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Defense-related protein activity in Tea (*Camellia sinensis* (L.) O. Kuntze) against 'red rust' disease-causing organism *Cephaleuros parasiticus* Karst.

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Abstract
'Red rust' disease in tea plants was caused by the green alga *Cephaleuros parasiticus* Karst. It grows as an endophyte in tea plants which causes severe destruction to leaves than stem regions. The objective of this work was to analyze different pathogen-related (PR) enzymatic secretion by the plant and the pathogen amid the illness assault. A total of two isolates and one standard culture of *Cephaleuros parasiticus* namely VCP27, MCP11, and UTEX2412 was used in this study. Carbohydrases, defensive enzymes, phenolic enzymes, and oxidative enzymes were studied with various incubation periods. All the enzymes exhibited the highest activity at 30 min of the incubation period, however, CMCase of carbohydrases has shown optimal activity at 60 min of incubation. The study has shown that *C. parasiticus* was adept of generating numerous pathogenic enzymes which demonstrated a maximal activity at 30th day harvest with the exception of protease enzyme which reveals the optimal activity at 40th day of harvest. The above-mentioned enzyme activity was studied both in non-infected and red rust disease infected tea leaves. These findings confirm the *C. parasiticus* capability of producing different enzymes such as carbohydrases, pectinase, protease, chitinase and guarded chemicals alongside phenolic and oxidative proteins. Based on the enzyme activity, the harmfulness of the pathogen was discovered and discussed. The study is very much useful to understand the host-pathogen interaction and to produce the red rust disease resistant crop.

Keywords: Red rust, *Cephaleuros parasiticus*, PR proteins, defensive enzymes, host-pathogen interaction, amylase, protease, poly phenol oxidase

1. Introduction
Tea productions anticipate a significant movement in the economy of many underdeveloped and developing countries in Asia and Africa. In India, almost 500,000 ha of tea estate is utilized for development and commercialization. Among them, around 97,600 ha is built-up by 123,000 small-scale industrialists. Also, there is a countless number of organizations owning substantial territories of tea. More than 50% of the workers are women in the Indian tea industry. The business produces good income and job for 10 million individuals in Asian and African nations.

Due to its abundant growing appearances, it provides stable agroclimate to nurture many microbes. The microbial disease prevalence is very high in monsoon months (June to November) and it reaches widespread growth (Baby 2001) [2]. In Southern India alone the crop loss was estimated of more than 50% beside it is severely affecting the quality of made tea (Baby 2001) [2]. Among the tea sicknesses, foliar infections assume the significant job, it specifically influences the wellbeing and yield of the plant (Muraleedharan and Chen 1997) [27]. Red rust is the leaf disease caused by the green alga *Cephaleuros parasiticus* Karst. The zoospores of the pathogen spread through wind and splash waters. It directly affects mature leaves for its growth and development. In a single leaf, it may be numerous or few, crowded or scattered spots will be there (Vidhysekaran and Parambaramani 1971) [47].

Most of the fungi, nematodes, and parasites of higher plants have the ability to breach the host cell surface directly. *Cephaleuros* cause infection through stomata; the zoospores of the alga enter by stomatal openings of the tea leaves by rainwater (Chowdary and Jose 1978) [11]. Tea plant itself has active defense mechanisms, it allows the resistance to different kinds of unfavorable conditions like an insect bite and other microbial attacks (Jackson and Tailor 1996) [16]. All defense-related compounds are proteinaceous in nature. That include enzymes such as β-1,3-glucanases and chitinases, oxidative compounds like peroxidases and polyphenol oxidases, phenolic compounds like Phenylalanine Ammonia Lyase (PAL) and Tyrosine Ammonia Lyase (TAL), inhibitors of proteases, α-amylases, and lectins (Ryan 1990) [41].

