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Induction of resistance in tea plants against *Sclerotium rolfsii* by application of amendments and associated changes in level of phenolic content

Indramani Bhagat

Department of Botany, Post Graduate Campus, T.U., Biratnagar, Nepal
**E-mail: drimbhagat@yahoo.com*

Abstract

Application of neem cake,oil cake and organic amendments including cow-dung, rabbit manure and chicken manure on four varieties of tea *Camellia sinensis* (L.) O. Kuntze were evaluated for induction of resistance against *Sclerotium rolfsii* Sacc. The emphasis was on the involvement of phenolics. It was observed that total phenol levels (total phenol and ortho-dihydroxy phenols) increased in treated inoculated tea root varieties with *S. rolfsii* than treated uninoculated tea root varieties. Oil cake and rabbit manure induced a rapid and distinct accumulation of phenolics in contrast to neem cake, cow dung and chicken manure.

***Key words*:** *Camellia sinensis*, total phenol, ortho-dihydroxy phenols.

Introduction

Tea is a hot beverage produced from the leaves of *Camellia sinensis* (L.).O.Kuntze. It is one of the important plant crops in Nepal. One of the important fungal pathogens, *Sclerotium rolfsii*, causing seedling blight disease in tea was found to be predominant in the nursery grown plants. The fungus is a soil borne rotting pathogen of very aggressive nature and causes considerable damage of young tea seedlings in the nursery which is very common in plains but rare in the hills.

S. rolfsii affects the lower stems and roots of tea seedlings at or near the soil line. During infection whitish mycelial growth of the fungus can be seen at the junction of the branch with the stem close to the soil level, which is the most favored point of attack. A dark brown lesion on a tea seedling's stem near the soil line is a very early indicator. With time, the disease progresses and a white mycelial web spread over the soil and the basal canopy of the plant, followed by the appearance of sclerotia of mustard seed size on the infected areas. In its advanced stage infection becomes prominent in the root system and subsequently the entire shoot withers and falls and finally the plants die. Seedling death usually occurs rapidly. The interaction between plants and their pathogens is complex and may be very specific to a given combination of the plant and the fungus. The biochemical mechanisms responsible for containment of fungal pathogens in the resistant interactions are undoubtedly multifold. Many biochemical changes occur in plants after infection, and some of these have been associated with the expression of defenses that are activated after infection (Chakraborty, 2005). Induction of resistance is one of the important methods, which is gaining worldwide importance and acceptance (Edreva, 2004). As polyphenols are the major constituents of tea plants, their role in the resistance mechanism was investigated after application of organic amendments. Changes in the levels of phenolic substances (total phenols and ortho-dihydroxy phenols) were determined in the untreated and treated varieties (TeenAli-17/1/54 and B-157) after inoculation with pathogen (*S. rolfsii*).

In the present investigation, an attempt was made to observe the effects of amendments of neem cake, oil cake, cow dung, chicken manure and rabbit manure in inducing resistance in tea plants against sclerotial blight disease has been demonstrated with special reference to the involvement in the levels of phenolic substances (total phenols and ortho-dihydroxy phenols) in the untreated and treated varieties (TeenAli-17/1/54,UP-3,K1/1,and B-157) after inoculation with pathogen (*S. rolfsii*).

Materials and Methods

Plant material

Four varieties of tea seedlings (UP-3, K1/1, TeenAli-17/1/54 and B-157) were grown in pots and were used for experimental purpose.

Fungal culture

Virulent culture of *Sclerotium rolfsii* Sacc was obtained from Immuno-Phytopathology Laboratory, Department of Botany, North Bengal University**.** This was originally isolated from Teen Ali-17/1/54 and after completion of Kock's Postulate, the organism was identified by Global Plant Clinic, Diagnostic and Advisory Service, CABI Bioscience UK and designated as Sr-1and its culture maintained at Immuno Phytopathology Laboratory, Department of Botanty, North Bengal University.

Maintenance of stock cultures

The fungus thus obtained was sub-cultured on PDA slants. After two weeks the culture has been stored under three different conditions (0°C, 4°C and at room temperature 28°C). The culture of *S.rolfsii* was examined at regular intervals to test its pathogenicity.

