Effect of *Raoiella Indica* Hirst (Acari: Tenuipalpidae) Infestation on Chlorophyll and Photosynthetic Efficiency of *Areca Catechue*  

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Abstract— *Areca catechu* L. is one of the most important plantation crop cultivated in Kerala. Recent reports indicate that phytophagous mites could infest the foliage and nuts of areca palm and induce substantial damage, leading to various levels of economic loss. Among the mites, *Raoiella indica* Hirst, popularly called Red Palm Mite (RPM) has been recognized as a serious pest of areca palm in various localities of India. Various life stages of this mite were found colonizing and feeding on the lower surface of palm leaves, by de-sapping and which in turn would lead to the formation of yellowish speckles and bronzing in later stages and the ultimate drying up of the leaves. Photosynthesis, being a vital process determining the plant vigour and productivity should be addressed with great concern. In the present study, attention has been focused to analyse the feeding impact of the red palm mite, on the photosynthetic machinery of areca palms through the quantitative estimation of chlorophyll 'a', 'b', 'total chlorophyll' pigments and also 'photosynthetic efficiency' of mite infested and uninfested leaves. Results of the study revealed that the feeding activity of the mite on the leaves of *A. catechu* induced drastic reduction in the levels of chlorophyll 'a', 'b', 'total chlorophyll' and 'photosynthetic efficiency'. The percentage loss in chl 'a', chl 'b' and 'total chlorophyll' were 62.21±0.63, 57.67±0.73 and 59.884±0.375 respectively in RPM infested Areca leaves. RPM infestation varied significantly in photosynthetic parameters such as minimum fluorescence (F0), maximum fluorescence (Fm), variable fluorescence (Fv), quantum yield of photosynthesis (Fv/Fm), performance index (PI) and the Area which has a negatively impact on photosynthetic efficiency of areca palms. These data obtained from chlorophyll analysis and photosynthetic efficiency were found significant at 0.05 level, up on statistical analysis using t-test, thereby establishing the pest status of RPM on *A. catechu*.  

Keywords— *Raoiella indica*; *Areca catechu*; Tenuipalpidae; chlorophyll; Photosynthetic efficiency; variable fluorescence; quantum yield of photosynthesis.
belonging to six to seven years of age were cultivated in separate gardens from 10 various localities for two months, April and May, 2015. Ten uninfested and infested leaf samples were collected randomly from each locality and kept in separate polythene bags. Samples were transported to the laboratory for subsequent microscopic observation under a Stereo Zoom Microscope (MVNSZ - 450) for recording the presence of various life stages of R. indica.

Qualitative and quantitative assessment of the damage induced by R. indica on the leaves of A. catechu was performed by rearing the species in the laboratory. Live specimens comprising 20–25 adults of R. indica were transferred to fresh uninfested leaves of A. catechu. Rearing of live mites were carried out in the laboratory by placing these leaf samples containing mite specimens on moistened cotton pads kept in petridishes. Regular observations were carried out under microscope to record the development of feeding symptoms on the leaf samples. Qualitative analysis of feeding damage at different life stages of R. indica was made by observing the development of chlorotic spots and yellowish white patches on the lower surface of the leaf lamina. Regular observations were made for 2 months (April–May) for confirmation of results.

Quantitative analysis of feeding damage was made by estimating the loss of chlorophyll ‘a’, ‘b’ and ‘total chlorophyll’ pigments in the leaf tissues of A. catechu infested by R. indica following method of Arnon (1949). For rating the damage, two categories of leaves were considered, namely heavily infested and uninfested (control). Chlorophyll was extracted from 2g of the leaf sample in 20ml of 80% acetone. The supernatant then transferred to a volumetric flask after centrifugation at 5000 rpm for 5minutes. The extraction repeated till the residue become colourless. Absorbance of the supernatant was read in a Shimadzu UV-VIS spectrophotometer (Model UV – 1601) at 645 nm, 663 nm and 750 nm against the solvent blank of 80% acetone for chlorophyll ‘a’, ‘b’ and ‘total chlorophyll’.

Leaf damage estimated, quantitatively, by measuring loss in photosynthetic efficiency of the leaves using the Handy Photo synthetic Efficiency Analyser instrument. Different parameters viz. Minimum fluorescence F0, maximum fluorescence Fm, variable fluorescence Fv, Fv/Fm, P Index and Area of infested and uninfested leaves were measured at room temperature on the fully expanded leaves by recording Chlorophyll fluorescence transient.

