

Insecticidal potential of certain common ferns on *Rugose spiralling* whitefly, *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae)

Sowmiya, D.,¹ Surya kumar, S.,² Rehanabanu, H.³ and Santhana Bharathi, N.⁴.

ABSTRACT

There is a search for plant based alternatives to synthetic chemicals to control pests in crop plants were started from any years back. In this study, the efficacy of common ferns to control the whitefly, *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae) in coconut plantation was investigated. The whiteflies are prevalent throughout the year in the coconut fields and cause severe losses to the farmers. Ferns such as *Adiantum caudatum*, *Hemionitis opposita*, *Pteris biaurita*, *Thelypteris meeboldii* and *Nephrolepis cordifolia* were evaluated for their efficacy in the control of the whitefly, *A. rugioperculatus*. The aqueous extracts of the ferns were screened at different concentrations viz., 1.0, 2.0, 3.0, 4.0 & 5.0% against *A. rugioperculatus* under laboratory conditions. Extracts from *H. opposita* and *Thelypteris meeboldii* showed the maximum mortality rate of 87% followed by *Nephrolepis cordifolia* (86%), *Adiantum caudatum* (83%) and *Pteris biaurita* (77%) at 5% concentration after 96 hours of treatment. The phytochemical screening of the extracts showed a qualitatively increased level of saponins in *H. opposita* and *T. meeboldii*. Reduced chlorophyll, carbohydrate and amino acid content were observed in the whitefly's infested leaves and increased protein content was observed in the infested leaves. Based on the study, the aqueous extracts of *H. opposita* and *T. meeboldii* can be recommended for the whitefly control in coconut plantations.

Key words: *Cocos nucifera*, bio-efficacy, biopesticide, whitefly, coconut plantation

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INTRODUCTION

The coconut tree (*Cocos nucifera*) belongs to the Family Arecaceae. It is a long-lived plant with a single trunk which is about 20-30 meters tall with smooth bark marked by ringed scars left by the fallen leaf bases. Coconut is widely distributed in tropical regions of the world, ranging between 20°N and 20°S latitude. The natural habitat of coconuts is found in coastal areas and on the fringes of deserts. The coconut is a tropical tree species mainly grown and harvested by small-scale farmers. The tree can live up to 100 years and in India, the coconut tree is considered the “tree of heaven” and its fruit is the “fruit of prosperity”. The coconut tree provides food, fuel, cosmetics, folk medicine and building materials, among many other uses. The major products of coconut

include coconut oil, coconut shell, raw kernel and tender coconut water. The tender coconut water and raw kernel are proved to contain antioxidant, hypoglycemic, immune-stimulant and antibacterial substances. Indonesia is the world's leading coconut producer with about 16.82 million metric tons of coconuts in 2020 followed by India with around 14.7 million metric tons of coconuts (Shahbandeh, 2022). Coconut plays a major role in the economy of India and Tamil Nadu. Pollachi plays a vital role in coconut production and is considered the “coconut capital” of Tamil Nadu. While coconut cultivation and commerce are gaining more attention in Pollachi, it is vulnerable to the threat posed by exotic pests/diseases. Mandal (2011) listed 116 exotic insect species in India. Among the insect pests, exotic whiteflies

introduced from Florida (USA) into Tamil Nadu and Kerala during 2016 caused a severe loss in coconut cultivation. Currently, there are 442 species of whiteflies belonging to 63 genera known from India.

Whiteflies inflict direct feeding injury on plants while sucking sap and serve as vectors of plant diseases. It drains sap from the underside of leaves producing a significant amount of dew which settles on the upper surface of the next lower leaf leading to the growth of black sooty mould. This is a secondary infection arising out of whitefly infection. Diseases like phytoplasma cause a 35% yield reduction and the losses may extend up to 80% in severe cases (Manimekalai *et al.*, 2010). Methods to control the insects include placing sticky yellow coloured polythene sheets which attract the insects and act as traps. Parasitoids, insects that eat rugose whiteflies such as *Chrysoperla zastrowi sillemi* and *Encarsia formosa* could be released in the affected fields as a biological control method.