Inoculum preparation

The sand maize meal medium was prepared in the ratio of 3:1 (sand : maize). In the prepared sand maize meal medium fungal pathogen (*S. rolfsii*) was inoculated and incubated at 28°C for 7 days. The inoculum was mixed with sterile soil at the ratio of 1:8. Fungus soil mixture (10 g) was mixed with the top soil of earthen pots containing tea seedlings and kept for development of disease reaction.

Preparation and application of organic amendments

Pot culture experiment was conducted with different organic amendments treated in the tomato seedlings. The earthen pots were filled with autoclaved 1kg of soil. Mustard oil cakes were allowed to decompose for a week in a clay pot covered with polythene. 100 ml of decomposed oil cake solution, 100 g powdered neem cake, 100 g cow dung, 100 g chicken and 100 g rabbit manure were added in pots containing sterilized soil separately and watered.

After one week, four varieties of tea seedlings (TeenAli-17/1/54, UP-3, K1/1,and B-157) were grown in soil amended with neem cake, oil cake, cow dung, rabbit and chicken manure separately before inoculation with 100 g *Sclerotium rolfsii* and watered lightly. Then these pots were inoculated with the test pathogen multiplied on sand-maize medium and watered lightly. The seedlings were planted in the earthen pots without amendments but inoculated with the pathogen and were maintained as control. Each treatment consisted of 10 plants, in triplicate and the values are an average of 30 plants.

As polyphenols are the major constituents of tea plants, their role in the resistance mechanism was investigated after of addition of amendments. Changes in the levels of phenolic substances (total phenols and ortho-dihydroxy phenols) were determined in the untreated and treated four varieties after inoculation with pathogen (*S. rolfsii*) in two months.

Results and Discussion

Biochemical changes in tea seedling associated with induction of resistance in tea plants

Results have been presented in table 1. It revealed that total phenol content increased in treated plants following inoculation than untreated inoculated plants. It has also been observed that total phenol levels increased in treated inoculated tea root varieties with *S. rolfsii* than treated uninoculated tea root varieties. Level of total phenol increased by 3.12%, 4.69% in T-17/1/54 following treatments with neem cake and oil cake respectively, whereas, 2.86% and 5.71% increased in total phenol was noticed in neem cake and oil cake treated B-157 respectively after inoculation with *S. rolfsii* as compared to healthy untreated control. Level of ortho-dihydroxyphenol was also determined in these varieties (TeenAli-17/1/54 and B-157) after treatment with neem cake and oil cake following inoculation with *S. rolfsii*. Results (Table 1) revealed that ortho-dihydroxy phenol decreased in untreated inoculated tea root varieties in comparison to uninoculated healthy control. Ortho-dihydroxy phenol levels increased in treated roots following inoculation with the pathogen than treated healthy plants. Similar pattern was noted in case of both the varieties tested. It is interesting to note that the plants grown in soil amended with neem cake and oil cake could resist the pathogen and changes in the level of total phenols as well as ortho-dihydroxy phenol can be correlated with the development of resistance in susceptible plants following such treatments.

Table 1. Total phenol and ortho-dihydroxy phenol contents in tea varieties after treatment with Neem cake and oil cake following inoculation with *Sclerotium rolfsii*

Tea variety		Phenol content (mg/g)[a]		Ortho-dihydroxy phenol content (mg/g)[a]	
		Healthy	Infected	Healthy	Infected
T – 17/1/54	Untreated	2.5±0.04	3.6±0.02	2.1±0.02	1.2±0.04
	Neem cake	2.8±0.02	4.8±0.01	2.0±0.03	2.7±0.03
	Oil cake	2.7±0.03	5.2±0.04	2.2±0.04	3.0±0.02
B-157	Untreated	2.5±0.01	2.6±0.02	1.1±0.02	0.6±0.04
	Neem cake	2.7±0.02	3.6±0.03	1.4±0.03	2.1±0.01
	Oil cake	2.8±0.03	3.7±0.04	1.5±0.01	2.2±0.02

[a]Average of 3 replicates, ±Standerd error

Changes in the level of phenolics were also determined in two varieties of tea plants (UP-3, B-157 and K 1/1) grown separately in soil amended with cow dung, rabbit manure and chicken manure following inoculation with *S. rolfsii*. Results revealed that total phenol content decreased in untreated plants of two susceptible varieties (UP-3 and B-157) following inoculation with the pathogen in relation to healthy control, whereas the resistant variety (K 1/1) responded against inoculation with the pathogen. In this case total phenol and ortho-dihydroxy phenol content increased in comparison with untreated healthy control (Tables 2, 4).

