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# Efficacy of Certain Common Ferns against Red Spider Mite *Oligonychus coffeae* and Tea Mosquito Bug *Helopeltis theivora* Infesting Tea

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**Abstract**

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In search for botanicals as an alternative remedy to synthetic chemicals in the pest control of tea plantations, ferns such as *Adiantum raddianum, Asplenium aethiopicum, Cyclosorus interruptus, Dicranopteris linearis, Diplazium polypodioides*, and *Pteridium aquilinum* were evaluated against the two major pests of tea, red spider mite *Oligonychus coffeae* Nietner and tea mosquito bug *Helopeltis theivora* Waterhouse, which are the foremost seasonal pests in tea fields, causing severe crop loss. Apart from synthetic chemicals, only the neem kernel extract is recommended in tea fields. The aqueous extracts of ferns were screened at different concentrations against these pests under laboratory and field conditions. The extracts of *P. aquilinum* and *D. linearis* showed good contact toxicity at a 5% concentration to *O. coffeae*. The acaricidal activity was observed in the order *P. aquilinum* > *D. linearis* > *C. interruptus* > *A. raddianum* > *D. polypodioides* > *A. aethiopicum*. Under field conditions, the extract of *D. linearis* and *P. aquilinum* showed a 50% reduction in the population of red spider mite and caused no phytotoxic effect to tea leaves. But their insecticidal activity was less pronounced against *H. theivora.* They exhibit antifeedant activity, which was sustained only for 24 hours. The photochemical screening of extracts showed a qualitatively increased level of saponins in *P. aquilinum*, *D. linearis*, and *C. interruptus*. The study shows that the aqueous extracts of *D. linearis* and *P. aquilinum* can be incorporated in the mite control programme in tea.

**Keywords**: botanicals; aqueous extracts; acaricidal activity; insecticidal activity

Tea occupies a major position among various plantation crops. It is grown in 50 different countries and the prime producers are India, Sri Lanka, China, Japan, Kenya, Malawi, Uganda, Georgia, Turkey, Iran and Argentina (Hazarika *et al*. 2009). Globally, tea cultivation doubled from 1.37 mil to 3.012 mil. ha in 1961–2009 (Ye *et al.* 2014). In India *Camellia sinensis* (L.) O. Kuntze (Fam: Theaceae) has been under cultivation for the past 100 years in 16 states covering an area of 579.35 thousand hectares occupying the second place in area and production next to China. More than 300 different types of pests were reported causing damage to various parts of the plant. It was estimated that 1034 species of arthropods and 82 species of nematodes infest tea plants (Chen & Chen 1989). Among the arthropod pests, Lepidoptera is the largest order comprising 32% of the pest species followed by Hemiptera with 27% (Muraleedharan 1991). Pest damage in tea often leads to a significant impact on productivity, both in

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the short and long term. It can result in almost total debilitation and thus in erosion of the capital assets (Radhakrishnan 2004). Annual crop losses in tea due to pests account for 8% of the production. In South Indian tea plantations the most serious pests of utmost importance are red spider mite (RSM), *Oligonychus coffeae* Nietner (Tetranychidae: Acarina), and tea mosquito bug *Helopeltis theivora* Waterhouse (Miridae: Hemiptera).

Red spider mite and tea mosquito bug are seasonal pests. The former occurs during summer months and the latter during rainy winter. RSM is a polyphagous pest that feeds on coffee, rubber, indigo, grape, cashew nut, citrus, mango, *Camella*, camphor, mulberry, oil palm, and many other tropical plants (Jeppson *et al*. 1975). It normally infests the upper surface of mature tea leaves, feeds along the midrib and veins and gradually spreads to the entire surface of the leaf, thereby changes the colour of the leaf to ruddy bronze. In severe infestation it damages the younger and older leaves and ultimately leads to defoliation and debilitation of the tea bush causing a crop loss of 14–18% (Radhakrishnan 2004). Both adults and nymphs of TMB suck the juice of young leaves and shoots with their needle-like rostrum. They cause drying up of shoots by their feeding behaviour and oviposition, which leads to severe crop losses ranging from 20% to 100% (Muraleedharan *et al.* 2001).

Several synthetic chemicals are under usage to control these pests in tea plantations. Due to various environmental concerns, there is an urge to find out the biopesticidal property from plants. The usage of substances from native plants in the form of oils, extracts from leaf, root, fruit, and seed has been evaluated since ages. Plants produce various bioactive compounds in the form of secondary metabolites such as alkaloids, glycosides, flavonoids, tannins, saponins for their survival against pests and diseases and also to attract the insects for their pollination. Different types of plant preparations such as powders, solvent extracts, essential oil, and whole plants are being investigated for their insecticidal activity including their action as fumigants, repellents, antifeedants, anti-oviposition and insect growth regulators (Isman 2000).

In India, so far 12 biopesticides have been registered under Central Insecticides Board, India comprising 80% of the neem formulation against various agricultural crops. In tea, only a neem kernel aqueous extract (NKAE) is registered for the control of RSM. In organically cultivated tea gardens of South India NKAE paraffinic oil and sulphur are the two recommended safe acaricides for the control of RSM and no other formulation is available for the tea mosquito bug. Despite increasing reports on the evaluation of medicinal plants in crop protection, only few are getting formulated and evaluated in laboratory, greenhouse, and field conditions and get approval for recommendation in plantation. Many plant products and their components of angiosperms are evaluated from time to time against various tea pests and diseases (Roobakkumar *et al.* 2010; Roy & Mukhopadhyaya 2012; Vasanthakumar *et al.* 2013). As a new approach, ferns were selected for study. They are a primitive group of vascular plants in which their existence dates back 350 million years ago. They were among the oldest herbs distributed widely in tropics. In India, about 800 fern species were reported and represented mostly from Western Ghats and Himalayas. Although 9300 species of insects were reported to use ferns as a food source (Crooper-Driver 1978), the insecticidal activity of ferns was reported by various authors (Jones & Firn 1978; Kubo *et al.* 1983; Rajendran & Reuben 1991; Amer & Mehlhorn 2006; Shukla & Tiwari 2011) and hitherto there is no report against tea pests.