In India, so far, 12 biopesticides have been registered under Central Insecticides Board, India comprising 80% of the neem formulation against various affected crops. In this study, Pteridophytes – the common ferns, were investigated for their efficacy in the control of white flies. Ferns are a primitive group of vascular plants whose existence dates back to 350 million years ago. They are called the reptiles of plants which are used as ornamentals and biofertilizers. They are also considered the bio-indicators of pollution and phyto-remediators. There are about 1200 species of ferns belonging to 305 genera, of which 191 genera representing 191 species are found in India (Giri *et al.*, 2021). They were reported mostly from the Western Ghats and the Himalayas. Although 9,300 species of insects were reported to use ferns as a food source (Crooper-Driver, 1978), the insecticidal activity of ferns have been reported by various authors (Jones and Firn, 1978; Kubo *et al.*, 1983; Rajendran and Reuben, 1991; Amer and Mehlhorn, 2006; Shukla and Tiwari, 2011) and hitherto there is no report against coconut pests.

The white flies are prevalently distributed throughout the year in the coconut fields and causing severe loss to the farmers. There is a search for a plant-based remedy against synthetic chemicals in the control of pests in crop plants. The ferns are commonly located on the slopes of lower hill tracks were cleared frequently during developmental works. Due to the activities they are voluntarily made extinct or endangered before understanding its medicinal value, from natural population.

This study was therefore conducted to evaluate the insecticidal potential of the common ferns against the whitefly *A. rugioperculatus*, in coconuts with the aim to formulate a bioinsecticide from the extracts of the ferns available on the slopes of road side of the Valparai hills against the white fly, a sucking pest of the coconut. Ferns such as *Adiantum caudatum*, *Hemionitis opposita*, *Pteris biaurita*, *Thelypteris meeboldii* and *Nephrolepis cordifolia* were evaluated for their efficacy to control the white fly *A. rugioperculatus*. The aqueous extracts of ferns were screened at different concentrations against the pest under laboratory conditions and proved to be an alternative eco-friendly insecticide to replace the chemical insecticides.

MATERIALS AND METHODS

The ferns *viz.*, *Adiantum caudatum*, *Hemionitis opposita*, *Pteris biaurita*, *Thelypteris meeboldii* and *Nephrolepis cordifolia* were collected from different areas in Valparai (latitude: 10°22'12"N and longitude: 76°58'12"E, 1050 m a.s.l.), Coimbatore district, Tamil Nadu, India. The collected plant materials were washed thoroughly three times in tap water and were shade dried for two weeks. As the leaflets turned slightly brown, they were removed separately leaving their stalk apart. Their leaflets were crushed using a laboratory blender and then sieved through a mesh (3 mm) to remove the coarse materials. The fine powder was then packed in an airtight container, labelled and stored until needed. The aqueous extracts were prepared by adopting an infusion method. 25 g of powdered plant material was dissolved in 100 ml of distilled water to have a

25% (w/v) aqueous extract. The set-up was kept overnight (12 h) in a mechanical shaker for complete extraction (Figure 1). The entire contents were filtered using a double-folded muslin cloth and the pure extract was collected, labelled and kept as a stock solution to prepare the required concentrations (Prabhakaran *et al.*, 2017).

Laboratory bioassay

The bioassay was carried out to assess the insecticidal potential of the fern extracts against the common coconut pest, *A. rugioperculatus*. The leaf disc method (Siegler, 1947; Ebeling and Pence, 1953) was adopted. Infected leaflets (triplicates) were collected from the experimental farm (Figure 2). It was cut down into 2 cm in diameter and placed with its ventral surface down over the wet cotton in a Petri dish (9 cm diameter). The infected leaves were sprayed with the selected fern aqueous extracts at 1.0, 2.0, 3.0, 4.0 and 5.0% (W/V) concentrations using a glass atomizer to ensure fine spraying (Figure 3). The mouth of the Petri dishes was covered with nylon mesh immediately after spraying the aqueous extract to contain the whiteflies. To ensure the insecticidal potential of the fern extract, the neem oil, Acetamiprid and water were kept as control and sprayed at 1.0, 2.0, 3.0, 4.0 and 5.0% concentrations. The mortality rate was assessed at 24 h intervals for 4 days (96 h).

Qualitative estimation of secondary metabolites from aqueous fern extract

The aqueous extracts of the selected ferns were tested using the standard procedures for the presence/absence of the phytochemical constituents *viz.*, alkaloids, glycosides, resins, saponins, volatile oils and terpenoids (Mir *et al.*, 2014); Phenols and flavonoids (Devi *et al.*, 2014); tannin, steroid and anthraquinones (Mahavidyalaya *et al.*, 2016).