Most of the plant pathogens were from the fungal background and majority of plant defense proteins are the derivatives of Chitinase (PR3) and β-1, 3-glucanase (PR2). This will arrest the pathogen from its constant expansion. These enzymes directly affect the cell wall and carbohydrate metabolism of the fungal pathogen. Due to the arrest of plant defense, the pathogen appears as necrotic spots (lesion) on the leaves. Plant pathogen also has the ability to degrade the cell wall of plants by producing some cellulolytic and pectinolytic enzymes during the host-pathogen interaction and these enzymes are called as pathogenic enzymes (Valueva and Mosolov 2004) [46]. The fungal pathogen *Cercospora theae* has produced a wide range of enzymes to depolymerize the significant component involved in structural integrity of the plant cell wall (Mythili Gnanamangai *et al.*, 2011) [28]. In the present study, an attempt was made to study the activity of pathogen-related (PR) proteins produced from the tea plants after infection with 'red rust' disease and the different cell wall degrading enzymes produced by the alga *Cephaleuros parasiticus* Karst, under laboratory conditions.

2. Methodology

2.1 Collection of Leaf samples

The 'red rust' disease infected leaves were collected from different tea growing districts of Tamil Nadu (Valparai (VCP27), Coimbatore District), Kerala (Munnar (MCP11) District) and Karnataka (Koppa District). Based on the disease susceptibility UPASI 7 (United Planters Association in Southern India) clone leaves have been collected for study (Ponmurugan *et al.*, 2010) [37]. The samples were collected in the month of February 2010. Leaf samples were compactly packed with polythene bags and stored 4ºC for prolong use.

2.2 Isolation of *Cephaleuros parasiticus* from collected leaf samples

The pathogen *Cephaleuros parasiticus* was isolated from the collected leaves by using Modified Bristol's medium (Yeast Extract 250mg, $CaCl_2.2H_2O$ 25mg, $MgSO_4.7H_2O$, 75mg, K_2HPO_4 75mg, KH_2PO_4, 175mg, NaCl 25mg and Trace metal mix ($Fecl_3$ 0.3mg, $MnSO_4.4H_2O$ 0.3mg, $ZnSO_4.7H_2O$ 0.2mg, H_3BO_3 0.2mg, $CuSO_4.5H_2O$ 0.06mg) 1 ml per litter media) (Park et.al. 2012) [35]. The *Cephaleuros virescens* culture has been procured from the University of Texas, Austin, USA (UTEX2412) as a standard culture.

2.3 Extraction of enzymes from healthy and infected leaf samples

2.3.1 Preparation of Acetone powder (Mahadevan and Sridhar 1974) [21]

5gm of leaves was weighed, cut into little pieces and grounded with chilled acetone (-20ºC) for 5 minutes. After filtering the crude extract, the moisture was removed repeatedly by washing with chilled acetone and diethyl ether. The resultant content was air-dried using the suction pump for 1 hour. The final powdered product was stored in a sealed container and kept in -20ºC for prolong use without any loss of enzyme activity. 0.1 gm of the acetone powder was revived with 5ml of 0.1 M chilled phosphate buffer at 4ºC for 10 minutes. The sample was centrifuged at 2000 g for 30 min. at 4ºC to expel the cell flotsam and jetsam. The supernatant was then filter sterilized and used as the enzyme source.

2.3.2 Extraction of enzymes from the pathogen

The algal isolates were cultivated in modified Bristol's agar plates for 45 days from which, 5mm disc of were taken. The discs were inoculated and cultivated into freshly prepared modified Bristol's media with the replacement of indigenous carbon and nitrogen sources in the medium. To evaluate cellulase, amylase, Invertase, and pectinase the corresponding carbonaceous substrates like carboxymethyl cellulose (CMC), starch, sucrose, and pectin were added in the medium at 1.5% of concentration. The protease action was estimated by adding casein in the media by replaced with its indigenous yeast extract (2.94 mM/Lit.) as a nitrogen source. Chitinase action was estimated by adding chitin as the substrate in the medium (1.5%). β-1, 3 glucanases was assayed by adding laminarin (1%) as the substrate. The culture flasks were incubating in a temperature controlled light chamber (23°C, 3000 lux) for 14 hours light and 10 hours dark. The enzyme activity was evaluated at 10 to 60 days of intervals. The culture filtrate was centrifuged at 10,000 rpm for 5 minutes and resulting supernatant was used as the enzyme source.

