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RESEARCH ARTICLE

MYCORRHIZAL ASSOCIATION AND PHYTOCHEMICAL ANALYSIS OF SELECTED FERNS OF IDUKKI DISTRICT

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SUMMARY Evaluation of mycorrhizal association and phytochemical analysis of eleven selected ferns from Idukki district of Western Ghats was carried out. Analysis of mycorrhizal association using root colonization assessment revealed the presence of fungal hyphae in all the ferns studied except *Diplazium brachylobum*. The root colonization percentage ranges from zero (*D. brachylobum*) to 91.73 ± 0.80 (*Angiopteris helferiana*). *Angiopteris helferiana* showed vesicular mycorrhizal association. Phytochemical evaluation of the rhizome extracts revealed the presence of various secondary metabolites such as alkaloids, cardioglycosides, phenolics, saponins, tannins, terpenoids, steroids, quinones, flavonoids and glycosides. Association with certain fungal species may enhance the presence of secondary metabolites.

Keywords: Fern, mycorrhiza, Idukki, phytochemical.

INTRODUCTION

Pteridophytes are a group of flowerless, seed-free, and spore bearing vascular plants having two definite evolutionary ancestral lines, i.e. lycophytes and ferns. Lycophytes and ferns are characterized by a life cycle with completely independent gametophyte and sporophyte generations in which the sporophyte is the dominant one (Haufler et al. 2016). Pteridophytes of India are represented by around 1000 species belonging to 191 genera which occur in different habitats and geographical zones. Eastern Himalayas has the richest fern diversity, followed by the Eastern and Western Ghats of India. Seventeen per cent of the total pteridophytic taxa in our country is categorized as endemic (Chandra et al. 2008, Dudani et al. 2012, Singh et al. 2012).

The majority of the vascular plants including pteridophytes are associated with arbuscular mycorrhizal fungi, which is one of the universal and age-old symbiosis among higher plants. Those mycorrhizal associations have facilitated plants to colonize terrestrial habitats. Associated fungi help the plants in uptake of nutrients and water; and thereby the survival in stressed conditions (Strullu-Derrien & Strullu 2007). Information on earlier studies of fungal association in Indian pteridophytes is very limited as

compared to angiosperms. The previous studies on mycorrhizal association from India were on ferns of Western Ghats (Muthukumar & Udaiyan 2000, Ragupathy & Mahadevan 1993), North-east India (Mishra et al. 1980) and Himachal Pradesh (Prashar et al. 2005).

The present work is aimed at analysing and evaluating the mycorrhizal association and phytochemical screening of eleven Polypodiales ferns namely, *Adiantum raddianum* C. Presl, *Angiopteris helferiana* C. Presl, *Blechnum orientale* L., *Christella dentata* (Forssk.) Brownsey & Jermy, *Dicranopteris linearis* (Burm. f.) Underw, *Diplazium brachylobum* Manickam & Irudayaraj, *Microlepia speluncae* (L.) T. Moore, *Nephrolepis cordifolia* (L.) C. Presl, *Pityrogramma austroamericana* Domin, *P. calomelanos* (L.) Link and *Tectaria coadunata* C. Chr of Idukki district.

MATERIALS AND METHODS

Ferns were collected from various localities of Idukki district, Kerala, and were authenticated by Dr. Raju Antony, Jawaharlal Nehru Tropical Botanical Garden & Research Institute, Palode, Thiruvananthapuram.

Fronds of the collected plant specimens were carefully removed and the rhizomes were rinsed with water to remove debris and soil particles on primary and secondary fine roots. The roots were then fixed in FAA. The fixed roots were processed for the assessment of fungal association. The roots were washed to remove the traces of FAA and cut into fragments of a cm each, cleared in 2.5 % KOH at 90° C, acidified with 5 N HCl and stained with trypan blue (0.05% in lactoglycerol). Most of the roots remained dark after clearing and were bleached in alkaline H_2O_2 prior to acidification. The roots were left overnight in trypan blue-lactoglycerol (0.05%) for staining. The stained roots were examined with a compound microscope for the presence of fungal structures and photographed with a digital camera. When arbuscules, vesicles, hyphae alone or in any combination of these were present, it is considered as mycorrhizal colonization. The root colonization percentage was measured using the standard equation and mycorrhizal root length colonization was quantitatively calculated (Biermann & Linderman 1981, McGonigle et al. 1990).

Extracts for the qualitative phytochemical analysis of rhizome were prepared using soxhlet extractor. 30 g of powdered samples were extracted with 150 ml methanol for 8–12 h at a temperature not exceeding the boiling point. The extracts were concentrated in vacuum using a rotary evaporator. Different qualitative chemical tests were performed on various extracts to detect the presence of phytoconstituents (Harborne 1998).

For testing alkaloids, 1 ml of the plant extract was mixed with 1 ml of 1% HCl, warmed and filtered. 2 ml of filtrate was treated with Mayer's reagent. Turbidity or precipitation, green colour indicates the presence of alkaloid. For testing cardioglycosides, 1 ml of the extract was mixed with 2 ml of glacial acetic acid to which added a

few drops of 5% ferric chloride. This was underlayered with 1 ml of concentrated sulphuric acid. Formation of a brown ring at the interface indicates the presence of cardioglycosides. To test phenolic compounds, 1 ml of extract was mixed with 2 ml of distilled water, 0.5 ml of sodium carbonate and Folin ciocalteau's reagent. Formation of a blue or green colour indicates the presence of phenol. For testing saponins, 1 ml of plant extract was dissolved in 2 ml of boiling water in a boiling tube, allowed to cool and shaken well to mix. The appearance of foam indicates the presence of saponins. To detect tannins, 2 ml of the test solution was mixed with 2 ml of ferric chloride. The formation of a blue-black or dark green colour indicates the presence of tannins. For testing terpenoids, 1 ml of extract was mixed with 2 ml of chloroform and 1.5 ml of concentrated sulphuric acid. Formation of a reddish brown colour indicates the presence of terpenoids. For testing steroids, 1 ml of plant extract was mixed with 2 ml of chloroform and 1 ml of sulphuric acid. Formation of a reddish brown colour at the interface indicates the presence of steroids. To detect quinones, 1 ml of extract was mixed with 1 ml of concentrated sulphuric acid. Formation of red colour indicates the presence of quinones. For testing flavonoids, 3 ml of extract was mixed with 4 ml of 1N sodium hydroxide. Formation of dark yellow colour indicates the presence of flavonoids. For testing glycosides, 2 ml of extract was mixed with 3 ml of chloroform and 1 ml of 10% ammonium solution. Formation of pink colour indicates the presence of glycosides.