The objective of the study focused on the evaluation of aqueous extracts of commonly available ferns in and around tea plantations on their lethal and sublethal dose against two important arthropod pests in tea *O. coffeae* and *H. theivora* under laboratory conditions. Considering the results of laboratory efficacy, the extracts were also evaluated under field conditions by adopting simple handling protocols for the benefit of planters.

## MATERIAL AND METHODS

***Plant materials.*** The ferns (Table 1) were collected from different areas of tea fields in Valparai (latitude: 10°22'12''N and longitude: 76°58'12''E, 1050 m a.s.l.), Coimbatore district, Tamil Nadu, India. The plant materials were washed three times in tap water and shade dried for two weeks. As the leaflets turned slightly brown, they were removed separately leaving their stalk apart. They were crushed using a laboratory blender (Remi Brand, Mumbai, India) and then sieved through a mesh size of 3 mm and the coarse material was removed. The fine powder was packed and labelled in an airtight polypropylene container and stored for further studies.

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Table 1. List of ferns selected for the study

| Sample No. | Name of the ferns | Common name | Family | Parts used | Month of collection (2013) |
|---|---|---|---|---|---|
| 1 | *Adiantum raddianum* C.Presl | Maidenhair fern | Adiantaceae | leaflets | Sept |
| 2 | *Asplenium aethiopicum* (Burm.) Bech. | – | Aspteriaceae | | Sept |
| 3 | *Cyclosorus interruptus* (Wild.) H.Itô | – | Thelypetridaceae | | Oct |
| 4 | *Dicranopteris linearis* (Brum.f.) Underw. | Forked fern | Gleichniaceae | | Oct |
| 5 | *Diplazium polypodioides* Blume | – | Athyriaceae | | Oct |
| 6 | *Pteridium aquilinum* (L.) Kuhn | Bracken fern | Dennstaedticae | | Sept |

***Preparation of aqueous extracts***. The aqueous extract was prepared by adopting an infusion method. In laboratory evaluation 25 g of powdered plant material was taken in a 250 ml conical flask having 100 ml of distilled water. The set up was kept overnight (12 h) in a mechanical shaker for complete extraction. Entire contents were filtered using a double folded muslin cloth and the pure extract was collected. The filtrate was a 25% extract, which was then labelled and kept as a stock solution. The required concentrate was prepared from the stock solution.

In a field study, 1 kg of plant powder was soaked in 5 l of tap water for 24 hours. The solution was filtered out with muslin cloth the next day and labelled as a stock solution. The spray solution of required concentration was prepared by diluting the stock solution.

**Laboratory evaluation of fern aqueous extracts**

*Red spider mite* – The adult mites of *O. coffeae* were obtained from a laboratory culture maintained in an incubator at 25 ± 2°C, 70–75% RH and 12L/12D photoperiod. The assay was carried out by the leaf disc method (Siegler 1947; Ebeling & Pence 1953). Leaf discs were prepared from mother leaves of the TRI 2043 clone from UPASI TRF experimental farm. Five leaf discs of 2 cm in diameter were prepared and placed with its ventral surface down over the wet cotton put in a Petri plate (9 cm diameter) and each disc represented a replicate. Ten adult unsexed mites were released on each disc with a spotting brush and allowed to settle. The aqueous extracts of ferns were sprayed at 1, 2, 3, 4, and 5% (w/v) concentrations using a glass atomiser (with constant pressure 2.5 g/cm$^2$; spray deposition of 4.75 ± 0.1 mg/cm$^2$) at a distance of one foot from the plate to ensure the fine spread of droplets.

The chemicals recommended for the control of red spider mite by Central Insecticides Board, India such as propargite 0.12% (Omite 57 EC 0.57 g a.i./ml), paraffinic oil (Tea spray oil®) 0.34% and NKAE 5% concentrations were kept as standards. The deionised water sprayed discs served as negative control. Observations were carried out after treatment at every 24 h interval for four days. The mites that did not show any response to the touch with brush were recorded as dead and the cotton trapped ones were not considered for analysis either.

*Tea mosquito bug* – The efficacy of fern extracts was also evaluated against unsexed adults of tea mosquito bug, *H. theivora*, obtained from a laboratory colony. Fresh shoots were collected from the unsprayed tea field. Seven shoots (3 leaves and buds) were taken in small bottles of 20 ml capacity having water inside them by wrapping with absorbent cotton to avoid drying of shoots. The aqueous extracts of ferns were evaluated at different concentrations (w/v) like in mites and the shoots were kept inside the transparent plastic jars of 5 l capacity. Ten adults of *H. theivora* were released inside the jars. The extracts were sprayed using a glass atomiser (with constant pressure 2.5 g/cm$^2$; spray deposition of 4.75 ± 0.1 mg/cm$^2$). The mouth of the jar was covered with nylon mesh immediately after spraying to avoid the escape of adults.

Five replicates were maintained for each treatment and deionised water sprayed leaves served as control. The recommended chemical Ekalux (Quinalphos 25 EC 0.25 g a.i/ml) at 0.22 % was kept as positive control. Their mortality rate was assessed at 24-h interval for four days (96 h). The number of feeding punctures in the leaves was also counted and recorded during the study. The antifeedant index was calculated for each treatment compared with the control by adopting the formula of Isman *et al.* (1990): Antifeedant index = (C – T/C + T) × 100; where C – number of feeding punctures in the control; T – number of feeding punctures in a treatment.

**Phytochemical screening**

The aqueous plant extracts were screened for the presence of saponins, alkaloids, tannins, and phenols

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qualitatively following the methods of Harborne (1973) and Trease and Evans (1989).