2.4 Activity of Carbohydrases

The activity of CMCase / β 1-4-endoglucanase, amylase, Invertase, and pectinase was measured from the VCP27, MCP11, and UTEX2412 isolates. According to Khare *et al.*, (1995) [20], Sinha *et al.*, (1995) [44], Zaldivar *et al.*, (2001) [48] using carboxymethyl cellulose, starch, sucrose and pectin as a substrate, the reducing sugars developed was calculated by using dinitrosalicyclic acid method (DNS). The reaction mixture was prepared as per (Okunowo *et al.*, 2010) [33] and the action was resolved at various time intervals ranging from 15 to 120 min. Absorbance was read at 540 nm using (U-2900E, Model No. 2JI-0003, UV-visible spectrophotometer, Hitachi, Tokyo, Japan). The release of reducing sugar concentration was calculated using glucose as the standard. Similarly, amylase and invertase activities were resolved using starch and sucrose as their substrate respectively and the absorbance was measured at 540 nm for estimation of reducing sugars according to Narayanan and Shanmugasundaram (2004) [29] The pectinase activity was estimated using D-Polygalacturonic acid as the standard for the enzyme source obtained from the pathogen (Miller 1959) [25].

2.5 Activity of defensive Enzymes

2.5.1 Protease

The protease activity was assayed by using the method of Srivastava and Prasad (1989) [45]. One micromole aromatic residues released from the enzyme source with Folin phenol reagent is measured at the absorbance of 660 nm.

2.5.2 Chitinase

2.5.2.1 Preparation of colloidal chitin

Colloidal chitin was prepared from chitin flakes (Himedia Laboratories, Mumbai, India) by using the method of Mathivanan *et al.*, (1998) [22]. Chitin flakes (100 grams) were crushed into powder using mortar and pestle (Borosil Glasswares, Mumbai, India). 100 ml of 10N HCl was added slowly to the flask and add 30 ml of 50% cold ethanol. The crude sample was kept it in a shaker for 24 hours at 150 rpm. Centrifuge the crude sample at 10, 000 rpm for 15 minutes and collect the resulting pellet. pH was adjusted to 7 using double distilled water. The subsequent blend was freeze-dried (Lark, India) and kept in 4ºC for further use.

2.5.3 Chitinase assay

3 ml of the enzyme solution was mixed with 10 ml of 50 mM sodium acetate buffer (pH 5.2) and incubated in water bath

40ºC for 0,30, 60, 90 and 120 minutes. Further 1.2ml of dinitrosalicylic acid (DNS) (Himedia Laboratories, Mumbai, India) was added and kept it boiling water bath for 5 minutes. 40% Rochelle salt (sodium potassium tartrate (Himedia Laboratories, Mumbai, India)) was added then, cooled up to room temperature and centrifuge at 2000 rpm for 5 minutes. The absorbance was measured with resulting supernatant at 575nm.

One unit of chitinase was characterized as the sum protein of compound, which produces one miniaturized scale mole of N-acetylglucosamine in 1 ml of response blend.

2.5.4 β - 1, 3 Glucanase

The enzyme activity was assayed calorimetrically by using dinitrosalicyclic acid method (Pan *et al.*, 1991) [34]. The reaction mixtures contain 1.5 ml of 1% laminarin and 1.5 ml of enzyme source and incubate this mixture at 40°C for 30 minutes. The reaction was arrested by adding 2 ml of dinitrosalicyclic acid and places this in a boiling water bath for 5 minutes. The subsequent response blend was cooled with an ice bath for 5 minutes and included 2 ml of 4% Rochelle salt (sodium potassium tartrate)