Table 2. Changes in the level of total phenol and ortho-dihydroxy phenol contents in tea roots grown in soil amended with organic additives following inoculation with *Sclerotium rolfsii*

Tea variety	Treatments	Phenol content (mg /g)[a]		Ortho-dihydroxy content (mg /g)[a]	
		Healthy	Infected	Healthy	Infected
UP – 3	Untreated	3.0±0.04	2.1±0.02	2.1±0.04	1.5±0.01
	Cow dung	3.1±0.03	3.2±0.03	2.4±0.02	2.6±0.03
	Rabbit manure	3.5±0.02	5.6±0.01	2.3±0.04	2.5±0.02
	Chicken manure	3.3±0.94	3.5±0.02	2.2±0.01	2.4±0.03
B – 157	Untreated	3.5±0.02	2.6±0.02	1.1±0.02	0.6±0.04
	Cow dung	2.6±0.03	3.9±0.02	1.8±0.01	1.9±0.05
	Rabbit manure	4.1±0.04	4.7±0.01	1.9±0.04	2.1±0.03
	Chicken manure	4.6±0.02	4.9±0.03	1.7±0.03	1.8±0.02
K-1/1	Untreated	2.8±0.02	6.7±0.03	0.9±0.01	2.3±0.03
	Cow dung	5.6±0.01	8.4±0.02	1.7±0.03	2.4±0.02
	Rabbit manure	4.4±0.03	8.5±0.04	3.0±0.04	4.5±0.05
	Chicken manure	4.1±0.02	4.9±0.04	1.7±0.01	2.6±0.02

[a]Average of 3 replicates, ±Standerd error

It was observed that total phenol levels increased in all the varieties tested following treatment with organic amendments. Rabbit manure responded markedly and in this case total phenol increased following inoculation with the pathogen in relation to treated healthy as well as untreated healthy control. Level of ortho-dihydroxy phenol increased markedly in soil amended with cow dung in case of UP-3 and B-157, whereas level of ortho-dihydroxyphenol increased in plants (B-157 and K 1/1) grown in soil amended with rabbit manure following inoculation with the pathogen.

In the experiments of different organic amendments were treated in different tea seedlings to observe growth promotion and percentage increase in shoot length in healthy and treated tea seedling varieties. Results revealed that the growth promotion and percentage increase in shoot length in tea seedlings treated with neem cake and oil cake were more in seedlings inoculated with *S. rolfsii* (after treatment) in comparison to the treated uninoculated tea seedlings. Total phenol and orthodihydroxy phenol contents were also increased in treated inoculated tea varieties with neem cake and oil cake. This is due to the *decomposition* of organic matter that helps in alteration of the physical, chemical and biological conditions of the soil and the altered conditions probably reduce the inoculum potential of soil-borne pathogens (Singh, 1983). In addition, the practice also improves soil structure, which promotes root growth of the host. Various antibiotics and phenols are released during decomposition, which induces resistance in the root system and increases over all growth of the plant. In case of organic amendments, *i.e*., cow dung, rabbit manure and chicken manure, the growth promotion as well as percentage increase in shoot length in different tea seedlings varieties were higher in uninoculated tea seedlings than the treated inoculated ones. But total phenol and orthodihydroxy phenol contents were higher in treated inoculated tea seedling varieties as in oil cake and neem cake treated tea seedling. This observation may correspond to the fact that microorganisms being present in soil phenol contents in treated inoculated tea seedling varieties with cow dung, rabbit manure and chicken manure. Many root pathogens have been successfully controlled by ploughing organic materials in the soil (Mehrotra & Tiwari, 1976; Chakraborty & Purkayastha, 1984; Malajczuk *et al.*, 1984; Tu, 1984; Ghaffar, 1993; Chakraborty, 2005). Increased aerobic activity of micro-organic increased the release of CO_2 which inhibits the growth of pathogen and helps to build up the crop health. Theses micro-organisms also release some enzymes, which help to improve the crop health and check the growth of pathogenic fungi (Anonymous, 2002). Similarly, the organic amendments also inhibited soil borne diseases either by antibiosis or by enhancing the population of selective antagonistic rhizosphere micro-flora which resulted in the reduction of population of soil-borne plant pathogens (Nargund *et al*., 1984; Chakrabarti & Sen, 1991; Hadar & Golodeeki, 1991; Kulkarni & Kulkarny, 1995).

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