*(a) Test for saponins – Foam test*: a drop of 1% sodium carbonate was added into a test tube containing about 5 ml of the aqueous extract. The mixture was shaken vigorously and kept for 3 minutes. Formation of honeycomb-like froth showed the presence of saponins.

*(b) Test for phenols – Ferric chloride test*: to 1 ml of the alcoholic solution of a sample, 2 ml of distilled water followed by a few drops of neutral 10% ferric chloride solution were added. Formation of blue or dark green colour indicated the presence of phenols.

*(c) Test for tannins – Lead acetate test*: to 5 ml of the extract, a few drops of 1% lead acetate solution were added. Formation of a yellow or red precipitate indicated the presence of tannins.

*(d) Test for alkaloids – Dragendorff's test*: about 10 g of the air-dried powder of the plant were extracted with 50 ml of diluted hydrochloric acid. The acidic filtrate was rendered alkaline with ammonium hydroxide and extracted with three successive portions (each 15 ml of chloroform). The chloroform extracts were evaporated till dryness and the residues were dissolved in 2 ml of diluted hydrochloric acid. When Dragendorff's reagent added to the residue solution, an orange precipitate was formed, which indicated the presence of alkaloids.

**Evaluation in an experimental farm with natural mite infestation**

Based on the laboratory assays, the acaricidal activity (observed high in laboratory tests) was evaluated under field conditions.

The field efficacy was carried out in organically a cultivated three-years-old (year from pruning) tea field, raised by seedlings with a spacing of 4 × 4 feet, located in Paralai estate, Valparai, Coimbatore Dist., Tamil Nadu, India. The infestation level of the red spider mite was calculated by randomly collecting 100 leaves in the field and the area was marked when the mite population was above the Economic Threshold Level (ETL) of four mites per leaf. Field records were checked to ensure there had not been any pesticide application for one month.

Randomised block designed (RBD) plots of six treatments and four replicates were laid out covering 50 bushes per plot. The plants such as *P. aquilinum* (T1) and *D. linearis* (T2), which showed higher mortality under laboratory conditions at 5% concentration were taken for the study and sprayed with 0.05% wetting agent (Triton X) for getting the sticky nature. The efficacy was compared with standard treatments, paraffinic oil (Tea spray oil) 0.34% (T3), NKAE (T4) (prepared as per standard procedure of UPASI Recommendation, 2010), Propargite 57 EC 0.12% (Omite) (T5), and water sprayed (T6) – negative control.

The mite population was calculated before spraying in each plot by randomly collecting 25 leaves and average mites per leaf were counted. Spraying was carried out twice after pretreatment at a weekly interval using a knapsack sprayer with a spray volume of 450 l/ha at a discharge rate of 450 ± 20 ml/minutes. The standards and spray volume were carried out as per recommendation for the management of *O. coffeae* in south Indian tea cultivation (Muraleedharan 1991). The field efficacy was observed for four weeks using the same sampling procedure.

The intrinsic population growth rate (ri) of mites was also calculated by using the formula of Stark and Banks (2003): $ri = \ln (N_f/N_i)/\Delta t$; where: $N_f$ – final number of live individuals in the sample; $N_i$ – initial number of live individuals in the pretreatment; $\Delta t$ – time (days) of observation after spraying.

The increase or decrease in ri of the mite population will show positive and negative values and zero refers to the stable population of mites during the observation period.

***Statistical analysis of data***. All the experiments were carried out statistically with randomised design. The adult mortality data was corrected with the control using Abbott's formula (Abbott 1925) in the laboratory bioassay and Henderson-Tilton's formula (Henderson & Tilton 1955) in the field evaluation. The results obtained in laboratory and field studies were statistically analysed by ANOVA and a post-hoc test was carried out by adopting Tukey's Multiple Range Comparison method ($P < 0.05$) to determine the significant difference between the treatments. All the analyses were performed in XLSTAT software package version 2015.

## RESULTS

The aqueous extracts of ferns showed acaricidal and insecticidal activity at different concentrations and exposure times. In general, when comparing their bioefficacy, the fern extracts are 100 times more lethal to red spider mite, *O. coffeae*, than to tea mosquito bug.

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Table 2. Efficacy of ferns against red spider mite, *O. coffeae* under laboratory condition (values are mentioned in mean ± SE)