2.6 Phenylalanine Ammonia Lyase (PAL) and Tyrosine Ammonia Lyase (TAL)

0.5 g of acetone powder was dissolved in 25 ml of Tris-HCl buffer (0.05M) pH 8.8 was kept in shaking condition for 30 minutes and filter using filter paper. The resulting filtrate was spun at 10,000 rpm for 10 minutes. Followed by NH_4SO_4 was included and again centrifuged at 10,000 rpm for 35 minutes. The supernatant was used as enzyme source. The total 7 ml reaction mixture contains 2 ml of enzyme source, 2ml of the substrate (0.1% of L-Phenylalanine / Tyrosine) and 3ml of Tris-HCl pH 8.8. It was kept hatching for 3 hours and the response was ceased by the expansion 5N HCl. The mixture was equally diluted with di-ethyl ether at 3 times. The sediment desiccated and dissolved in 3ml of 0.05N sodium hydroxide. Enzyme activity was measured at 268 nm for PAL and 330 nm for TAL using UV spectrophotometer (U-2900E Hitachi, Tokyo, Japan). cinnamic acid and p-coumaric acid was used as standards for PAL and TAL activities (Narayanaswamy 1980) [30].

2.7 Peroxidase (PO) and Poly Phenol Oxidase (PPO)

0.5g of acetone powder was soaked in sodium phosphate buffer pH 6.8 and kept incubation for 24 hours under refrigeration temperature and pass through clean and dry filter paper. The filtrate was directly used as enzyme source. The total 5ml reaction mixture contains 1ml of 125µmoles of sodium phosphate buffer pH 6.8, 1ml of 50 µmoles of pyrogallol, 1ml of 50 µmoles of H_2O_2 (for peroxidize assay alone not for polyphenol oxidase) and 2ml of enzyme source. The response was permitted to persist for 5 minutes and it was halted by the expansion of 0.5ml of 5% H_2SO_4. Enzyme activity was measured at 420 nm PO and PPO were assayed by using the method reported by Patra and Mishra (1979) [36].

2.8 Catalase

Catalase activities in healthy and infected leaves were assayed using the modified method of Kar and Mishra (1976) [18]. 1g of acetone powder was soaked in sodium phosphate buffer pH 6.8 and kept incubation for 3 hours and filter through filter paper. The filtrate was used as an enzyme source. The total 5ml of the reaction mixture contains 1ml of 300 µmoles of sodium phosphate buffer pH 6.8, 1ml of 100 µmoles of H_2O_2 and 1ml of the enzyme source. Incubate for one minute and the enzyme action was arrested by adding 10 ml of 2% (v/v) H_2SO_4. Titrate the mixture against a 0.1M solution of $KMNO_4$. It produces purple color and the color persists up to 15 seconds.

2.9. Enzyme activity

Catalyst action for carbohydrases was estimated by micromole of sugar discharged per min. against glucose as a standard. One unit movement is the measure of protein discharging 1 pmole of decreasing sugar from the substrate (CMC, Starch, Sucrose, Pectin, and Chitin). Protein movement for phenolic catalysts was characterized as the one unit action. It is the measure of chemicals (PAL or TAL) discharged 1 µmole of the basic compound (Cinnamic corrosive or p-Coumaric corrosive) from the substrate (Phenylalanine or Tyrosine). One unit of peroxidase or polyphenol oxidase movement was characterized as the measure of purpurogallin framed which expanded the absorbance by 0.1 min' under test condition. One unit of catalase action is resolved as the measure of catalyst that separates 1 µmole of H_2O_2 every moment.

3. Results

3.1 Activity of Carbohydrases in *C parasiticus* isolates

The results of CM Case activity showed that all the isolates exhibited its activity during 10th, 20th, 30th, 40th, 50th and 60th day of enzyme harvest. The maximal activity was obtained at 20th day enzyme harvest as shown in which exhibited significant variation at (p<0.05). In healthy and red rust disease infected leaves, CMCase activity was determined and found to be high in infected leaves at (p<0.05) and exhibits significant variation on comparing with healthy leaves.

The isolates of *C. parasiticus* exhibiting amylase activity at various intervals of enzyme harvest were determined. The amylase activity was high at p<0.05 on the 30th day of harvest In healthy and red rust disease infected leaves, CMCase activity was determined and found to be high in infected leaves at (p<0.05) and exhibits significant variation on comparing with healthy leaves.