Quantitative estimation of primary metabolites from healthy and infected coconut leaves

In general, the insect in its larval stage feeds on the sap of coconut leaves. Adult insects create white-coloured formulations on the underside of leaves, which then turn black due to fungal formation. This causes a drop in production by 20%. To ensure the production

loss is due to the infection of whitefly in the coconut palm, chlorophyll (Arnon, 1949), carbohydrate (Yemm and Willis, 1954), protein (Lowry *et al.*, 1951) and free amino acid (Mahesha, 2019) were estimated for healthy and infected leaf.

Statistical analysis of data

The obtained data were analysed using ANOVA and a post hoc test was carried out by adopting Duncan Multiple Range Test (DMRT) to determine the significant difference between the treatments. SPSS V 16 software was used for all the analyses.

RESULTS AND DISCUSSION

Extracts of ferns such as *H. opposita* and *T. meeboldii* showed the maximum mortality rate of 87% followed by *N.cordifolia* (86%), *A.caudatum* (83%) and *P.biaurita* (77%) at 5% concentration after 96 hours of treatment (Table 1). The treatments such as *T. meeboldii*@ 3%, & 5%, *H. opposita* @ 5% and Acetamiprid 20% produced on par mortality against the whitefly after 24 hrs of application. Similarly, *T. meeboldii* @ 5% and *P. biaurita* @ 5% produced significantly high mortality on the insect after 48 hrs of application. Likewise, *T. meeboldii* @ 5%, *P. biaurita*@ 5%, *H. opposita* @ 5%, *N. cordifolia*@ 5% and *A. caudatum* @ 5% achieved significantly high mortality of the whitefly after 72 and 96 hrs of application (Table 1). The LD₅₀ and LD₉₀ values of the evaluated ferns are presented (Table 2). Acetamiprid 20% and neem oil 2% achieved 67% and 40% of adult mortality after 96 hrs of application. All evaluated ferns produced significant adult mortality than the acetamiprid and neem oil while applied at the 5% concentration. The whitefly's mortality was positively correlated with increasing concentrations (1-5%) and the time interval (24-96 hrs after treatment).

The preliminary phytochemical analysis results are listed in Table 3. The aqueous extract of *Adiantum caudatum* revealed ten phytochemicals such as alkaloids, volatile oils, glycoside, phenol, tannin, saponin, flavonoids, steroids, anthocyanin and triterpenoids and the aqueous extract of *Nephrolepis cordifolia* revealed the presence of minimum

Table 1. Toxicity of certain common ferns to Rugose spiraling whitefly, *Aleurodicus rugioperculatus* under laboratory conditions