| Plant extracts | Time (h) | Mortality (%) | | | | |
|---|---|---|---|---|---|---|
| | | 1% | 2% | 3% | 4% | 5% |
| *Adiantum raddianum* | 24 | 0.0 ± 0.0$^{a}$ | 0.0 ± 0.0$^{a}$ | 0.0 ± 0.0$^{b}$ | 0.0 ± 0.0$^{b}$ | 0.0 ± 0.0$^{c}$ |
| | 48 | 0.0 ± 0.0$^{a}$ | 0.0 ± 0.0$^{a}$ | 0.0 ± 0.0$^{b}$ | 0.0 ± 0.0$^{d}$ | 0.0 ± 0.0$^{d}$ |
| | 72 | 0.0 ± 0.0$^{b}$ | 0.0 ± 0.0$^{c}$ | 0.0 ± 0.0$^{d}$ | 8.0 ± 4.8$^{d}$ | 34.0 ± 2.4$^{d}$ |
| | 96 | 0.0 ± 0.0$^{b}$ | 0.0 ± 0.0$^{b}$ | 0.0 ± 0.0$^{d}$ | 8.0 ± 4.8$^{d}$ | 34.0 ± 2.4$^{d}$ |
| *Asplenium aethiopicum* | 24 | 0.0 ± 0.0$^{a}$ | 0.0 ± 0.0$^{a}$ | 0.0 ± 0.0$^{b}$ | 0.0 ± 0.0$^{b}$ | 0.0 ± 0.0$^{c}$ |
| | 48 | 0.0 ± 0.0$^{a}$ | 0.0 ± 0.0$^{a}$ | 0.0 ± 0.0$^{b}$ | 0.0 ± 0.0$^{d}$ | 0.0 ± 0.0$^{d}$ |
| | 72 | 0.0 ± 0.0$^{b}$ | 0.0 ± 0.0$^{c}$ | 14.0 ± 4.0$^{c}$ | 18.0 ± 1.2$^{c}$ | 28.0 ± 2.0$^{e}$ |
| | 96 | 0.0 ± 0.0$^{b}$ | 0.0 ± 0.0$^{b}$ | 14.0 ± 4.0$^{b}$ | 22.0 ± 2.0$^{c}$ | 28.0 ± 2.0$^{d}$ |
| *Cyclosorus interruptus* | 24 | 0.0 ± 0.0$^{a}$ | 0.0 ± 0.0$^{a}$ | 0.0 ± 0.0$^{b}$ | 0.0 ± 0.0$^{b}$ | 24.0 ± 2.4$^{b}$ |
| | 48 | 0.0 ± 0.0$^{a}$ | 0.0 ± 0.0$^{a}$ | 0.0 ± 0.0$^{b}$ | 12.0 ± 1.4$^{c}$ | 50.0 ± 1.4$^{c}$ |
| | 72 | 0.0 ± 0.0$^{b}$ | 0.0 ± 0.0$^{c}$ | 12.0 ± 2.0$^{c}$ | 20.0 ± 1.4$^{c}$ | 56.0 ± 1.4$^{c}$ |
| | 96 | 0.0 ± 0.0$^{b}$ | 0.0 ± 0.0$^{b}$ | 24.0 ± 2.0$^{b}$ | 38.0 ± 3.74$^{b}$ | 62.0 ± 5.8$^{c}$ |
| *Dicranopteris linearis* | 24 | 0.0 ± 0.0$^{a}$ | 0.0 ± 0.0$^{a}$ | 0.0 ± 0.0$^{b}$ | 0.0 ± 0.0$^{b}$ | 64.0 ± 1.5$^{a}$ |
| | 48 | 0.0 ± 0.0$^{a}$ | 0.0 ± 0.0$^{a}$ | 0.0 ± 0.0$^{b}$ | 30.0 ± 2.4$^{b}$ | 70.0 ± 2.4$^{b}$ |
| | 72 | 0.0 ± 0.0$^{b}$ | 18.0 ± 1.4$^{b}$ | 22.0 ± 3.7$^{b}$ | 44.0 ± 1.2$^{b}$ | 72.0 ± 1.6$^{b}$ |
| | 96 | 0.0 ± 0.0$^{b}$ | 24.0 ± 2.4$^{a}$ | 32.0 ± 3.7$^{b}$ | 58.0 ± 2.0$^{a}$ | 80.0 ± 3.16$^{b}$ |
| *Diplazium polypodioides* | 24 | 0.0 ± 0.0$^{a}$ | 0.0 ± 0.0$^{a}$ | 0.0 ± 0.0$^{b}$ | 0.0 ± 0.0$^{b}$ | 0.0 ± 0.0$^{c}$ |
| | 48 | 0.0 ± 0.0$^{a}$ | 0.0 ± 0.0$^{a}$ | 0.0 ± 0.0$^{b}$ | 0.0 ± 0.0$^{d}$ | 0.0 ± 0.0$^{d}$ |
| | 72 | 0.0 ± 0.0$^{b}$ | 0.0 ± 0.0$^{c}$ | 0.0 ± 0.0$^{d}$ | 0.0 ± 0.0$^{e}$ | 0.0 ± 0.0$^{f}$ |
| | 96 | 0.0 ± 0.0$^{b}$ | 0.0 ± 0.0$^{b}$ | 0.0 ± 0.0$^{d}$ | 0.0 ± 0.0$^{e}$ | 24.0 ± 2.0$^{d}$ |
| *Pteridium aquilinum* | 24 | 0.0 ± 0.0$^{a}$ | 0.0 ± 0.0$^{a}$ | 24.0 ± 1.4$^{a}$ | 40.0 ± 2.6$^{a}$ | 68.0 ± 1.4$^{a}$ |
| | 48 | 0.0 ± 0.0$^{a}$ | 0.0 ± 0.0$^{a}$ | 28.0 ± 2.4$^{a}$ | 44.0 ± 1.8$^{a}$ | 86.0 ± 1.8$^{a}$ |
| | 72 | 14.0 ± 1.2$^{a}$ | 24.0 ± 2.2$^{a}$ | 32.0 ± 1.6$^{a}$ | 58.0 ± 1.4$^{a}$ | 94.0 ± 2.4$^{a}$ |
| | 96 | 18.0 ± 2.8$^{a}$ | 26.0 ± 4.0$^{a}$ | 40.0 ± 4.4$^{a}$ | 60.0 ± 2.4$^{a}$ | 100.0 ± 0.0$^{a}$ |
| Control – water sprayed | 24 | 0.0 ± 0.0$^{a}$ | 0.0 ± 0.0$^{a}$ | 0.0 ± 0.0$^{b}$ | 0.0 ± 0.0$^{b}$ | 0.0 ± 0.0$^{c}$ |
| | 48 | 0.0 ± 0.0$^{a}$ | 0.0 ± 0.0$^{a}$ | 0.0 ± 0.0$^{b}$ | 0.0 ± 0.0$^{d}$ | 0.0 ± 0.0$^{d}$ |
| | 72 | 0.0 ± 0.0$^{b}$ | 0.0 ± 0.0$^{c}$ | 0.0 ± 0.0$^{d}$ | 0.0 ± 0.0$^{e}$ | 0.0 ± 0.0$^{f}$ |
| | 96 | 0.0 ± 0.0$^{b}$ | 0.0 ± 0.0$^{b}$ | 0.0 ± 0.0$^{d}$ | 0.0 ± 0.0$^{e}$ | 0.0 ± 0.0$^{e}$ |
| Neem kernel aqueous extract (NKAE) 5% | 24 | | | 60.0 ± 1.2 | | |
| | 48 | | | 74.0 ± 1.2 | | |
| | 72 | | | 80.0 ± 1.4 | | |
| | 96 | | | 86.0 ± 2.4 | | |
| Paraffinic oil 0.34% | 24 | | | 64.0 ± 0.8 | | |
| | 48 | | | 72.0 ± 1.4 | | |
| | 72 | | | 80.0 ± 2.0 | | |
| | 96 | | | 84.0 ± 2.2 | | |
| Propargite 0.12% | 24 | | | 100.0 ± 0.0 | | |
| | 48 | | | 100.0 ± 0.0 | | |
| | 72 | | | 100.0 ± 0.0 | | |
| | 96 | | | 100.0 ± 0.0 | | |