OBSERVATIONS

Mycorrhizal root colonization assessment

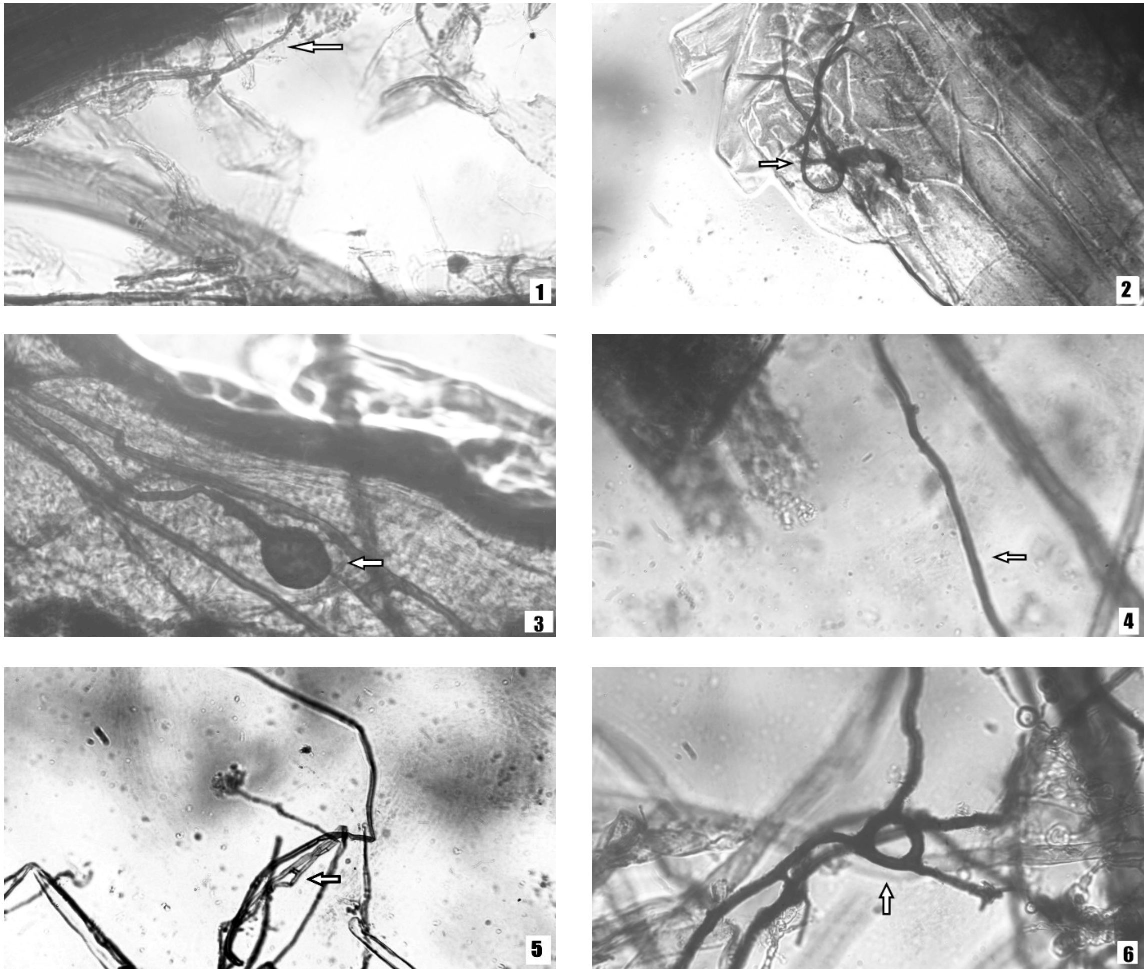
Out of eleven ferns examined, ten showed the presence of hyphal colonization only. Of them five selected taxa have been depicted in Figs 1–6. Vesicular and arbuscular colonizations were absent in all specimens except *Angiopteris helferiana* (Fig. 3). *Angiopteris helferiana* showed the highest percentage of mycorrizhal association (91.73 ± 0.80) whereas *Diplazium brachylobum* showed no mycorrizhal association (Table 1).

Qualitative phytochemical analysis

Phytochemical analysis carried out in methanol extracts of eleven fern specimens indicated the presence of various secondary metabolites. Extracts of rhizomes showed the existence of various phytoconstituents such as alkaloids, cardioglycosides, phenols, saponins, tannins, terpenoids, steroids, quinones, flavonoids and glycosides (Table 2).

DISCUSSION

The majority of ferns studied here possess mycorrhizal association ranging from 0–91.73%. Out of eleven species studied, ten are found to have hyphal colonization while, *Angiopteris helferiana* showed vesicular mycorrhizal colonization. *Diplazium brachylo-bum* is found to be non-mycorrhizal. *Angiopteris helferiana* has the highest percentage of fungal colonization (91.73 ± 0.80). *Adiantum raddianum* also possess a good range of root colonization (84.37 ± 0.99). The



Figs 1–6: Portions of cleared roots showing fungal structures (arrows) 1. *Adiantum raddianum*, hyphae. 2. *Angiopteris helferiana*, hyphae. 3. *Angiopteris helferiana*, vesicle. 4. *B. orientale*, hyphae. 5. *P. calomelanos*, hyphae. 6. *T. coadunata*, hyphae.

results of the present study contradict the previous studies (Muthukumar & Prabha 2013, Muthukumar et al. 2014) where, *D. linearis*, *Adiantum raddianum*, *P. calomelanos*, *C. dentata* and *N. cordifolia* from various localities of Tamil Nadu have the presence of arbuscular mycorrhizal association. The current findings reveal that those fern species from Idukki generate hyphal association only instead of arbuscular association. It is considered that mycorrhizal association can be influenced by a wide range of host, fungal and environment factors and the variation in the extent of colonization is not surprising.

TABLE 1: Assessment of mycorrhizal root colonization of different ferns.

Fern	Locality	RC (%)	RC type
Adiantum raddianum	Anachal	84.37 ± 0.99	H
Angiopteris helferiana	Thodupuzha	91.73 ± 0.80	H,V
B. orientale	Kalvari Mount	12.66 ± 0.76	H
C. dentata	Nedumkandam	29.42 ± 0.59	H
D. linearis	Kulamavu	17.16 ± 1.25	H
D. brachylobum	Kulamavu	0	-
M. speluncae	Mathikettan Shola	28.55 ± 0.91	H
N. cordifolia	Kalayanthani	4.65 ± 0.43	H
P. austroamericana	Munnar	17 ± 1	H
P, calomelanos	Kalayanthani	54.05 ± 1.04	H
T. coadunata	Anachal	8.46 ± 0.15	H

RC%, Root colonization percentage (Values= Mean ± Standard deviation). H, hyphal; V, vesicular.

TABLE 2: Qualitative phytochemical analysis of different ferns.

Phytoconstituent	Extract										
	1	2	3	4	5	6	7	8	9	10	11
Alkaloids	-	+	-	+	-	+	+	-	-	-	-
Cardioglycoside	+	+++	++	+++	++	-	+	++	++	-	++
Phenol	++	++	+++	+++	++	+	+	-	+++	-	+++
Saponin	+++	++	+++	+++	+++	++	+	++	+++	-	+++
Tannin	+	+++	+++	+++	+++	+	++	++	+++	-	+++
Terpenoid	++	+++	+++	+++	+++	++	++	+	+++	+	+++
Steroid	+	+++	++	++	++	+	+	+	+++	-	+++
Quinone	++	+++	+++	+++	+++	+	++	+	+++	-	+++
Flavonoid	-	+++	+++	+++	+++	+	+	-	+++	-	+++
Glycoside	+++	+++	+++	+++	+++	-	-	+++	++	-	++

1, *Adiantum raddianum*; 2, *Angiopteris helferiana*; 3, *B. orientale*; 4, *C. dentata*; 5, *D. linearis*; 6, *D. brachylobum*; 7, *M. speluncae*; 8, *N. cordifolia*; 9, *P. austroamericana*; 10, *P. calomelanos*; 11, *T. coadunata*. (+++, Strongly positive; ++, Moderately positive; +, Positive; -, Negative).

Previous studies on the comparative phytochemical analysis of *P. calomelanos*, *C. dentata*, *T. coadunata* reported the presence of various secondary metabolites such as alkaloids, cardioglycosides, phenolics, saponins, tannins, terpenoids, steroids, quinones, flavonoids and glycosides. The present study also is in agreement with the earlier findings. The plant extracts exhibited significant antibacterial activity against *Rhodococcus pyrinidivorans, Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Escherichia coli, Listeria monocytogenes* and *Xanthomonas campestris*. The presence of antimicrobial activity of these species may be due to the presence of one or more phytoconstituents such as alkaloids, glycosides, flavonoids, steroids, saponins etc. (Manhas et al. 2018, Thomas et al. 2020, Thomas et al. 2021a, b).

In the present study, qualitative phytochemical screening in eleven ferns had revealed the presence of secondary metabolites. Association with certain fungal species may enhance the presence of secondary metabolites thereby its medicinal properties. Studies of fungal association in ferns are essential for the conservation and survival of endemic fern species.

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RESEARCH ARTICLE

DIFFERENTIAL RESPONSE OF THE TWO ACCESSIONS OF BLACK PEPPER CULTIVAR KARIMUNDA TO '*PHYTOPHTHORA FOOT-ROT'* DISEASE

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SUMMARY Assessment of response of the 2 accessions of the high yielding, popular Black pepper cultivar Karimunda to the most dreaded disease of the crop '*Phytophthora* foot-rot' ('Quick wilt') was carried out, employing standard protocol. The data were analysed using ANOVA and HSD Post hoc multiple comparison tests. The study revealed that Disease Severity Indices (DSI) of the 2 accessions differ significantly from each other. Hence, it is inferred that the 2 accessions of the cultivar Karimunda, Karimunda-I and Karimunda-II, differ remarkably in their capability to resist the disease. The results of the study indicate that the differential response to the disease exhibited by the 2 accessions is due to difference in their genetic makeup, and suggest that the Karimunda-I genome may possesses certain genes/alleles other than those of Karimunda-II genome, and the presence of which might have led to the increased disease resistance by Karimunda-I. The added resistance of Karimunda-I is prospective from the point of view of both cultivation and crop improvement of Black pepper.