Treatments	Concentrations	Mortality %			
		24 HAT	48 HAT	72 HAT	96 HAT
<i>Adiantum caudatum</i>	1%	13±3.3 ^{bc}	23±3.3 ^{cd}	33±3.3 ^{cdef}	33±3.3 ^{cd}
	2%	17±3.3 ^{bcd}	23±3.3 ^{cd}	30±0.0 ^{cde}	34±3.3 ^{cd}
	3%	17±3.3 ^{bcd}	27±3.3 ^{de}	30±5.8 ^{cde}	37±3.3 ^{cd}
	4%	23±3.3 ^{cde}	27±3.3 ^{de}	43±3.3 ^{efgh}	57±3.3 ^{gh}
	5%	23±3.3 ^{cde}	40±5.8 ^{fgh}	63±3.3 ^j	83±3.3 ^{jk}
<i>Nephrolepis cordifolia</i>	1%	13±3.3 ^{bc}	15±2.8 ^{bc}	22±1.6 ^{bc}	25±2.8 ^{bc}
	2%	17±1.6 ^{bcd}	30±2.8 ^{def}	37±3.0 ^{defg}	42±4.4 ^{def}
	3%	23±3.3 ^{cde}	37±3.3 ^{efgh}	43±3.3 ^{efgh}	50±5.8 ^{efg}
	4%	23±3.3 ^{cde}	37±3.3 ^{efgh}	57±3.3 ^{hij}	73±6.6 ^{ij}
	5%	23±3.3 ^{cde}	33±3.3 ^{defg}	67±3.3 ^j	86±3.3 ^k
<i>Hemionitis opposita</i>	1%	7±3.3 ^{ab}	10±5.7 ^{ab}	17±3.3 ^{ab}	17±3.3 ^{ab}
	2%	17±3.3 ^{bcd}	33±3.3 ^{defg}	47±3.3 ^{fghi}	50±5.7 ^{efg}
	3%	27±3.3 ^{de}	37±3.3 ^{efgh}	47±3.3 ^{fghi}	57±3.3 ^{gh}
	4%	27±3.3 ^{de}	40±0 ^{fgh}	57±3.3 ^{hij}	77±3.3 ^{ijk}
	5%	30±5.7 ^e	43±3.3 ^{gh}	67±3.3 ^j	87±3.3 ^k
<i>Pteris biaurita</i>	1%	13±3.3 ^{bc}	23±3.3 ^{cd}	27±3.3 ^{bcd}	37±3.3 ^{cd}
	2%	17±3.3 ^{bcd}	30±0 ^{def}	37±3.3 ^{defg}	43±3.3 ^{def}
	3%	17±3.3 ^{bcd}	27±3.3 ^{de}	33±8c ^{def}	53±3.3 ^{fg}
	4%	27±3.3 ^{de}	40±0 ^{fgh}	60±5.7 ^{ij}	67±3.3 ^{hi}
	5%	27±3.3 ^{de}	47±3.3 ^h	67±3.3 ^j	77±3.3 ^{ijk}
<i>Thelypteris meeboldii</i>	1%	17±3.3 ^{bcd}	27±3.3 ^{de}	33±3.3 ^{cdef}	40±0 ^{de}
	2%	23±3.3 ^{cde}	40±0 ^{fgh}	50±5.7 ^{ghi}	53±3.3 ^{fg}
	3%	30±0 ^e	43±3.3 ^{gh}	46±6.7 ^{fghi}	53±3.3 ^{fg}
	4%	27±3.3 ^{de}	40±5.7 ^{fgh}	47±3.3 ^{fghi}	67±3.3 ^{hi}
	5%	33±3.3 ^e	47±3.3 ^h	67±3.3 ^j	87±3.3 ^k
Acetamiprid 20 %	20%	33±3.3 ^e	43±3.3 ^{gh}	60±5.8 ^{ij}	67±3.3 ^{hi}
Neem oil	2%	17±3.3 ^{bcd}	23±3.3 ^{cd}	33±3.3 ^{cdef}	40±5.8 ^{de}
Untreated Control	-	0±0 ^a	3±2.7 ^a	5±0.5 ^a	5±0.5 ^a

HAT= Hours after treatment; Mean±SD followed by the same letter in the column is not significant at 5% level in DMRT

Table 2. LD₅₀ and LD₉₀ values of common ferns on *Aleurodicus rugioperculatus* at 5% concentration after 96 hours of application

Treatments	LD ₅₀	95% confidential limit		LD ₉₀	95% confidential limit		Chi-Square value
		LCL	UCL		LCL	UCL	
<i>Adiantumcaudatum</i>	0.427	-	1.11	1.234	0.807	12.9	2.769
<i>Nephrolepiscordifolia</i>	0.354	0.86	0.528	0.896	0.666	1.891	1.106
<i>Hemionitisopposita</i>	0.355	0.139	0.502	0.822	0.633	1.465	1.028
<i>Pterisbiaurita</i>	0.319	-17.97	0.682	1.19	0.763	154.09	0.586
<i>Thelypterismeeboldii</i>	0.241	-3.606	0.49	1.072	0.702	16.46	1.383

Table 3. Phytochemical analysis of aqueous extracts of common ferns

Phytochemicals	<i>Adiantum caudatum</i>	<i>Nephrolepis cordifolia</i>	<i>Hemionitis opposita</i>	<i>Pteris biaurita</i>	<i>Thelypteris meeboldii</i>
Alkaloids	+	+	+	+	+
Volatile oils	+	+	+	+	+
Glycoside	+	-	+	-	+
Phenol	+	-	+	+	+
Tannin	+	-	+	+	+
Saponin	+	-	+	+	+
Terpenoids	-	-	-	-	-
Flavonoids	+	+	-	+	+
Steroids	+	+	+	+	-
Anthroquinones	-	-	-	-	-
Anthocyanin	+	-	+	-	-
Triterpenoids	+	-	+	-	+