$^{a–f}$mean followed by same alphabets are not differ significantly according to Turkey's Multiple Range comparison test ($P < 0.05$); comparison was carried out among the plant extracts corresponding to respective concentration and hours of observation with control

In red spider mite *O. coffeae*, *D. linearis*, and *P. aquilinum* showed acute toxicity compared to other plants at a 5% (w/v) concentration at the end of 96 h of observation, which is shown in Table 2. We found that the efficacy rose significantly over time at a considerable rate. In case of *D. linearis* and *P. aqui-*

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Table 3. Antifeedant activity against tea mosquito bug, *Helopeltis theivora* under laboratory condition

| Plant extracts | Time (h) | Antifeedant index rate* | | | | |
|---|---|---|---|---|---|---|
| | | 1% | 2% | 3% | 4% | 5% |
| *Adiantum raddianum* | 24 | −1.16 ± 1.79$^{a}$ | 3.57 ± 1.86$^{a}$ | 6.67 ± 1.54$^{a}$ | 10.15 ± 1.78$^{b}$ | 16.42 ± 1.09$^{b}$ |
| | 48 | −1.92 ± 1.11 | 1.88 ± 0.7$^{a}$ | 2.85 ± 0.31$^{a}$ | 3.07 ± 0.5$^{a}$ | 5.15 ± 1.66$^{b}$ |
| | 72 | 0.2 ± 0.01 | 0.7 ± 0.1$^{a}$ | 1.0 ± 0.0$^{a}$ | 1.20 ± 0.5$^{a}$ | 1.2 ± 0.82$^{b}$ |
| *Asplenium aethiopicum* | 24 | −1.54 ± 1.86$^{a}$ | 0.14 ± 2.59$^{a}$ | 8.98 ± 1.46$^{a}$ | 14.06 ± 1.88$^{ab}$ | 18.59 ± 2.92$^{b}$ |
| | 48 | −1.07 ± 0.56$^{a}$ | −0.37 ± 0.6$^{a}$ | 3.27 ± 1.7$^{a}$ | 6.4 ± 1.88$^{a}$ | 7.8 ± 1.2$^{b}$ |
| | 72 | 0.1 ± 0.07$^{a}$ | −1.2 ± 0.6$^{a}$ | 0.5 ± 0.2$^{a}$ | 2.0 ± 0.26$^{a}$ | 2.0 ± 1.3$^{b}$ |
| *Cyclosorus interruptus* | 24 | 5.14 ± 3.87$^{a}$ | 1.6 ± 1.52$^{a}$ | 12.92 ± 1.24$^{a}$ | 17.49 ± 1.85$^{ab}$ | 27.33 ± 2.37$^{ab}$ |
| | 48 | −0.54 ± 1.4$^{a}$ | 0.9 ± 0.57$^{a}$ | 5.13 ± 0.2$^{a}$ | 5.95 ± 0.31$^{a}$ | 14.27 ± 1.46$^{ab}$ |
| | 72 | −1.2.0 ± 0.4$^{a}$ | 0.01 ± 0.3$^{a}$ | 1.0 ± 0.05$^{a}$ | 2.0 ± 1.2$^{a}$ | 2.0 ± 1.7b |
| *Dicranopteris linearis* | 24 | −2,32 ± 1.36$^{a}$ | 5.16 ± 1.89$^{a}$ | 13.56 ± 1.19$^{a}$ | 20.19 ± 1.02$^{ab}$ | 34.13 ± 1.84$^{a}$ |
| | 48 | −0.22 ± 1.04$^{a}$ | 2.31 ± 0.8$^{a}$ | 6.31 ± 0.6$^{a}$ | 8.85 ± 1.9$^{a}$ | 22.64 ± 0.52$^{a}$ |
| | 72 | −0.1 ± 0.0$^{a}$ | 0.2 ± 0.01$^{a}$ | 1.01 ± 0.09$^{a}$ | 2.7 ± 1.2$^{a}$ | 8.0 ± 1.39$^{a}$ |
| *Diplazium polypodioides* | 24 | 0.76 ± 1.57$^{a}$ | 0.46 ± 0.6$^{a}$ | 9.22 ± 1.0$^{a}$ | 13.78 ± 2.74$^{ab}$ | 18.59 ± 1.09$^{b}$ |
| | 48 | −0.98 ± 0.2$^{a}$ | −0.6 ± 0.2$^{a}$ | 4.05 ± 0.52$^{a}$ | 6.64 ± 0.99$^{a}$ | 7.62 ± 0.41$^{b}$ |
| | 72 | −1.2 ± 0.4$^{a}$ | 0.02 ± 0.0$^{a}$ | 0.70 ± 0.02$^{a}$ | 0.09 ± 0.01$^{a}$ | 1.07 ± 0.09$^{b}$ |
| *Pteridium aquilinum* | 24 | 1.75 ± 0.75$^{a}$ | 6.85 ± 1.18$^{a}$ | 16.38 ± 1.72$^{a}$ | 25.57 ± 2.22$^{a}$ | 40.08 ± 1.14$^{a}$ |
| | 48 | 1.27 ± 0.38$^{a}$ | 5.29 ± 0.99$^{a}$ | 4.04 ± 0.24$^{a}$ | 12.04 ± 1.6$^{a}$ | 22.05 ± 1.18$^{a}$ |
| | 72 | 0.8 ± 0.12$^{a}$ | 1.4 ± 0.2$^{a}$ | 1.02 ± 0.09$^{a}$ | 2.08 ± 1.2$^{a}$ | 8.0 ± 1.6$^{a}$ |
| Quinalphos 0.22% at 24 h | | | | 76.34 ± 1.07 | | |

