acid changes in sunflower infected by necrotrophic fungi. Solomon and Oliver (2001) [17] noted an increase in nitrogen level of tomato leaf epoplast during infection by *Cladosporium fulvum.* Gupta and Naquvi (1979) [18] studied the changes in amino acid contents of coriander plants infected with *Protomyces macrosporus.*
The increased protein content at the early stages of infection may be attributed to increased catabolic reactions or decrease in proteolytic degradation or both. The pathogen itself may also contribute the proteolytic enzymes. (Kosuge and Gilchrist 1976, Solomon *et al.*2003 and Berger *et al.*2007). [19-21]
The present study provides a precise account of the total protein contents of healthy and diseased tissues of *Coriandrum sativum* infected by *Protomyces macrosporus in vivo* and *in vitro* conditions.

2. Materials and Methods
Total protein contents were estimated following the method of Lowry *et al.* (1951) [22]. Fresh material was used for analysis of normal and galled tissues *in vivo* conditions. Three stages of galled stem young, mature, old and normal and galled fruits were analysed. For *in vitro* biochemical estimations thirty days old normal and galled callus and dual cultures were used.
500 mg. of normal and galled plant material was extracted in 10 ml of 5%TCA (Trichloro acetic acid). The mixture was then centrifuged at 2000 rpm for 20 minutes. Supernatant was discarded. The residue was dissolved in 0.1N NaOH. 0.1 ml of this solution was taken and subsequently made up to 1.0 ml by adding distilled water.

The following reagents were prepared
A. Alkaline sodium carbonate solution (20% Na_2Co_3 in 0.1N NaOH)
B. Copper sulphate- Sodium potassium tartrate solution (0.5% of CuSo4.5H_2O in 1% sodium potassium tartrate) was prepared fresh.
C. Alkaline copper reagent - (50 ml of reagent A was mixed with 1.0 ml of reagent B just before use).

To 1.0 ml of dissolved residue 5.0 ml of alkaline copper reagent was added and allowed to stand for 10 minutes. To

this 0.5 ml of Folin ciocalteu reagent (diluted with equal volume of distilled water) was rapidly added and mixed thoroughly.

The optical density was measured at 750 nm in a spectrophotometer after 10 minutes. For blank ethanol was used. The amount of protein in the sample was calculated with a standard curve prepared from egg albumin. Protein was expressed in terms of mg/g fresh weight of the tissues.

3. Results and Discussions

Present studies revealed high protein content in galled tissues as compared to normal both *in vivo* and *in vitro* conditions (Table 1, Fig 1). Mature gall showed higher amount of protein as compared to young and old galls. In *in vitro* state dual culture showed high protein as compared to gall callus. However, maximum amount of total proteins were recorded in galled fruits.

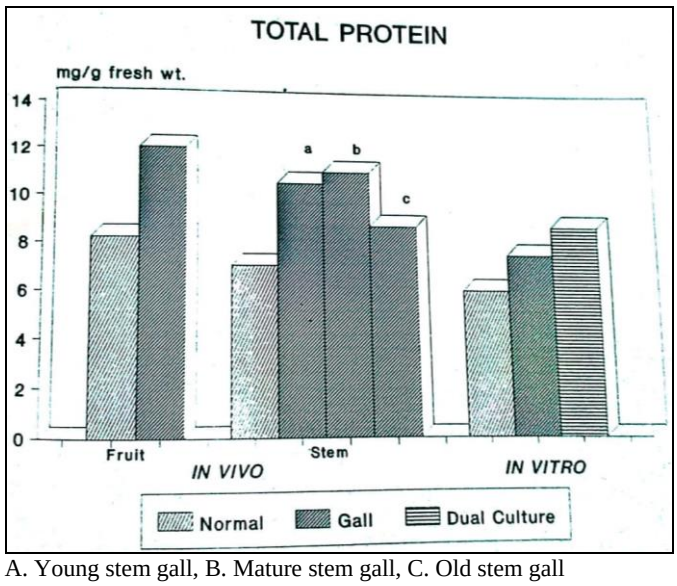
There are several reports indicating a net increase in total nitrogen content of various plants infected with fungi. (Rangaswamy and Natrajan 1966, Shekhawat and kothari 1971, Garg and Mandhar 1975 and Jain 1978) [23-26]. Increased protein levels were found to be associated with the diseased tissues as compared to normal ones was reported in various cases.(Novak and Galston 1955, Shaw and Colotelo 1961, Shekhawat 1980, Begnami 2010, Asgarpanha 2012 and Bhat *et al*.2014.) [27-32]

Higher activities of peroxidase, catalase, glucose-6-phosphate dehydrogenase and alpha amylase were resultant of more protein synthesis.

In addition, isozyme formation in the diseased tissues in case of peroxidase supported this view. Stahamann and Demorst (1972) [33] reported appearance of new is peroxidase on account of de novo protein synthesis in diseased tissues. Shaw (1963) [34] concluded that final outcome of host parasite interaction, particularly in case of obligate parasites, might depend on the ability of host to synthesize new proteins.

Table 1

S. No.	Type of tissue		Total Protein Contents (mg/g fresh wt.)
(A) *In vivo*			
1	Normal stem		7.0
2	Galled stem	(a) Young	10.5
		(b) Mature	11.0
		(c) Old	8.9
3	Normal fruit		8.0
4	Galled fruit		12.0
(B) *In vitro*			
5	Normal callus		6.0
6	Gall callus		7.5
7	Dual culture		8.0



A. Young stem gall, B. Mature stem gall, C. Old stem gall

Fig 2: Total protein contents of normal and galled tissues of *Coriandrum sativum in vivo* and *in vitro*

4. Conclusion

Total protein contents were found to be more in galled tissues as compared to normal both *in vivo* and *in vitro* conditions. Maximum protein contents were found in galled fruits and minimum protein contents were estimated in normal callus (Table 1, Plate 1).

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