III. RESULTS AND DISCUSSION

Results of field studies helped to recognize RPM infestation on the lower surface of the leaves of areca palm, near the midrib or veins. On Stereo zoom microscopic observation, the infested leaves collected from the field, disclosed the presence of large number of yellow and white coloured spots. Later these feeding punctures were found coalesced to form light brown coloured areas (Figure.1. B). Similar damage symptoms were found developed by this species on various palms earlier (Flechtman & Etiene, 2004 & Beard et al., 2012). Feeding activity of large number of various life stages of the mite resulted in the development of localised yellow colouration on leaf lamina (Fig.1.A,C&D). On progressive feeding, these yellow patches coalesced to become bronze coloured, leading to the withering of the leaves. Leaves bearing such symptoms were found to harbour 20–25 adults, 20–30 nymphs and 20–25 larvae of R. indica.

In the present study, symptoms of chlorotic patches were evident when population of the mite was high. Such heavily infested leaf tissues, when subjected to quantitative analysis by the estimation of chlorophyll content, revealed drastic reduction in both ‘a’, ‘b’ and ‘total chlorophyll’ contents (Table 1; Figs. 2,3 &4). As shown in Table-1 and Fig. 2 heavily infested leaves has 0.47± 0.01mg/ gm chlorophyll ‘a’ content. When compared to uninfested leaves, the infested leaves showed 62.21±0.63 % loss of chlorophyll ‘a’. The reduction of chlorophyll ‘b’ in infested leaves could be recorded as 57.67±0.73% when compared to uninfested leaves and the infested leaves had 0.72±0.01mg chlorophyll ‘b’/gm (Table.1. & Fig.3.). The reduction of ‘total chlorophyll’ in infested leaves could be recorded as 59.88±0.375% when compared to uninfested leaves and the infested leaves had 1.180±0.009 mg ‘total chlorophyll’/gm (Table.1.& Fig.4.). These data when subjected to statistical analysis (t-test) were found significant at 0.05 levels (Table.2).

In the case of uninfested and infested leaves the mean values for Fv/Fm were 0.804 and 0.473 respectively (Table.3. & Fig.5). The low value of Fv/Fm is an indication of plant’s stress due to abiotic or biotic factors (Shigeto and Makoto, 1998) and the reduction of Fv/Fm value in infested plants reflects the decrease in PS II activity (Schansker et al., 2006). The area above the fluorescence curve between F0 and Fm is proportional to the pool size of the electron acceptors QA on the Photosystem II and this area reduced in the infested plant (Joliot and Joliot, 2002). The reduced area in the infested leaves (Table 3 & Fig.5.) might be due to blocking of the electron transfer between reaction centers to the quinine pool due to infestation by RPM.

Earlier studies on Coleus owing to infestation by another species of tenuipalpid mite viz. Brevipalpus obovatus (Meena & Sadana 1983) also could also establish similar loss in chlorophyll contents. Chlorophyll, being one of the
major parameter for determining the photosynthetic efficiency of the plant, (Maithra & Sen1988) the feeding impact of RPM should be considered seriously for chartering appropriate control strategies.

IV. CONCLUSION
The heavy loss of chlorophyll pigments and the significant variations in the photosynthetic parameters as evidenced during the present study disclosed the potential of R. indica to affect adversely the general health, growth rate and biomass of the host plant, A. catechu and thereby leading to reduction in the economic utility of the plant.

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### Table 1: Quantitative loss in Chlorophyll pigments induced by the feeding activity of *R. indica* on *A. cathcetu*