*antifeedant rate calculated based on the number of feeding punctures in leaf; values are mentioned in mean ± SE; mean followed by same alphabets are not differ significantly according to Turkey's Multiple comparison test ($P < 0.05$)

*linum* for 24 h from application, the efficacy was 64 and 68%, respectively, at a 5% concentration, i.e. similar to the standards NKAE and paraffinic oil. The synthetic sulphite ester formulation Omite (propargite 0.57 g a.i./ml) showed 100% mortality at 24 h of observation. But on continuous observation (96 h) the efficacy for the two extracts was raised to maximum mortality of 100% in *P. aquilinum* and slightly lower to 80% in *D. linearis.* While the chronic toxicity of *C. interruptus* rose significantly from 24% (24 h) to 62% (96 h) at an increased exposure of the toxicant.

The insecticidal activity of the aqueous extracts of ferns was not reflected against *H. theivora* in the laboratory. The tea mosquito bug showed 100% survival rate against all the extracts evaluated and showed no mortality even upto 72 h of laboratory observation. They showed lower feeding activity to the extracts for 24 h (Table 3) and the punctures caused by them were comparatively less numerous than on water sprayed leaves. But they increased their feeding activity in consequent hours of observation. While the standard treatment Ekalux 0.22% showed 100% mortality after 24 hours.

As a preliminary study on their chemical constituents the ferns were also screened qualitatively for their phytochemical properties which were found to be varied in saponins, tannins, and phenols (Table 4). The presence of major compounds such as alkaloids and saponins was varied among the plants causing acaricidal property. The extracts of *D. linearis*, *P. aquilinum*, and *C. interruptus* showed the increased intensity of saponins by forming dense froth compared to other plants in the qualitative test.

Based on the acaricidal action observed in a laboratory study, the field evaluation of *D. linearis* and *P. aquilinum* extracts under natural mite infestation caused a significant reduction in the mite population on par with NKAE and paraffinic oil (Table 5) and brought down the mite infestation level below ETL after the first

Table 4. Photochemical screening of aqueous fern extracts

| Plants | Tannins | Phenols | Alkaloids | Saponins |
|---|---|---|---|---|
| *Adiantum raddianum* | + | + | − | + |
| *Asplenium aethiopicum* | + | + | − | + |
| *Cyclosorus interruptus* | + | + | + | +++ |
| *Dicranopteris linearis* | + | + | + | +++ |
| *Diplazium polypodioides* | + | + | + | + |
| *Pteridium aquilinum* | + | + | + | +++ |

+ presence; +++ high intensity froth formation; − absence

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Table 5. Field bioefficacy of ferns against red spider mite, *O. coffeae* (values are in mean ± SE from the values of three replicates)

| Treatments | Pretreatment* | After I spray | | | | After II spray | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | I week* | ri | II week* | ri | III week* | ri | IV week* | ri |
| *Dicranopteris linearis* 5% | 6.87 ± 0.24$^{a}$ | 4.60 ± 0.16$^{bc}$ (39.76) | –0.06 ± 0.00 | 6.21 ± 0.18$^{b}$ (14.92) | –0.01 ± 0.0 | 2.93 ± 0.45$^{b}$ (52.03) | –0.11 ± 0.02 | 4.89 ± 0.38$^{b}$ (11.85) | –0.02 ± 0.01 |
| *Pteridium aquilinum* 5% | 6.09 ± 0.25$^{a}$ | 4.83 ± 0.26$^{b}$ (28.64) | –0.03 ± 0.01 | 6.08 ± 0.33$^{b}$ (6.037) | 0.0 ± 0.0 | 2.59 ± 0.11$^{bc}$ (56.69) | –0.12 ± 0.01 | 4.83 ± 0.7$^{b}$ (12.93) | –0.02 ± 0.0 |
| Neem kernel aqueous extract 5% (recom. dose) | 7.05 ± 0.33$^{a}$ | 4.43 ± 0.20$^{bc}$ (49.99) | –0.07 ± 0.01 | 5.57 ± 0.11$^{c}$ (31.72) | –0.02 ± 0.0 | 2.08 ± 0.24$^{cd}$ (64.39) | –0.14 ± 0.02 | 3.85 ± 0.24$^{c}$ (24.58) | –0.03 ± 0.01 |
| Paraffinic oil 0.34% (recom. dose) | 6.39 ± 0.30$^{a}$ | 2.16 ± 0.28$^{c}$ (69.59) | –0.16 ± 0.02 | 3.8 ± 0.15$^{d}$ (44.03) | –0.04 ± 0.0 | 1.15 ± 0.17$^{d}$ (69.23) | –0.17 ± 0.02 | 2.56 ± 0.12$^{d}$ (69.0) | –0.03 ± 0.0 |
| Propargite 0.12% (recom. dose) | 6.64 ± 0.36$^{a}$ | 0.71 ± 0.18$^{d}$ (90.38) | –0.33 ± 0.03 | 1.19 ± 0.31$^{e}$ (83.13) | –0.13 ± 0.0 | 0.11 ± 0.02$^{d}$ (90.6) | –0.34 ± 0.01 | 0.35 ± 0.09$^{e}$ (67.08) | –0.09 ± 0.02 |
| Control | 6.19 ± 0.29 | 6.88 ± 0.24$^{a}$ | 0.02 ± 0.01 | 7.31 ± 0.25$^{a}$ | 0.01 ± 0.01 | 7.19 ± 0.10$^{a}$ | 0.0 ± 0.0 | 6.53 ± 0.39$^{a}$ | –0.01 ± 0.0 |