The Invertase activity from the *C. parasiticus* isolates produces maximal activity at the 30th day of harvest Invertase action was observed to be an increment in sound leaf when contrasted with the tainted leaves at (p<0.05). This was the only enzyme present low in infected leaves when compared to the defensive enzymes. It clearly indicates that the pathogen did not use sucrose for its metabolic growth and development. The optimum activity of pectinase was obtained in the 30th day of harvest The pectinase activity was very low in *vitro* grown *C. parasiticus* when compared to the *in vivo* activity. The pectolytic activity in red rust infected leaves was increased more than two-fold than that of healthy leaves. Invertase action was observed to be an increment in sound leaf when contrasted with the tainted leaves at (p<0.05).

3.2 Activity of chitinase activity

As the pathogen was coming from the algal background, the pathogen itself has the ability to produce chitinase under *in vitro* conditions. It shows the maximum rate of chitinase production at 40th day of harvest except for MCP11, it gives the optimum activity at the 30th day of harvest The chitinase activity was found to be low in healthy leaf when compared to the tained leaves at (p<0.05). In plant defense mechanism chitinase is one of the major defensive enzymes. In the plant protection system, chitinase is one of the significant guarded

catalysts. Majority of the plant pathogens are from the fungal background because this reason plant itself produces chitinase enzyme and it digests the cell wall of the fungal pathogen. The similar action happened on the algal pathogen.

3.3 Activity of Phenylalanine Ammonia Lyase (PAL) and Tyrosine Ammonia Lyase (TAL)

C. parasiticus infected leaves exhibit the rise of PAL activity when compared to healthy leaves. The significant raise and decrease of the PAL action in infected and healthy leaves were noticed in at (p<0.05). As like PAL activity, TAL activity also exhibits more than that of healthy leaves. The occurrence of TAL activities in the healthy and infected tissues is not significant at (p<0.05).

3.4 Activity of Peroxidase (PO), Poly Phenol Oxidase (PPO) and catalase

Peroxidize, polyphenol oxidases, and catalases are the oxidative defensive enzymes present in the plants. These three enzyme activities in infected tea leaves are increased to two-fold when compared to the activities of healthy tea leaves The varieties in the oxidative catalyst exercises were altogether higher in unwell tissues at p<0.05.

4. Discussion

The present work was carried out to study the activity of defense-related proteins in red rust disease infected tea plants by comparing activity with healthy tea plants.

Since chlorophyll is the primary pigment in the algae, it can able to synthesize starch molecules for its requirement of carbon nutrition. This is the reason that, more accumulation of sugar molecule in the infected leaf tissues. The dark metabolisms of algae are essentially similar to that of non-photosynthetic organisms (Danforth 1962) [13].

Glucose, fructose, galactose, and sucrose are the carbon source that was effectively used by the green alga was studied by Neish (1961) [31] and Samejima and Myers (1958) [42]. The sugar utilized by the algae was specifically for an active structurally specific energy process (Ayhan Demirbas 2001) [1]. The increase of fructose content in the infected leaves was due to the fulfillment of algal filaments in the plant's cells. The general increase of total soluble sugar content in the infected tissues may be due to the accumulation of algal fructose.

Most of the plant pathogens like *Curvularia* sp., *Fusarium solani,* and *Myrothecium* sp. are able to produce CMCase (Banerjee 1990 [5], Moreira *et al.*, 2005 [26], Nitharwal *et al.*, 1991) [32]. This enzyme will cleave the cellulose present in the plant cell wall especially in secondary cell walls have shown that suggesting CMC as the substrate for the Cellulose is the polymer glucose units connected with β 1,4 glycosidic linkages. The cellulase activity was marginally higher in *C. parasiticus* infected tissues than healthy tissues. Cellulose content was observed to be high in parasitic alga infected plant tissues because of the algal cell wall containing cellulose content (Baldan *et al.,,* 2001) [4]. Activities of the CMCase on comparing between healthy and infected leaves were observed to be significantly high which were a contrast to work of Moreira *et al.,* (2005) [26] in which plant proteinases had been implicated in the inhibition of CM Case, other cellulases and hemicellulase production by phytopathogens.

Even though amylase has no significant role in the pathogenesis, during the disease incidence of the parasitic algae amylase content will be increased. Starch is the reserve food material for green algae (Meeuse 1962) [23].