Keywords: *Piper nigrum,* Black pepper, cv. Karimunda, *Phytophthora* foot–rot, genetic variability.

INTRODUCTION

Piper nigrum L. is one of the most important spice crops of the world, and its dried fruits - Black pepper - is acclaimed as 'King of spices'. Until a few decades ago, India was the leading producer of Black pepper in the world, and the country lost its claim in 1990s, when Vietnam became the largest producer. The current average yield of Black pepper in India is ca. 321 kg/ha, whereas in other Black pepper producing countries such as Malaysia, Thailand and Vietnam, average yield of the crop ranged from 1100–3594 kg/ha. (Parthasarathy et al. 2008). One of the major causative factors leading to low productivity of Black pepper in India is pests and diseases of the crop, of which '*Phytophthora* foot-rot', known as 'Quick wilt' disease of Black pepper is the main cause of crop loss in the country. The most

appropriate strategy for overcoming the present day setback of decreased yield of Black pepper is crop improvement and popularisation of the resultant high yielding cultivars, with special reference to increased capability to resist severe diseases of the crop. In this backdrop, the present study has been undertaken for assessing the response of the 2 accessions of cv. Karimunda, a high yielding and popular cultivar of Black pepper to '*Phytophthora* foot-rot' disease.

MATERIALS AND METHODS

Two accessions of the Black pepper cv. Karimunda – Acc. No. FGB/P/024 (Karimunda-I) and Acc. No. FGB/P/029 (Karimunda-II) were subjected to screening studies for assessing the degree of their resistance to '*Phytophthora* foot-rot' disease. The accessions, Karimunda-I and Karimunda-II were procured from farmers' fields at Vellayani and Peroorkada (Thiruvanathapuram District) respectively, and they were maintained in the Prof. (Dr.) P. M. Mathew Field Gene Bank (FGB) of Black pepper at the Department of Botany, University of Kerala. Ten 3-month-old, 3- or 4-noded polybagged plants of Karimunda-I (Fig.1a) and Karimunda-II (Fig.1b) were raised vegetatively using stem cuttings of the plants maintained in the FGB. The polybagged plants were nurtured in identical environmental conditions in the Nursery of the FGB, and they were subjected to screening studies against *Phytophthora capsici* – the causative organism of 'Quick wilt' disease. Disease Severity Indices (DSI) of the 2 accessions, a measure used to assess the degree of resistance exhibited by the genotypes of Black pepper as regards to '*Phytophthora* foot-rot' disease were estimated (Eikemo et al. 2001). Voucher specimens of the accessions, Karimunda-I and Karimunda-II were deposited in the Herbarium of the Department of Botany, University of Kerala (KUBH).

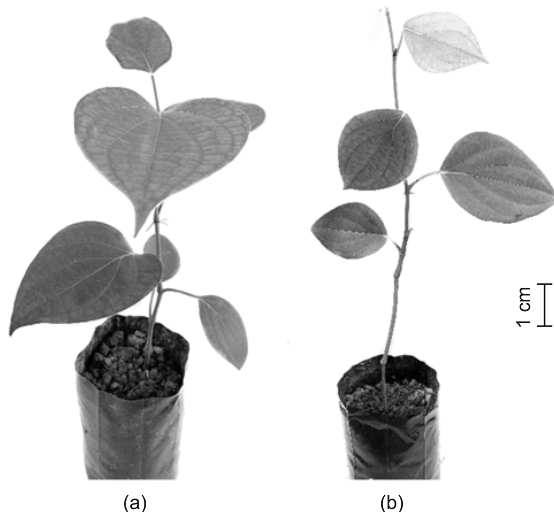

Fig. 1: 3-month-old, polybagged plants of the 2 accessions of *Piper nigrum* cv. Karimunda (a) Karimunda-I, (b) Karimunda-II.

Phytophthora capsici inoculum

The isolates of *P. capsici* (Strain No. 05-06) maintained in the *Phytophthora* repository at ICAR-IISR, Kozhikode were procured and used for the screening purpose. The isolate was subcultured in Carrot agar and grown for 72 h day: night period for inoculation.

Ariel inoculation (stem and leaf)

Rooted cuttings were inoculated by making a pin

prick with a sharp-edged needle at the centre of the third internode from the tip of the stem and placed over it an inoculum disc of 5 mm size taken from the actively growing margin of 72 h-old colony (Sarma et al. 1994, Bhai et al. 2010). Simultaneously with stem inoculation, the third and fourth leaf from the tip of the plant was also inoculated at the centre of the abaxial side of the leaf by placing an inoculum disc. The inoculated portions on the stem and leaves were covered with a wet cotton pad and tied with a polythene strip to keep the inoculum in place, without drying. The inoculated cuttings were incubated for 72 h and maintained at a temperature of 25–28^0 C and 75–90% RH in a polyhouse. After 72 h, diameter of lesions on the upper surface of the inoculated leaves were measured. In the case of inoculated stem, cotton pads were removed, and length of lesions were measured after splitting the stem.

The leaf lesions were scored on a 0–4 scale as, 0 for no lesion, 1 for 1–5 mm lesion, 2 for 6–10 mm, 3 for 11–15 mm and 4 for >15 mm lesion size. Simultaneously, the stem lesion length was scored on a 0–4 scale as, 0 for no lesion, 1 for 1–5 mm lesion, 2 for 6–20 mm, 3 for 21–30 mm and 4 for >30 mm lesion length. Based on the lesion scoring, the DSI of the leaf and stem were calculated using the formula proposed by Kim et al. (2000).

Root inoculation

The 3-month-old rooted cuttings in polybags were inoculated with 72 h-old culture of *P. capsici* in the form of mycelial discs. Five mycelial discs of 10 mm diameter were incorporated into the root zone of the plants and observed for infection/mortality. Root/collar infection will be manifested as decay of the collar portion of the root, which extends upwards resulting in the collapse of the plant. The presence of the inoculum in the soil was determined by the baiting method proposed by Anandaraj & Sarma (1991). The number of plants died within a period of 7 d after inoculation was taken as the measure for calculating the DSI of the root, using the following formula:

$$\frac{\text{No. of plants died within seven days}}{\text{Total No. of plants inoculated}} \times 100$$

The average DSI of an accession is calculated using the DSI of leaf, stem and root and it is rated as < 30% as resistant, 30–40% as moderately resistant and > 40% as susceptible.

Statistical analysis

One way Analysis of Variance (ANOVA) was used to compare the average DSI values of Karimunda-I and Karimunda-II and Turkey HSD Post hoc multiple comparison test was done for grouping the 2 accessions based on their DSI values.

OBSERVATIONS

The Disease Severity Indices of the two accessions of the Black pepper cultivar Karimunda, Karmunda-I and Karimunda-II showed considerable variation as regards to DSI of their

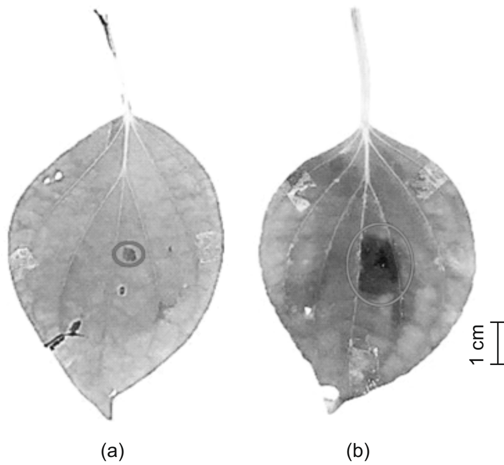

Fig. 2: Photographs showing lesions on the leaves of the 2 accessions of Black pepper cv. Karimunda after 72 h of inoculation. (a) lesion on the leaf of Karimunda-I. (b) lesion on the leaf of Karimunda-II.

leaf, stem and root as well as the average infection rate (Table 1). Photographs showing remarkable variation in the severity of infection expressed as lesions on the leaves of Karimunda-I and Karimunda-II after 72 h of inoculation are given in Fig. 2a and 2b respectively. ANOVA of the average DSI of the 2 accessions revealed that they differ significantly at 1% level. Turkey HSD Post hoc multiple comparison test showed that the mean DSI of the 2 accessions belong to 2 different subsets.