*average No. of mites per leaf; ri – Intrinsic growth rate of mite population; values in parenthesis indicates bioefficacy compared with control using Henderson-Tilton formula (1955); $^{a-e}$mean followed by same alphabets in superscript are not differ significantly according to Turkey's Multiple Comparison test ($P < 0.05$)

spraying. They did not cause any phytotoxic symptoms by causing any injury on leaf surface, leaf tips, wilting, necrosis, vein clearing, epinasty, and hyponasty. After two continuous applications of extracts at a weekly interval, the mite population was reduced to 50% when compared with the unsprayed control. The bioefficacy increased by 20–30% after two sprays of plant extract.

## DISCUSSION

Among various control measures to bring down the population of mites in a tea field 90% is achieved through chemicals only. In an organic tea field the spraying of inorganic acaricides (lime sulphur and paraffinic oil) and botanical extract (NKAE) is recommended to avoid a major economic loss. Availability of few chemicals and botanicals for recommendation in organic fields causes negative consequences on non-target organisms and also involves development of resistance against them. It can be largely eliminated by search for new botanicals with a potential for the development of botanical acaricides in tea fields (Pavela 2015). The ferns *P. aquilinum* and *D. linearis* dominate with 70% among various ferns in tea fields of Valparai. In our present study, the bioefficacy of aqueous extracts of ferns on their acaricidal and insecticidal property was clearly proved for the first time against *O. coffeae.* In earlier studies on using botanicals against RSM, Roobakkumar *et al.* (2010) observed 100% mortality at 96 h after the spray of Pongam oil at a 5% concentration; the aqueous leaf extracts of *Morinda tinctoria* (Roxb) and *Pongamia glabra* (Vent) (Vasanthakumar *et al.* 2013) and dry pericarp of the fruits of *Terminalia chebula* (Roy *et al.* 2014) caused ovipositional deterrence and 100% adult mortality at a similar concentration. The acaricidal effect of ferns observed in the present study was similar to those reports. The extracts of *D. linearis* and *P. aquilinum* cause 20–100 times higher acaricidal property compared to insecticidal property. The toxicity increases with an increase in time. The exposure of the pest to the treated plant for a long period may then enhance its biological efficacy because some plant constituents may exhibit chronic toxicity (Pavela 2015). Apart from causing adult mortality, they also exhibit antifeedant activity in insects at a minimal level. The ferns would constitute a better resourceful weed in organic tea gardens where there is lacuna in alternative strategies to chemicals. Due to the cost and

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availability of neem kernels the locally available plants can substitute for one or two rounds of the mite control programme.

The relative toxicity of ferns against mites and insects might be attributed to the difference in anatomy and sclerotisation of two different taxa, detoxification mechanism in which the tea mosquito bug was reported earlier on the presence of detoxifying enzymes in their saliva. They form the first resisting barrier against any xenobiotics. Among many angiosperms evaluated against *H. theivora* in the course of time, only few were reported to cause antifeedant property. Dolul and Debnath (2010) observed a good antifeedant effect from the methanolic extracts of *Heliotropium indicum* flowers; the aqueous extracts of *Acorus calamus* caused a less than 50% reduction in their field infestation (Prabhakaran & Radhakrishnan 2014).

Most of the botanicals showed good contact and fumigant toxicity against biting and aquatic insects. Even in lepidopteran pests of tea the oils of *Ocimum basilicum*, *Rosmarinus officinalis*, *Cinnamomum zeylanicum*, *Cuminum cyminum*, and *Pelargonium graveolens* displayed strongest repellency against the male and female adults of *Ectropis obliqua* (Zhengqun *et al.* 2014). In some other insect species such as house fly *Musca domestica* adults and mosquito *Aedes albopictus* the methanol extracts of five fern species, *Cupressus funebris* (leaves and stems), *Cycas acuminatissima* (roots), *Keteleeria fortunei* (leaves and stems), *Onychium japonicum* (whole plant), and *Pinus taiwanensis* var. *Daming shanensis* (leaves and stems) showed more than 50% mortality under laboratory conditions (Huang *et al.* 2010). Moreover, *H. theivora* is a solitary, winged insect and feeds on the leaves by sucking with its well-adapted rostrum rather than the colonial and apterous form of mites that feeds by scrapping and sucking. The mites have a sufficient chance to get contact with sprayed extracts. The synthetic chemicals widely recommended to control tea mosquito are of stomach poison and systemic action in nature. The fern extracts have failed in that property and are not able to cause adult mortality in *H. theivora* which would make the field evaluation as eccentric.

Even though the efficacy observed in ferns was comparable with standard chemicals in both red spider mite and tea mosquito bug, their acute toxicity was comparable with NKAE and paraffinic oil. The former create an oily coating on the leaf and control the mite population by disrupting the respiratory mechanism and the latter cause disruption in moulting and oviposition of the mite. It also suggests that propargite showed far more superior to aqueous extracts and its potential effect is known in controlling the pest population by its contact toxicity. The selection of these standards is due to their widespread usage in south Indian tea plantations. Moreover, using the same positive control facilitates the comparison of the data and evaluation of the potential of new products (Isman & Grieneisen 2014; Zanardi *et al.* 2015).