+ = Present, - = Absent

Table 4. Estimation of chlorophyll content of healthy and infected leaves

Sample	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll (mg/g)
Healthy leaf	21.19849	38.61286	59.79016
Infected leaf	21.95849	29.35022	51.29162

phytochemicals such as alkaloids, volatile oils, flavonoids and steroids. Quantitative estimation of total chlorophyll content was lesser (51.29 mg/g) in *A. rugioferulatus* infested leaves than the chlorophyll content (59.79 mg/g) of the healthy leaves which is not infested by *A. rugioferulatus* (Table 4). Similarly, the amount of carbohydrate present in the healthy leaves of coconut was higher (16.8 mg/g) than the amount of carbohydrate (11.6 mg/g) in the infested leaves. The amount of protein present in the infested leaves of coconut was higher (16 mg/g) than the amount of protein (14.6 mg/g) in the healthy leaves. The amount of amino acids present in the infested leaves of coconut was higher (0.025 mg/g) than the amount of amino acids (0.02 mg/g) in the healthy leaves.

DISCUSSION

Many researchers have reported the insecticidal potential of several ferns against various agricultural pests. In this study, all the evaluated fern extracts produced highly significant mortality against the whitefly, *A. rugioferulatus*. Murasing *et al.* (2019) evaluated the alcoholic extracts of three ferns *Diplazium culentum*, *Christella parasitica* and *Blechnum orientale* against diamondback moth, *Plutella xylostella* in cabbage and found that the fern, *D. esculentum* was more toxic to the larvae of *P. xylostella*. They also reported that the fern, *D. esculentum* altered the larval feeding behavior of *P. xylostella* leading to reduced larval and pupal weight, prolonged pupation period, malformed pupae and adults under in vitro conditions. An increase in larval mortality was also observed with the increase in the concentrations. A similar observation was made in this study. Sahayaraj and Selvaraj (2013) evaluated three fern extracts *viz.*, *Pteridium aquilinum*, *Hemionitis arifolia* and *Christella parasitica* on *Helicoverpa armigera* (Hubner) and *Spodoptera litura* (Fab) under groundnut field condition. The highest population reduction was recorded in *P. aquilinum* treated plots followed by *H. arifolia* and *C. parasitica*. The high yield and cost-benefit ratio was recorded high in *P. aquilinum* followed by *H. arifolia* and *C. parasitica*. This insecticidal potential of ferns may be due to the presence of

toxic proteins. Shukla *et al.* (2016) identified the expression of insecticidal proteins, Tma12 from an edible fern, *Tectaria macrodonta* (Fee) which act as an insecticide to whitefly and interferes with its lifecycle at sublethal doses. Other than the insects, Prabhakaran *et al.* (2017) evaluated *Dicranopteris linearis* and *Pteridium aquilinum* against red spider mite, *Oligonychus coffeae* in tea and found that 5% fern concentration showed 50% reduction in the population of red spider mite after 96 hours of application and also found it caused nil phytotoxic effect on tea leaves.

In the present study, among the twelve recorded phytochemicals, *A. Caudatum* had a maximum of ten phytochemicals, *N. cordifolia* had four phytochemicals, *H. opposita* had nine phytochemicals, *P. biaurita* had seven and *T. meeboldii* had eight phytochemicals. In these phytochemicals, saponin was found to be high in *T. meeboldii* and *H. opposita*, and it acts as natural insecticide. Geyter *et al.* (2007) reported that plant saponins act as natural insecticides to control pests and the application of saponin produced a high mortality rate, lowered food intake, weight reduction, and retardation in development and decreased reproduction. In our study, the aqueous extract of *H. Opposita* had the highest saponin content which indicates that the mortality of whitefly was due to the sensitivity of the insect to saponin. Similarly, Prabhakaran *et al.* (2017) found that a high amount of saponin was found in *Cyclosorus interruptus*, *D. linearis* and *P. aquilinum* and minimum amount of saponin was found in *Adiantum raddianum*, *Asplenium aethiopicum* and *Diplazium polypodioides*. The minimum amount of tannins and phenols were found in all the fern extracts. Alkaloids were present in all the aqueous extracts except *A. raddianum* and *A. aethiopicum*. Mir *et al.* (2013) also evaluated the preliminary phytochemical screening of acetone, ethanol, methanol and aqueous extracts of 34 pteridophyte species collected from Shopian district, Kashmir, India and reported that the aqueous extract of the ferns was found to contain flavonoids, terpenoids, saponins and phenolic compounds.