DISCUSSION

The cv. Karimunda possesses certain ideal agro-morphological traits, enabling easy maintenance of its stands by farmers in their fields on relatively small-sized living standards (Anchana et al. 2020). Two accessions of the cv. Karimunda, Karimunda-I and Karimunda-II, were subjected

TABLE 1: DSI of the 2 accessions of Black pepper cv. Karimunda, Karimunda-I and Karimunda-II.

Sl. No.	Acc. No.	Acc. Code	DSI % Leaf infection	Stem infection	Root infection	Average
1.	FGB/P/024	Karimunda-I	80	50	70	66.66
2.	FGB/P/029	Karimunda-II	100	90	100	96.66

to screening studies against '*Phytophthora* foot-rot'disease. The study showed that the average DSI of the 2 accessions differ from each other despite the accessions belong to the same cv. Karimunda (Table 1). Analysis of variance (ANOVA) of the DSI of the 2 accessions revealed that the variation in DSI of the accessions is significant at 1% level. Turkey HSD Post hoc multiple comparison test also showed that the DSI of the 2 accessions belong to two different subsets and corroborated the result of ANOVA that the variation in DSI of the 2 accessions is significant. Therefore, it may be inferred that the 2 accessions, Karimunda-I and Karimunda-II, differ remarkably from each other in their capability to resist '*Phytophthora* foot-rot' disease. The results of the study indicate that the differential response to the disease exhibited by the 2 accessions is due to difference in their genetic makeup. Hence, it is assumed that Karimunda-I genome possesses certain genes/alleles other than those present in Karimunda-II genome, and the presence of which might have led to the increased disease resistance exhibited by Karimunda-I. The concept of polygenic and quantitative inheritance of disease resistance in crop plants supports this postulation.

The better performance of the Karimunda -I than Karimunda-II with regard to disease resistance is significant from the point of view of cultivation of the crop as well as breeding for resistant lines of the cv. Karimunda against '*Phytophthora* foot-rot' disease. The present findings assume added significance since cv. Karimunda is a high yielding cultivar, cultivated throughout Kerala and elsewhere. The cv. Karimunda is generally considered to be highly susceptible to '*Phytophthora* foot-rot' disease. The DSI value (96.66%) exhibited by the accession, Karimunda-II, fully corroborate the high susceptibility of cv. Karimunda to the 'Quick wilt' disease. In contrast, the DSI (66.66%) exhibited by the accession Karimunda-I suggest that there is intracultivar variation in cv. Karimunda as regards to resistance to '*Phytophthora* foot-rot' disease. Mammootty et al. (2008) have also reported that 2 different accessions of cv. Karimunda expressed significant variation in mortality rate against '*Phytophthora* foot-rot'. Intracultivar morphological variation has also been reported in the high yielding, hybrid cultivar of Black pepper, Panniyur-I (Ravindran, personal communication).

Intracultivar genetic variability in Black pepper is an unexplored area, which is potential enough to be utilised for appropriate interventions for increasing crop production. Such interventions include (i) identification and selection of suitable intracultivar genotypes appropriate for cultivation in various farmlands, which may differ in micro agro-ecosystems, and (ii) utilisation of such genotypes as putative parents in breeding disease resistant lines of the crop, especially for pyramiding disease resistant genes/alleles in an ideal cultivar. In this backdrop, the present finding of added resistance exhibited by Karimunda-I is prospective from the point of view of both cultivation and crop improvement in Black pepper. Intracultivar phenotypic variations are getting originated and evolved in agricultural ecosystems, and the changes acquired in their genetic makeup are the result of mutations or natural hybridizations followed by farmer's selection. Hence, identification of intracultivar forms in diverse, elite cultivars of Black pepper is significant from the point of both conservation and effective utilisation of genetic resources of the crop. This also finds importance for meeting the conventional challenges in crop production such as biotic and abiotic stresses as well as the newly emerging problems associated with the change in climatic patterns.

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RESEARCH ARTICLE

GC-MS AND FTIR ANALYSIS OF LEAF EXTRACT OF *PSEUDERANTHEMUM BICOLOR* (SCHRANK) RADLK. EX LINDAU

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SUMMARY Leaves of *Pseuderanthemum bicolor* were extracted with methanol, chloroform and ethyl acetate. The extracts were subjected to screen the phytochemical constituents through Gas Chromatography Mass Spectrometry (GC-MS) and Fourier-Transform Infrared Spectroscopy (FTIR) analysis. The GC-MS analysis of crude extracts revealed the presence of 38 phytochemical compounds, the major compounds were, N,N-dimethyl-2-aminoethanol, 2-propanone. 1(dimethyl amino), 2-propanone, 1-dimethyl amino, methane diamine, N,N,N,N'-tetramethyl, betaine hydrochloride, hexatriacontane, di-N-decyl sulfone, 1H-tetrazol-5-amine, 2,3-epoxy hexanol, 3-decyn-2-ol, methylene chloride, octadecanal, squalene, heptacosane, 2,4-dimethyl-7-oxo-4,7-dihydro, 3,7,11,15-tetramethyl-2-hexadecane-1-ol, decane,1-iodo. FTIR studies confirmed the presence of functional groups of phytocompounds N-H, O-H, C=C, C-H, C-O, CH_3 with their respective peaks. From the present findings, it can be concluded that *P. bicolor* leaves contain effective bioactive compounds of pharmacological importance.

Keywords: *Pseuderanthemum bicolor,* FTIR, GC-MS, bioactive compounds, NIST.

INTRODUCTION

Plants are the major source used in traditional medicine and have stood up to the test of time and reveal many novel compounds for preventive and curative medicine to modern science (Sharmila et al. 2019). Ancient traditional medicinal practices such as, Indian ayurveda, unani and Chinese systems are mainly based on a large diversity of medicinal plants (Karthika et al. 2016). Plants are the rich source of phytochemical constituents which are biologically active. Generally, the secondary metabolites are considered as phytochemical constituents. They are organic compounds having definite structural arrangements and properties. Although these compounds have no direct involvement in the normal growth and development or reproduction, they act as competitive weapons against other living organisms (Vanitha et al. 2011). Several studies have shown that many ornamental plants also have medicinal properties. Phytochemical constituents of ornamental plants are pharmaceutically important. *Pseuderanthemum bicolor,* commonly known as "limang-sugat"belongs to the family Acanthaceae, an important ornamental undershrub. Traditionally, the decoction of aerial parts of the plant used for aphthae, also used as a cicatrizant for wounds and ulcers. The Gujjar tribe of sub-Himalayan tract, Uttarakhand, India, used the plant paste on boils. Leaves are fried in mustard oil and applied externally on cracked feet. The pharmacological studies suggest

antimicrobial, antipyretic, antiinflammatory, hepatoprotective, CNS depressant and anti-convulsant properties (Godofredo Stuart 2019). The present work was carried out to identify the phytochemical constituents and functional groups in the methanol, chloroform and ethyl acetate extracts of the leaf of *P. bicolor* by GC-MS and FTIR technique respectively to ascertain the medicinal properties of the plant.

MATERIAL AND METHODS

Pseuderanthemum bicolor plants were collected from Gudemaranahalli, Magadi taluk, Rama-nagaram district (13.051951˚N, 77.263177˚E) and got authenticated by late Dr. K. Gopala-krishna Bhat, Taxonomy Research Centre, Department of Botany, Poornaprajna College, Udupi, Karnataka. Voucher specimen was deposited in the Herbarium of Department of Botany, Bangalore University, Bengaluru.