The plant extracts/bioproducts exhibit reduced bioefficacy under field conditions, which was not similar to the laboratory assay. It happens due to the photodegradation of active compounds by visible or UV or IR rays from sunlight or any other agroclimatic conditions. The findings are coherent with Sarmah *et al.* (2009), who observed 40% lower efficacy in field than *in vitro* study against *O. coffeae* on spraying the aqueous extracts of *Acorus calamus* (L.), *Xanthium strumarium* (L.), *Polygonum hydropiper* (L.), and *Clerodendron infortunatum* (Gaertn). A similar response was observed against various mite species under *in vivo* conditions (Hosamani *et al.* 2007; Anita 2010; Hossain *et al.* 2013; Qayyoum *et al.* 2013; Tehri & Gulati 2014). The above-mentioned factors corroborate the low persistence of fern extracts in our study. Even though the bracken fern *P. aquilinum* proved its carcinogenic compound ptaquiloside (Gomes *et al.* 2011), it is used in traditional medicine in many countries of the world for its substantial antibacterial, antifungal, antiviral, and cytotoxic properties (Wink 2015); it was also evaluated for its insecticidal activity by various authors. Jbilou *et al.* (2008) reported 66% mortality in *Tribolium castaneum* caused by *P. aquilinum.* Sahayaraj and Selvaraj (2013) observed the field efficacy of three fern extracts in the order *Pteridium aquilinum* > *H. arifolia* > *C. parasitica* against *Helicoverpa arrnigera* (Hubner) and *Spodoptera litura* (Fab.) infesting groundnut. Moreover, the DNA damage caused by *P. aquilinum* was proved at a higher concentration of 40 mg/ml in rats (Gomes *et al.* 2011); while the concentration evaluated in our study was at a lower level. On comparing with standard treatments, *D. linearis* and *P. aquilinum* require continuous application at a seven-day interval for better control. This property will help to avoid any residues during harvest or in made tea and can be utilised with a reduced safe harvest interval. It also reduces the possibility of resistance evolution in arthropods (Georghiou & Taylor 1977) and reduces any harmful effect on naturally available

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predators in the field due to their short action period, which helps to build up ecological equilibrium in the agroecosystem (Zanardi *et al.* 2015).

Screening of major phytochemicals in the aqueous extracts of ferns showed the presence of saponins that varied among them and which were higher in intensity in *D. linearis* and *P. aquilinum*. It indicates that the mortality of *O. coffeae* was due to the sensitivity of mites to saponins. The group of heteroside compounds exhibits steroidal structure and possesses a defensive role (Appelbaum 1969) and is able to interrupt larval development (Ishaaya *et al.* 1969). They inhibit digestive proteases by easily dissolving in water and form a foaming solution by their tension activity (Chaieb 2010). The sensitivity of RSM to ferns was higher than that of tea mosquito bug. It was proved by Oleszek *et al*. (1999), who observed a reduction in the survival rate of *Tetranychus urticae* mite by 85% on the spray of aqueous solution of lucerne saponins 0.1–0.2%. The saponins of lucerne also caused mortalities on eggs of *T. urticae.* The most commonly observed effects of saponins are increased mortality, lowered food intake, weight reduction, retardation in development, and decreased reproduction (De Geyter *et al.* 2007). They have the ability to dehydrate the insect tissues in gut, which was reflected in the antifeeding property of *H. theivora*. Even though saponins were represented in *C. interruptus* during the qualitative test, they caused lower mortality than *D. linearis* and *P. aquilinum*. Thus it indicates that some other metabolites or difference in the chemical nature of saponins among ferns might be responsible for its acute and chronic toxicity.

The ferns used in this study were reported for their various medicinal properties such as respiratory problems, hair growth, vermicide, rheumatism, gynaecology, etc. and also as a source of phytoecdysteroids. These phytoecdysteroids provide them the defensive nature to insect attack by disrupting their moulting (Galbraith & Horn 1966). Ainge and Lorimer (2002) isolated the alkaloid huperzine A from *Lycopodium varium* as a major antifeedant and insecticidal component and showed mortality in carpet beetle, *Anthrenocerus australis* at 110 ppm. Many insecticidal lectins have been isolated from ferns. The enzyme thiaminase derived from ferns and moss has been demonstrated for insect resistance activity. Thiaminase exhibited deterrence in feeding on sprayed plants against southern armyworm *Nephrolepis exaltata* (Hendrix 1977). The crude protein extracts of several ferns and mosses caused 70–100% mortality of *Spodoptera frugiperda* and *Helicoverpa zea* (Markham *et al.* 2006). Many insect resistant secondary metabolites such as ferulic acid, hydrolysable tannins, terpenes, and alkaloids (Schaufelberger & Hostettmann 1983; Asakawa 1990) and ecdysones (Jones & Firn 1978; Lafont & Horn 1989) were reported in them. These properties of the ferns *D. linearis* and *P. aquilinum* have constituted them to cause acaricidal property, which would be the first report against *O. coffeae.* The adult mortality is due to interference with ecdysteroid metabolism and inhibition of ecdysis triggering hormones (ETH) (Hincapie *et al.* 2011) and by their intrinsic chemical constituents. These variations in metabolites of *P. aquilinium, D. linearis*, and *C. interruptus* caused considerable acute toxicity in *O. coffeae.*

## CONCLUSION

In view of agronomic efficiency in the tea plantation pest and weed control there are two major constraints. In mitigating these, weeds can be used for the pest and disease control to some extent. The present study clearly states that the species such as *D. linearis* and *P. aquilinum* possess acaricidal and insect repellent activity when applied at a concentration of 5%. Emphasis can be laid on utilising these extracts for further studies in exploring their chemical compounds for the development of new botanical acaricides. This would offer safe and effective alternative strategies to synthetic chemicals for the pest control programme in organic tea fields. On utilising these plants, recycling of weeds would achieve for the small farm holders having limited resources for pest management. Such knowledge throws light on using weeds as botanical insecticides by conserving the pesticide load in tea fields and utilising naturally available resources.

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