The reduced chlorophyll, carbohydrate and amino acid contents were observed in the present study due to the infection of whiteflies. Decreased chlorophyll content certainly plays a vital role in the photosynthetic efficiency of plants. Arun *et al.*, 2021 reported the decline of chlorophyll and carotenoid content up to 50% and 34% respectively, during the infection of *A. rugioperculatus* and sooty mold in coconut leaves. In the present study, the total chlorophyll content showed a decline of 14% in the infected leaves in comparison with that of the healthy leaves. The fungal cover over the leaves reduced the light availability for the absorption by the pigments, causing an inefficient energy harvest which leads to the degradation of the chlorophyll pigments in the infected plant. Baron *et al.*, 2012 reported a decrease in the photosynthetic efficiency of plants due to fungal infection. Prolonged infection in the coconut leaves by the sooty mold severely damages the leaf tissue by preventing photosynthesis, turning the cells dead due to the death of photosynthate and ultimately the infected leaves dry up prematurely, which is similar to the observation in the present study. Arun *et al.*, 2021 reported that the total sugar content was increased by 25% in the infected leaves over that of the normal leaves. But in the present study, the carbohydrate content of the whitefly and sooty mold infected leaf was decreased over that of the healthy leaf. The total carbohydrate at 0.5 concentration of healthy leaves contain 16.8 milligrams/grams whereas 0.5 concentrations of infected leaves contains 11.6 milligrams/grams. Morkunas and Ratajczak (2014) reported the role of sugar signalling in plant defense responses against fungal pathogens. In most fungal pathogen plant systems, a high level of sugars in plant tissues enhances plant resistance. This agrees with the result of the present study. Arun *et al.*, 2021 reported that the soluble total free amino acid content in the infected leaf was increased by 126% over that of the normal leaves. Similarly in the present study, the total soluble total free amino acids content was increased by 25% in the infected leaves. The total free amino acid content at 0.5 concentrations of healthy leaves contains 0.02

milligrams/grams whereas 0.5 concentration of infected leaves contains 0.025 milligrams/grams of protein. Hayat *et al.*, 2012 reported that in many plants, stress acclimation was associated with an increase in the levels of specific amino acids. This increase in amino acids aids in the maintenance of the nitrogen metabolism and thus could impart stress tolerance to coconut.

Arun *et al.*, 2021 reported that the total soluble protein content in the infected leaf was increased by 114% over that of the normal leaves. Similarly in the present study, the protein content was increased in the infected leaves than in the healthy leaves. The total protein content at 0.5 concentrations of healthy leaves contains 14.6 milligrams/grams whereas 0.5 concentrations of infected leaves contain 16 milligrams/grams of protein. The leaves of the coconut plant accumulated more proteins as part of the defense against the whitefly and sooty mold infection. Earlier reports were indicating that the increasing levels of proteins helped the plants in maintaining their growth under various stressful environments (Agastian *et al.*, 2000; Ferreira *et al.*, 2007). The increased soluble protein content was evidenced in the increased activity.

Comparatively the extracts of *Hemionitis opposita* and *Thelypteris meeboldii* at 5.0% concentration were reported to be toxic and control the white flies considerably under laboratory condition. Hence, the ferns are considered and suggested to be good alternative and eco-friendly insecticide to chemical insecticides to agricultural sector.

Conflict of Interest

Authors have no conflict of interest.

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- Sowmiya, D.^{1*}, Surya Kumar, S.², Rehanabanu, H.³ and SanthanaBharathi, N.⁴**
- ^{1,3}Department of Botany, PSGR Krishnammal College for Women, Coimbatore, Tamil Nadu, India
- ²PG and Research Department of Botany, Nallamuthu Gounder Mahalingam College, Pollachi, Tamil Nadu, India
- ⁴Division of Entomology, UPASI TRF tea Research Institute, Valparai, Tamil Nadu, India
- Corresponding author
E-mail: d.sowmiya22@gmail.com