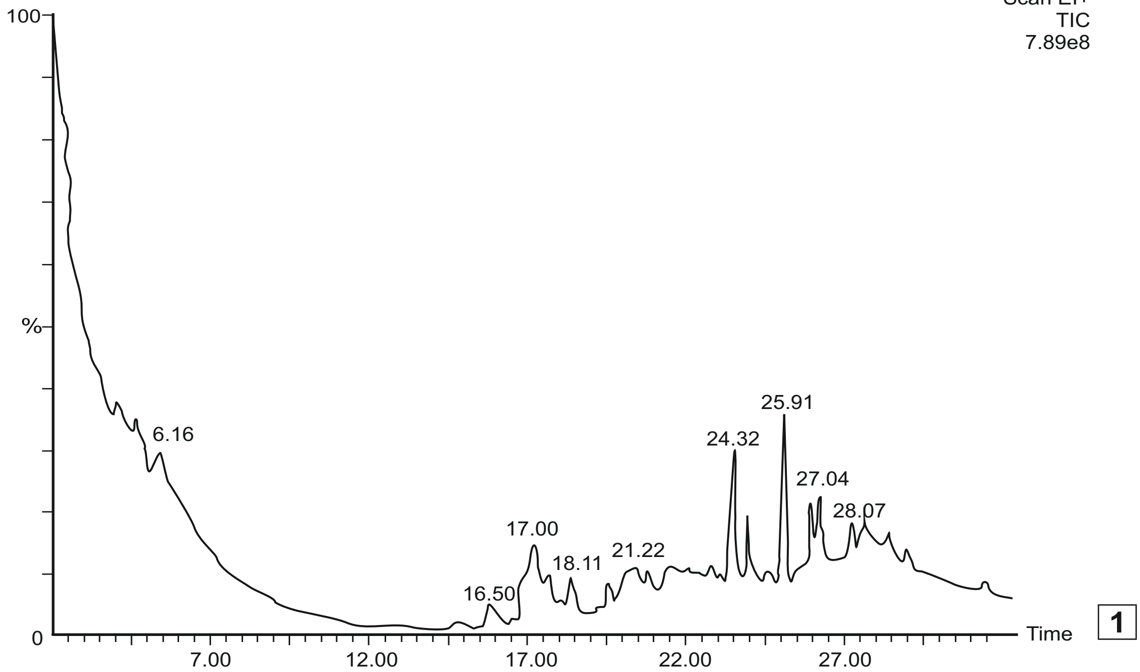
Fresh leaves were collected and washed under the running tap water and shade dried for 8–10 d. The dried leaves were ground into fine powder using mixer grinder. 15 g of powder was mixed with 90 ml of organic solvents viz., methanol, chloroform and ethyl acetate for 4 h in water bath at 50˚ C. The contents were filtered through the Whatman No.1 filter paper. The extract obtained was allowed to evaporate. The condensed extracts were stored in microcentri-fuge vials at 4˚ C till further use (Leena Palakkal et al. 2017).

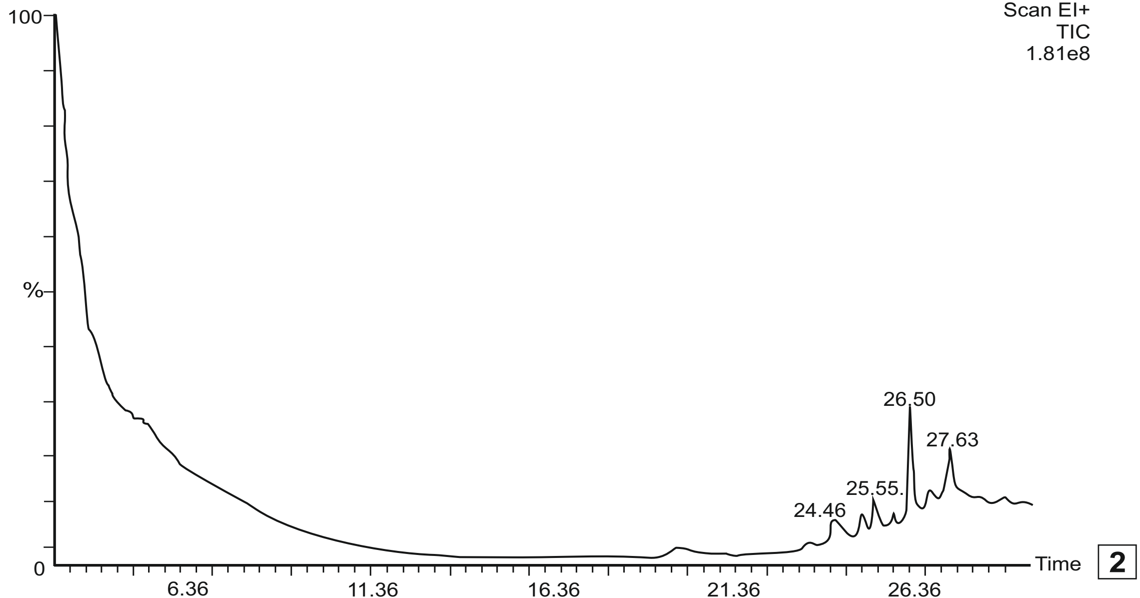
For GC-MS analysis, 3 different leaf extracts viz., methanol, chloroform and ethyl acetate were sent to sophisticated instrumentation facility, Vellore Institute of Technology (VIT) University, Vellore. The Clarus 680 gas chromatograph-(Perkin Elmer) comprising of AOC 20i auto-sampler and a gas chromatograph interfaced to a mass spectrometer (GC-MS) was used to analyse the chemical composition of leaf extracts. It consists of a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethyl-polysiloxane, 30 m × 0.25 mm ID × 250 µm df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. During the chromatographic run, the injector temperature was adjusted at 260° C. 1 µl of plant sample was administered into the instrument through injector, the oven temperature was regulated at 60° C for 2 min, followed by 300° C at the rate of 10° C/min, and it was clenched at 300° C for 6 min. The mass detector temperature was set at 230° C for transfer line, while the ion source temperature was also set at 230° C. Mass spectra of ionization mode electron impact were taken at 70 eV, a scan time of 0.2 s and scan interval of 0.1 s, and fragments from 40 to 600 Da had been recorded. The relative percentage of spectrum of the components was calculated by comparing its average peak area to the total areas. The database of spectrum of known components was stored in the GC-MS NIST (2008) library (Mc Lafferty 1986, Kalaiselvan et al. 2012, Sithara et al. 2017, Otuokere et al. 2016).

Dried powder of 3 different solvent extracts of *P. bicolor* leaves were subjected to FTIR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet in order to prepare translucent sample discs. The powdered sample of each leaf extract was loaded in FTIR spectroscope (Shimadzu, IR Affinity 1, Japan), with a scan range from 400–4000 cm^{-1} with a resolution of 4 cm^{-1} (Ashok Kumar & Rama-swamy 2014, Sithara et al. 2017). The absorbance thus obtained was analysed using origin pro (v 8.5) software and the peaks were constructed.

OBSERVATIONS

GC-MS analysis revealed the occurrence of 38 phytocompounds from 3 different leaf extracts of





Figs 1&2: GC-MS spectra of leaf extracts of *P. bicolor.* 1. Ethyl acetate extract, 2. Chloroform extract.