The obtained results for amylase activity were in agreement to the study of Singh *et al.,* (2009) [43] who reported that the activity of amylase was irrespective to the cultivars and stage of infection in leaf blight disease caused *Hordeum vulgare* in barley plants, indicating that amylase had no significant role in the mechanism of pathogenesis. Similar results were observed in the present study on determining the amylase activity in healthy and infected leaves which doesn't exhibit any statistically significant variation.

The up control of invertase action has all the earmarks of being a typical reaction to different biotic and abiotic stresses (Roitsch *et al.,* 2003) [40]. They additionally demonstrated that an expansion in extracellular invertase action prompts converse regulation of photosynthesis and starches amid pathogen contamination. A role for invertase in the age of hexoses which supply vitality for resistance responses as well as may go about as signs initiating barrier quality articulation was all around depicted by Singh *et al.,* (2009) [43].

The algal cell wall itself has pectin molecules as like higher plants (Dainty *et al.,* 1960) [12]. Here, *C. parasiticus* tainted tissues have more gathering of pectic substances because of the nearness of gelatin content in the phone mass of the parasitic alga. Pectic proteins that can part the β 1, 4 bonds between the galacturonic corrosive moieties in the pectic division of the cell divider remain the main compound affirmed to cause plant tissue maceration. The above results on pectinase activity in *Cercospora theae* were in agreement with Bahkali *et al.,,* (1997) [3] and Heale and Gupta (1972) [15] who reported similar findings in the in vitro and in vivo of some fungi causing leaf spot and soft rot diseases like *Fusarium oxysporum, Rhizoctonia Solani, A. macrospora and P. debaryanum*. The lytic enzymes like chitinases reported in the present study were suggested to have hydrolytic action and hence degrades the cell wall of many pathogenic fungi (Mathivanan *et al.,* 1998) [22].

Chitinases are well-known to be induced in many plants in responses to infection with fungal pathogens and were correlated to induced resistance by Ji and Kuc (1996) [17]. Movement of extracellular pumAe protease from *U. maydis* by developing in YNB medium containing ammonium sulfate demonstrated particular movement that started to increment after 12 h of development and achieved its greatest action after 24 h was accounted for by Mercado-Flores *et al.,* (2003) [24]. Proteolytic enzymes have a potential importance in Plant pathogenesis by disrupting the host plant metabolism and initiating disease process. A few proteases are critical in the development of spores, germination of spores, in the pathogenesis of a few microorganisms and in post-translational direction were accounted for by Rendueles and Wolf (1988) [39]. In agreement to the above studies, our results showed higher protease activity by the pathogen and diseased plants.

Significantly high PAL activity in the incompatible interactions was also demonstrated in woody kiwifruit vines (Reglinski *et al.,* 2001) [38] and tea plants (Chakraborty and Chakraborty 2005) [8]. The movement of TAL is not very much shown in dicots (Reglinski *et al.,* 2001) [38]. TAL movement was unquestionably lower than PAL in the present discoveries like the outcomes got in wheat tissues by Guerra *et al.,* (1985) [14]. Numerous investigations have exhibited the signs of phenolic mixes in plant guard instrument.

In general, plant phenolics have a diverse range of biological activity, depending on their structure, the degree of polymerization, stereoisomeric differences, etc. Given all the above properties, it is obvious that plant phenolics play an

important role in defense (Chakraborty and Chakraborty 2005) [8]. In the present study, an increase in phenolic enzyme activity was observed during host-parasite interaction disease attack which in turn affects the contents of defensive phenolics. Like the present investigate oxidative chemicals, past works likewise shown that these oxidative catalysts are engaged with the protection job (Chakraborty *et al.*, 2002) [7] with the opposition in numerous plants (Chits and Rajamani 2010) [10].

An immediate response of plants to injury, in most cases, is the accelerated accumulation of oxidative enzymes required for scavenging toxic radicals (Kessler and Baldwin 2002) [19]. Plants were accounted for to have proficient cancer prevention agent protection framework because of the nearness of pathway for catalase and peroxidase (Saffar *et al.*, 2009). On the other hand, Bi *et al.*, (1997) reported insect pest damage in cotton cultivars caused activation of several oxidative enzymes, including peroxidase, diamine oxidase, and lipoxygenase within the tissue. Consequences of the present investigation firmly bolster the above perception of Bi *et al.*, (1997) [6].