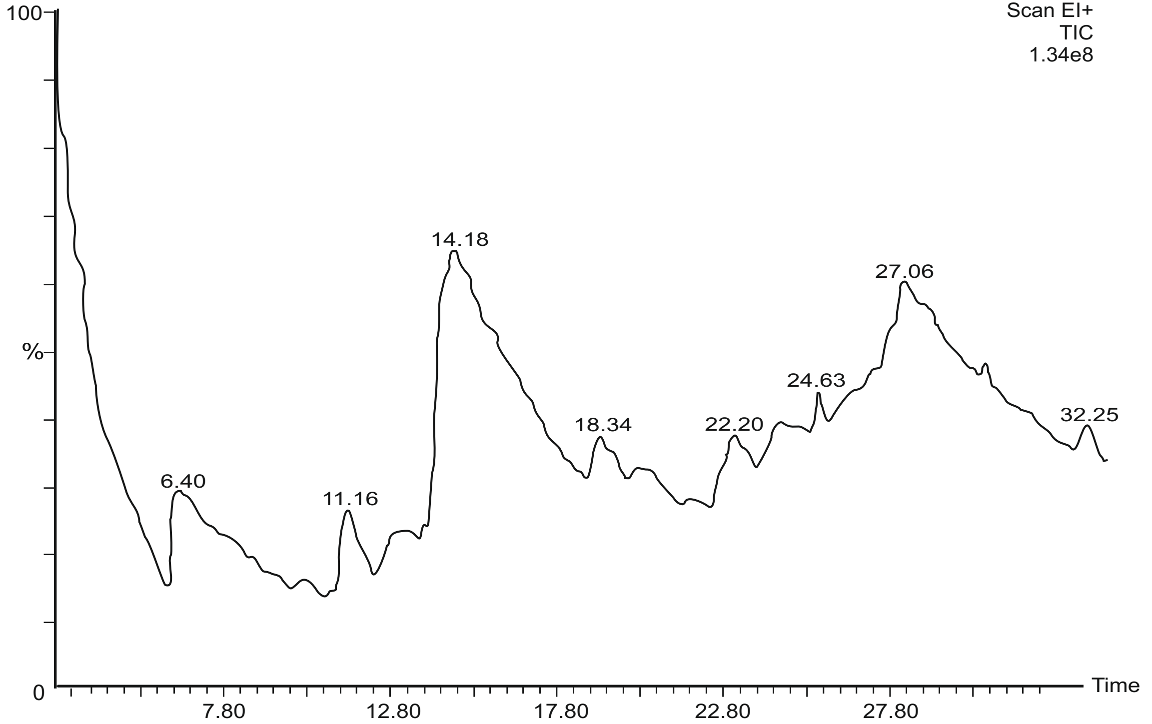


Fig. 3: GC-MS spectrum of methanol leaf extract of *P. bicolor.*

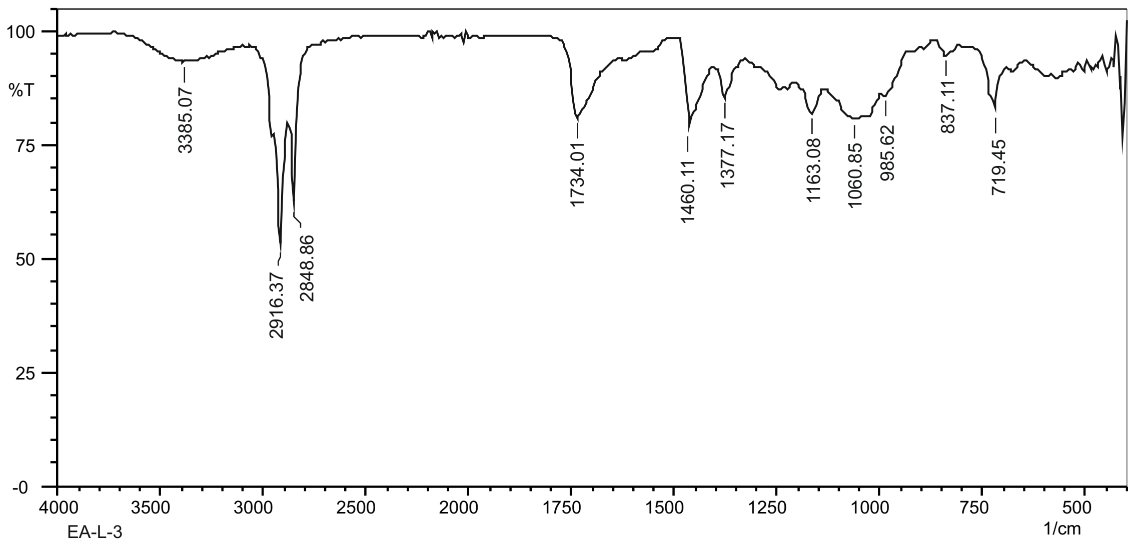
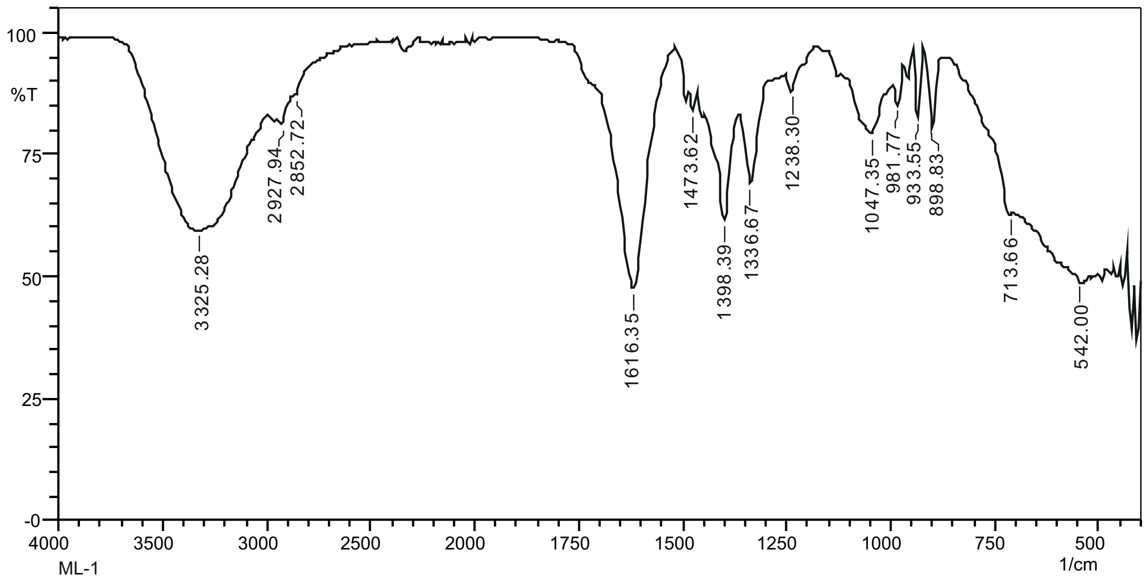
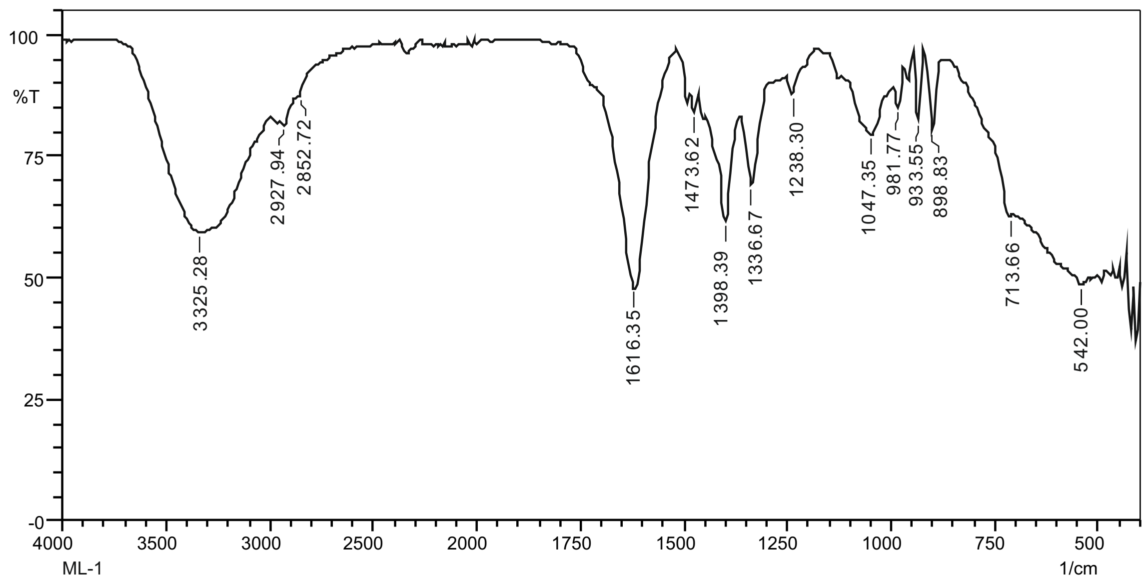


Fig. 4: FITR spectrum of ethyl acetate leaf extract of *P. bicolor.*





Figs 5&6: FTIR spectra of leaf extracts of *P. bicolor.* 5. Chloroform extract, 6. Methanol extract.

TABLE 1: Phytochemical analysis of *P. bicolor.*

Extract	Retention time (min)	Compound	Peak area %
Methanol	6.325	Betaine hydrochloride	7.231
	13.873	2-propanone, 1-(dimethylamino)-	18.654
	13.953	N,N-dimethylglycine	4.102
	13.993	2-propanone, 1-(dimethylamino)-	8.489
	14.163	N,N-dimethyl-2-aminoethanol	23.497
	14.318	L-alanine, N-methyl-	5.521
	14.418	2-propanol, 1-(dimethylamino)-	6.508
	14.563	Methanediamine,N,N,N',N'-tetramethyl-	7.649
	16.154	Dimethylaminomethyl-isopropyl-sulfide	3.254
	22.271	N,N-dimethyl-10-undecen-1-amine	4.643
	24.637	1,6;2,3-dianhydro-4-deoxy-.beta.-D-ribo-hexopyranose	5.349
	27.038	Pseduosarsasapogenin-5,20-dien methyl ether	5.618
	27.273	2-[1,2-dihydroxyethyl]-9-[.beta.-D- ribofuranosyl]	4.128
Chloroform	3.038	Methylene chloride	4.350
	4.104	Methylene chloride	1.968
	24.472	(2s,3s)-(-)3 propyloxiranemethanol	3.953
	25.212	3-decyn-2-ol	5.025
	25.542	1H-tetrazol-5-amine	12.664
	26.073	2-T-butylperoxy-2-ethylbutan-1-ol, butyrate ester	3.288
	26.483	Hexatriacontane	48.874
	27.123	2,3-epoxyhexan	5.526
	27.613	Di-n-decylsulfone	14.353
Ethyl acetate	16.569	Decane, 1-iodo-	6.319
	17.654	Hexadecane	5.765
	17.834	1-decanol, 2-ethyl-	2.440
	17.984	Octadecanal	20.802
	18.445	3,7,11,15-tetramethyl-2-hexadecen-1-ol	6.420
	19.095	Heptadecane, 2,6-dimethyl-	3.069
	20.320	Hydroxylamine, o-decyl-	3.091
	24.307	Squalene	10.474
	24.752	Hexatriacontane	4.926
	25.903	Heptacosane	10.352
	26.713	2,4-dimethyl-7-oxo-4,7-dihydro-triazolo(3,2-c) triazine	6.901
	26.783	2-propenoic acid, oxybis (methyl-2,1- ethanediyl) ester	4.692
	27.028	Dotriacontane	3.623
	28.063	1-hexyl-2-nitrocyclohexane	2.874
	28.434	2,2-dibromocholestanone	5.731
	29.794	Hexadecane, 1,16-dichloro-	2.521

TABLE 2: FTIR analysis of Functional groups in leaf extracts of *P. bicolor.*

Extract	Functional group	Absorption frequency
Methanol	Alcohol	3325.28
	Alkane	2927.94
	Alkane	2852.72
	Aromatic	1473.62
	Alkane	1398.39
	Nitriles	1336.67
	Carbonyl	1238.30
	Alkyl halide	1047.35
	Alkene	981.77
	Alkyl halide	713.66
	Alkyl halide	542.00
Chloroform	Alcohol	3387.00
	Alkane	2918.30
	Aldehyde	2848.86
	Aldehyde	1735.93
	Aromatic	1452.40
	Nitro compound	1375.25
	Alkyl and aryl halides	1163.08
	Alkyl and aryl halides	1083.99
	Alkyl and aryl halides	1022.27
	Aromatic	835.18
	Halogen compound (Chloro)	719.45
	Halogen compound (Iodo)	567.07
	Halogen compound (Iodo)	491.85
Ethyl acetate	Alcohol	3385.07
	Alkane	2916.37
	Aldehyde	2848.86
	Aldehyde	1734.01
	Aromatic	1460.11
	Nitro compound	1377.17
	Alkyl and aryl halides	1163.08
	Alkyl and aryl halides	1060.85
	Alkanes	985.62
	Alkyl and aryl halides	837.11
	Alkyl and aryl halides	719.45

P. bicolor. Ethyl acetate, methanol and chloroform extracts showed the presence of 16, 13 and 9 phytocompounds respectively (Table 1). Among these, hexatriacontane is present in both chloroform and ethyl acetate extracts. The identification of phytochemical compounds were confirmed on the basis of retention time, peak area and molecular weight and formula. Phytoconstituents obtained through GC-MS analysis was interpreted by using the database of (NIST) library (Punniyakotti & Vijaya Anand 2018, Ponnamma & Manjunath 2012, Keerti Gautam et al. 2013). GC-MS chromatogram of all 3 leaf extracts are shown in Figs 1–3.

The FTIR spectrum characterization of the crude extracts, revealed the presence of significant functional groups belonging to the major classes of secondary metabolites like alkaloids, phenols, flavonoids, terpenoids, glycosides and steroids (Figs 4–6, Table 2).

DISCUSSION

Leaf extracts of *P. bicolor* revealed the presence of 38 phytochemicals. Among these, hexatriacontane is common in both chloroform and ethyl acetate extracts, but absent in methanol extract. Soosairaj & Dons (2016) recorded hexatriacontane in ethanol leaf extract of *Justicia tranquebariensis*. Octadecanol was reported by Yakubu et al. (2018) in ethyl acetate fraction of the whole plant extract of *Dyschoriste perrottetii*. The results obtained during the present investigation are in conformity with those of Soosairaj & Dons (2016) and Yakubu et al. (2018). Heptocosane is an important phytocompound reported in ethanol leaf extract of *Barleria courtallica* (Sujatha et al. 2017).

Squalene was obtained in methanolic leaf and whole plant extracts of *Justicia adhatoda* (Jayapriya & Shobha 2015) and *Andrographis neesiana* (Sivapriya et al. 2019). It was also reported in ethanol leaf extract of *Ruellia patula* (Karthika et al. 2016) and *Barleria courtallica* (Sujatha et al. 2017). During the present investigation also, squalene is reported for the first time in *P. bicolor*. From the above studies, it is clear that squalene is found to occur in many members of the family Acanthaceae. The identified compounds may have medicinal value and possess various pharmaceutical applications. The present findings will give scope for identifying new phytocomponents for establishing potential source of useful drugs. FTIR analysis confirmed the presence of diverse functional groups and it has been proved to be an effective and sensitive tool for detection of biomolecular composition (Sharmila et al. 2019). The results of the present study will pave the way to identify other bioactive compounds in *P. bicolor* to resolve their efficacy by in vivo studies and to develop novel safer drugs.

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RESEARCH ARTICLE

KARYOLOGY OF GALL FORMING APHID SPECIES ON *SORBARIA* SP. AND *PRUNUS PADUS* FROM KULLU, HIMACHAL PRADESH

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SUMMARY Present study was undertaken to investigate the gall forming aphids of Kullu, Himachal Pradesh. Gall aphids were collected from the 2 different host plants i.e. *Sorbaria* sp. and *Prunus padus*. Two gall forming aphid species *Myzus sorbi* and *Rhopalosiphum padi* were found to infest these host plants. Galls were found on the leaves of hosts plants. *M. sorbi* formed leaf roll or leaf-curl on the leaves of host plant *Sorbaria* sp. *R. padi* formed pocket galls on the leaves of host plant, *Prunus padus,* feeding on the young leaves which become longitudinally rolled or folded to enclose the aphid colony. The diploid chromosome number in *M. sorbi* was found to be 12. In *R. padi* the diploid chromosome number was found to be 8. The karyotypes in both the species have been analysed. The chromosomes are holocentric. Alates migrated in the months of May and June to numerous grasses and cereals.

Keywords: *Myzus sorbi*, *Rhopalosiphum padi,* gall aphids, holocentric chromosome karyotype.

INTRODUCTION

Aphids, the plant lice or green flies which they often called represent a large group of small soft bodied sap sucking homopteran insects (Ghosh 2008). These are the most destructive insect pests of the cultivated plants in the temperate regions (McGavin 1993). These insects are also responsible for transmitting a number of plant virus diseases (Kennedy et al. 1962). There are About 5000 valid species of aphids found around the world (Blackman & Eastop 2015, Favret 2020). Out of 4700 known aphid species world-wide, approximately 10–20% are gallicolous (Chakrabarti 2007). About 700 are gall forming aphid species which induce galls on coniferous and deciduous trees (Blackman & Eastop 1994).