Chatterjee and Ghosh (2008) [9] studied the biochemical changes in mesta plants infected with yellow vein disease. It has been accounted for that distinctive compounds like catalase, corrosive phosphatase, peroxidase, esterase, polyphenol oxidase and superoxide dismutase which uncovered lower exercises of catalase, corrosive phosphatase, and peroxidase catalysts improved exercises of esterase, polyphenol oxidase and superoxide dismutase in unhealthy plants when contrasted with solid ones.

Table 1: Clonal Susceptibility of tea plants against red rust disease in Annamalai hills.

S. No.	Planting districts	Clonal susceptibility*		
		High	Moderate	Low
1.	Annamalai (2090 ha)	UPASI-3 UPASI-8 UPASI-9 UPASI-27 TRI-2024 BSS-1	UPASI-2 UPASI-12 UPASI-13 UPASI-15 UPASI-26 TRI-2023 CR-6017 ATK-1	UPASI-1 UPASI-11 UPASI-14 UPASI-17 UPASI-18 TRI-2025

*UPASI 1-27 – United Planters Association of Southern India (clone number)
*TRI - Tea Research Institute India, (Clone number)
*BSS - Biclonal Seed Stock (Clone number)
*CR - Crigmore (Clone number)
*ATK - Athikunna (Clone number)

Table 2: Activity of Pathogen Related Proteins in Tea leaves after infection with red rust disease*.

S. No.	PR Proteins	Specific Activity of PR proteins in U/mg						
		UPASI – 9		UPASI – 2		TRI -2025		
		Healthy Leaves	Infected Leaves	Healthy Leaves	Infected Leaves	Healthy Leaves	Infected Leaves	CD at P=0.05
Carbohydrases								
1.	CMCase	54.21±0.06	55.93±0.22	45.17±0.14	47.33±0.19	34.12±0.21	35.26±0.16	0.80
2.	Amylase	50.55±0.16	55.61±0.05	40.07±0.23	43.57±0.27	35.72±0.17	37.01±0.31	1.09
3.	Invertase	52.38±0.14	48.22±0.04	43.74±0.25	44.06±0.13	34.86±0.26	35.21±0.18	0.78
4.	Pectinases	17.52±0.19	36.29±0.21	33.62±0.14	48.27±0.24	39.18±0.21	53.04±0.26	0.84
Defensive Enzymes								
5.	Protease	93.21±0.10	103.5±0.10	104.18±0.22	113.36±0.12	106.46±0.21	117.58±0.16	0.32
6.	Chitinase	25.89±0.07	39.37±0.02	42.41±0.18	50.13±0.29	48.37±0.18	53.76±0.21	0.23
7.	β-1,3 Glucanase	55.76±0.13	72.95±0.04	67.30±0.23	74.16±0.14	68.84±0.20	73.07±0.32	0.19
Phenolic Enzymes								
8.	PAL	39.04±0.34	59.57±0.24	53.46±0.18	66.17±0.30	58.56±0.22	65.73±0.27	2.60
9.	TAL	30.85±0.30	58.88±0.31	49.84±0.25	60.63±0.17	54.92±0.34	59.18±0.36	2.40
Oxidative Enzymes								
10.	PO	0.084±0.004	0.110±0.001	1.08±0.016	2.03±0.023	1.84±0.026	2.15±0.020	0.02
11.	PPO	0.082±0.003	0.055±0.022	1.83±0.027	2.04±0.018	2.46±0.034	2.57±0.027	0.12
12.	Catalase	98.17±0.06	76.96±0.06	113.57±0.31	116.38±0.19	117.06±0.24	117.38±0.27	0.58

* - Average of three trials in triplicate as mean with standard deviation
Similar alphabets in a row denote the differences are statistically not significant and vice versa at $P<0.05$

Table 3: Carbohydrases activity of *Cephaleuros parasiticus* at different days of intervals under *in vitro* condition*.