Galls which are a kind of swelling or growths on the external tissue of plants are the abnormal plant structures induced by various organisms, in particular by the insects (Mani 1964). Weis et al. (1988) stated that exact mechanism of gall formation by insects is still unknown. Galls provide nutrition, optimum temperature and protect the gall aphids from their enemy. Martinez (2009) reported that temperature inside the gall is low during the hottest hour of the day and it is far below the lethal temperature for aphids on *Pistacia*.

Although there is a lot of studies on chromosomes of many aphid species done by

earlier workers, there is a very little information available on chromosomes of gall aphids from Himachal Pradesh. Therefore, an attempt has been made here to expand the chromosomal account of gall forming aphid species from this region. The present paper deals with the karyotypes of two gall forming aphid species viz., *Myzus sorbi* Takahashi and *Rhopalosiphum padi* (Linnaeus).

MATERIALS AND METHODS

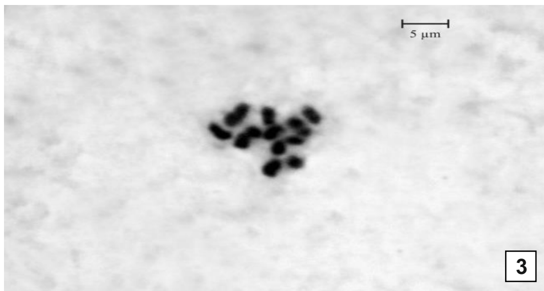
The gall forming aphids were collected from Kullu district of Himachal Pradesh situated at 1965 msl in the months of April and May from leaf galls of *Sorbaria* sp. and *Prunus padus* plants. Galls were observed in the months of April and May for shape, size and colour at regular intervals. For chromosomal study, only apterous, parthenogenetic and viviparous females were used. The embryos were taken out by puncturing the posterior end of abdomen. Then, pretreated in 0.7% sodium citrate solution for 30 min. The pretreated embryos were then fixed in 1:3 acetic acid-ethanol solution for about 15–20 min at room temperature. After fixation, embryos were placed on a glass slide in a drop of 45% acetic acid for 3–5 min. A cover slip was put on the material. Staining of slides was done with 2% Giemsa. Well spread metaphase plates were selected and lengths of chromosomes were measured using ocular micrometer. Photomicrography was done by LEICA DML-S2 microscope fitted with LEICA DFC-320 camera. From actual lengths, the total complement length (TCL) and relative lengths of chromosomes were calculated for each species.

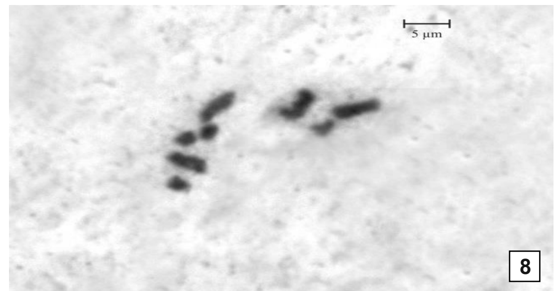
For identification of species, keys developed by Blackman & Eastop (1984) were used. The whole mounts of aphids were prepared. Specimens were gently boiled in 95% alcohol for 2–5 min and then, alcohol was pipetted out and 10% KOH solution was added up to 1 cm depth. Thereafter, specimens were again boiled till they become transparent. Then, KOH solution was pipetted out and the specimens were washed to remove all the KOH using 2–4 changes of distilled water. After that aphids were dehydrated by taking through series of progressively higher grades of alcohol from 30% to 100%. Then, these aphids were put into clove oil for 20–30 min for clearing. After clearing, 1 or 2 aphids were transferred to a drop of fairly thin Dibutylphthalate Xylol (D.P.X.) on a clean slide and the appendages were arranged. A clear cover slip was then dipped in xylene and carefully placed onto specimens so as to spread the mountant evenly without trapping air bubble. The slides were then dried in oven at 60˚ C for about one wk. Photography of whole mount slides was done under LEICA M205 C binocular microscope.

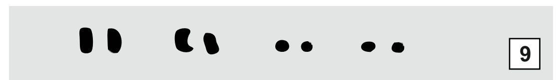
OBSERVATIONS

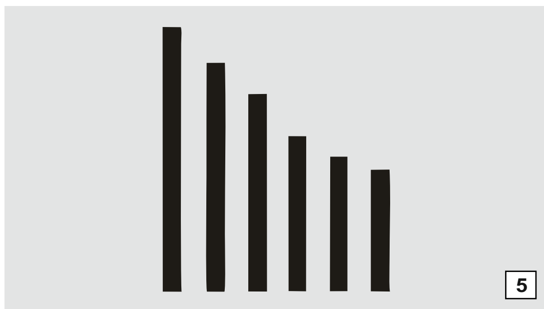
M. sorbi

This aphid species induced leaf roll or leaf-curl enclosing a cavity on the leaves of host plant *Sorbaria* sp. in the month of April and May. Leaves were heavily infested. Bunches of leaf gall and leaf roll were seen on the branches of host plant. Galls were open and can be regarded as pseudogalls (Figs 1, 2). Colour of galls was whitish green. In the present study, Light green colour of parthenogenetic females was observed.









Figs 1–10: 1–5. *M. sorbi.* 1. Galls. 2. Alate. 3. Somatic chromosomes. 4. Karyotype. 5. Idiogram. 6–10. *R. padi*. 6. Galls 7. Alate. 8. Somatic chromosomes. 9. Karyotype. 10. Idiogram.

The diploid chromosome number was found to be 12 (Figs. 3, 4). The chromosomes are holocentric. The mean length of chromosomes ranged from 0.97 µm ± 0.11 S.E. in shortest chromosome to 2.24 µm ± 0.25 S.E. in the longest chromosome. Total complement length was 18.35 µm ± 1.57 S.E. The relative lengths of chromosomes were calculated from actual length data and these ranged from 5.51 ± 0.60 S.E. to 12.00 ± 0.80 S.E. The idiogram was constructed based on relative length data (Fig. 5) and it showed gradual decrease in lengths. Chromosome record of this species is the first report from Himachal Pradesh.

R. padi

This species formed elongate, pocket galls on the leaves of host plant, *P. padus* in the month of April (Figs. 6, 7). Galls were open pockets formed by feeding action of aphids which folded the leaves to ventral side. Galls were formed on the single as well as both edges of leaves. The colour of galls varied from whitish green to red. Galls matured in the month of May and leaves bearing these got dry and eventually fell down.

The diploid chromosome number was found to be 8 (Figs 8, 9). The chromosomes are holocentric. The mean length of chromosomes ranged from 0.97 µm ±0.11 S.E. in shortest chromosome to 3.28 µm ±0.28 S.E. in the longest chromosome. Total complement length was 15.60 µm ±1.15 S.E. The relative lengths of chromosomes were calculated from actual length data and it ranged from 6.40 ±0.67 S.E. to 21.15 ±1.15 S.E. The idiogram was constructed based on relative length data (Fig. 10). It showed gradual decrease in the length of chromosomes.

DISCUSSION

The genus *Myzus* comprises about 55 aphid species. Probably most of them are palaearctic and mostly Asian in origin. Primary hosts of heterocious species are Rosaceae usually *Prunus.* Biology and secondary host association are diverse in this genus (Basu & Raychaudhuri 1976, Blackman & Eastop 1994). Some polyphagous species use trees as their host plant. *M. sorbi* formed leaf roll or leaf-curl on the leaves of host plant *Sorbaria* sp. (Blackman & Eastop 1994). In the present study, similar galls were reported from *Sorbaria* sp. The diploid chromosome number was found to be 12. The mean length of chromosomes ranged from 0.97 µm to 2.24 µm with total complement length 18.35 µm. Khuda-Bukhsh & Pal (1983) reported the same chromosome number for this species.

The diploid chromosome number in *R. padi* was found to be 8. The mean length of chromosomes ranged from 0.92 µm to 3.28 µm. Total complement length was 15.60 µm. Same diploid chromosome number was reported by many earlier workers (Sun & Robinson 1966, Robinson & Chen 1969, Mayo & Starks 1972, Kurl & Misra 1979, Chen & Zhang 1985, Kar & Khuda-Bukhsh 1989, Hales & Cown 1990, Kuznetsova & Gandrabur 1991, De Barro 1992, Valenzuela et al. 2009, Monti et al. 2010, Blackman & Eastop 2015, Gavrilov et al. 2015).

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