S. No.	Isolates	Specific Activity of Amylase in different days (U/mg)					CD at P=0.05
		10	20	30	40	50	
Amylase							
1.	VCP27	21.92±0.36	25.61±0.13	34.71±0.45	30.16±0.02	26.84±0.29	0.83
2.	MCP11	19.56±0.92	22.80±0.18	36.58±0.21	32.94±0.27	28.63±0.31	0.37
3.	UTEX2412	22.21±0.06	25.72±0.35	33.75±0.17	32.16±0.04	30.94±0.49	0.80
CMCase							
1.	VCP27	3.39±0.4	4.42±0.24	4.2±0.17	3.41±0.12	2.67±0.13	0.63
2.	MCP11	2.67±0.24	3.85±0.05	3.12±0.19	2.81±0.13	2.30±0.11	0.41
3.	UTEX2412	2.27±0.03	3.37±0.09	2.70±0.29	2.31±0.06	2.01±0.05	0.35
Invertase							
1.	VCP27	21.41±0.65	25.6±0.15	34.74±0.38	30.03±0.27	25.43±0.28	1.02
2.	MCP11	19.14±0.36	22.81±0.20	41.13±0.05	36.42±0.11	32.10±0.13	0.47
3.	UTEX2412	21.59±0.27	25.72±0.23	34.60±0.14	30.11±0.07	24.12±0.14	0.52
Pectinase							
1.	VCP27	2.36±0.36	4.99±0.81	6.58±0.29	5.73±0.26	4.73±0.38	0.96
2.	MCP11	4.2±0.23	4.85±0.22	6.36±0.26	5.30±0.15	4.86±0.61	0.84
3.	UTEX	4.42±0.21	3.708±0.09	6.308±0.05	5.421±0.22	4.71±0.01	0.26

* - Average of three trials in triplicate as mean with standard deviation
Different alphabets in a row denote the differences are significantly different and vice versa at $P<0.05$
VCP27- Valparai 27
MCP11- Munnar 11
UTEX- University of Texas, Austin (USA) for *Cephaleuros virescens* 2412

Table 4: Defensive enzyme (protease and chitinase) activity of *Cephaleuros parasiticus* at different days of intervals*.

S. No.	Isolates	Specific Activity in different days of incubation period (U/mg)					CD at P=0.05
		10	20	30	40	50	
Chitinase							
1.	VCP27	7.09±0.03	11.14±0.04	13.20±0.05	12.13±0.06	9.17±0.12	0.19
2.	MCP11	6.87±0.05	9.31±0.05	12.18±0.33	11.06±0.35	8.93±0.01	0.13
3.	UTEX	6.93±0.22	11.17±0.15	13.36±0.16	12.11±0.05	11.21±0.09	0.41
Protease							
1.	VCP27	5.85±0.28	10.86±0.34	12.62±0.25	13.87±0.17	11.34±0.25	0.54
2.	MCP11	6.46±0.18	13.46±0.22	14.18±0.03	14.74±0.31	12.93±0.30	0.47
3.	UTEX	6.30±0.15	12.89±0.39	13.67±0.57	16.41±0.25	13.19±0.03	0.75
β-1, 3 Glucanase							
1.	VCP27	2.85±0.37	3.17±0.79	5.62±0.25	4.89±0.18	3.92±0.12	0.43
2.	MCP11	2.33±0.22	4.02±0.16	5.78±0.03	5.01±0.26	3.98±0.20	0.41
3.	UTEX	2.77±0.30	3.64±0.25	5.63±0.57	4.93±0.31	3.86±0.11	0.35

* - Average of three trials in triplicate as mean with standard deviation

Different alphabets in a row denote the differences are significantly different and vice versa at $P<0.05$

VCP27- Valparai 27

MCP11- Munnar 11

UTEX- University of Texas, Austin (USA) for *Cephaleuros virescens* 2412

5. Conclusion

From the above study, it was revealed that enzymes amylase, invertase, pectinase, and CM Case has no role in plant pathogenesis. Due to the presence of parasitic algae in the plant tissues, these compounds accumulated. Protease, chitinase, PAL, TAL, PO, PPO and catalase were actively involved in inducing pathogenesis in imparting defense in tea plants against *C. parasiticus* during infection.

6. References

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