| Chlorophyll | S. No. | Milligram Chlorophyll/gram tissue | Loss in chlorophyll | % chlorophyll loss |
|-------------|--------|-----------------------------------|---------------------|--------------------|
|             |        | Uninfested | Infested |                  |                    |
| Chlorophyll a | 1       | 1.31       | 0.52     | 0.79              | 60.30              |
|              | 2       | 1.23       | 0.45     | 0.78              | 63.41              |
|              | 3       | 1.22       | 0.47     | 0.75              | 61.48              |
|              | 4       | 1.22       | 0.46     | 0.76              | 62.30              |
|              | 5       | 1.12       | 0.41     | 0.71              | 63.39              |
|              | 6       | 1.41       | 0.40     | 1.01              | 71.63              |
|              | 7       | 1.10       | 0.43     | 0.67              | 60.91              |
|              | 8       | 1.08       | 0.56     | 0.52              | 48.15              |
|              | 9       | 1.30       | 0.51     | 0.79              | 60.77              |
|              | 10      | 1.49       | 0.45     | 1.04              | 69.80              |
| Mean ± SEM  |        | 1.25±0.01  | 0.47±0.01| 0.78±0.02 | 62.21±0.63 |
| Chlorophyll b | 1       | 1.90       | 0.63     | 1.27              | 66.84              |
|              | 2       | 1.69       | 0.68     | 1.01              | 59.62              |
|              | 3       | 1.88       | 0.75     | 1.13              | 59.93              |
|              | 4       | 1.67       | 0.73     | 0.94              | 56.56              |
|              | 5       | 1.46       | 0.70     | 0.76              | 52.39              |
|              | 6       | 1.69       | 0.73     | 0.96              | 56.88              |
|              | 7       | 1.80       | 0.63     | 1.17              | 65.14              |
|              | 8       | 1.80       | 0.64     | 1.16              | 64.58              |
|              | 9       | 1.39       | 0.80     | 0.59              | 42.91              |
|              | 10      | 1.85       | 0.89     | 0.96              | 51.83              |
| Mean ± SEM  |        | 1.71±0.02  | 0.72±0.01| 1.00±0.02 | 57.67±0.73 |
| Total Chlorophyll | 1       | 3.201      | 1.145    | 2.055             | 64.210             |
|              | 2       | 2.912      | 1.127    | 1.785             | 61.299             |
|              | 3       | 3.091      | 1.221    | 1.870             | 60.500             |
|              | 4       | 2.883      | 1.187    | 1.697             | 58.840             |
|              | 5       | 2.581      | 1.106    | 1.475             | 57.137             |
|              | 6       | 3.092      | 1.121    | 1.971             | 63.740             |
|              | 7       | 2.885      | 1.056    | 1.830             | 63.412             |
|              | 8       | 2.865      | 1.196    | 1.669             | 58.261             |
|              | 9       | 2.689      | 1.299    | 1.390             | 51.691             |
|              | 10      | 3.335      | 1.343    | 1.993             | 59.747             |
| Mean ± SEM  |        | 2.953±0.023| 1.180±0.009| 1.773±0.022 | 59.884±0.375 |
| Table 2: Statistical analysis using t-test |
|-------------------------------------------|
| **Chlorophyll a** | Levene’s Test for Equality of Variances | t-test for Equality means | 95% Confidence Interval of the df |
| | F | Sig | T | Df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | Lower | Upper |
| Equal Variance assumed | 6.210 | .023 | 17.352 | 18 | .000 | .78200 | .04507 | .68732 | .87668 |
| Equal Variance not assumed | 17.352 | 11.543 | .000 | .78200 | .04507 | .68337 | .88063 |
| **Chlorophyll b** | Equal Variance assumed | 3.92 | .061 | 16.458 | 18 | .000 | .99500 | .06046 | .86798 | 1.12202 |
| Equal Variance not assumed | 16.458 | 12.893 | .000 | .99500 | .06046 | .86428 | 1.1257 |
| **Total Chlorophyll** | Equal Variance assumed | 6.707 | .018 | 22.811 | 18 | .000 | 1.773 | .07774 | 1.6099 | 1.9366 |
| Equal Variance not assumed | 22.811 | 11.646 | .000 | 1.773 | .0774 | 1.6033 | 1.9432 |

| Table 3: Quantitative difference in Photosynthetic parameters induced by the feeding activity of R.indica on A.catechu |
|-----------------------------------------------|
| Sl. No. | F0   | Fm   | Fv   | Fv/Fm | P index | Area |
| Uninfested |       |      |      |       |         |  |
| 1.       | 662  | 3274 | 2612 | .798  | .789   | 44500 |
| 2.       | 623  | 3264 | 2641 | .809  | 1.38   | 46800 |
| 3.       | 654  | 3355 | 2701 | .805  | 1.0219 | 48300 |
| 4.       | 674  | 3343 | 2669 | .798  | 0.784 | 44300 |
| 5.       | 662  | 3431 | 2769 | .807  | 1.027 | 47600 |
| 6.       | 634  | 3454 | 2820 | .816  | 1.112 | 48200 |
| 7.       | 634  | 3414 | 2780 | .814  | 1.048 | 47800 |
| 8.       | 670  | 3484 | 2814 | .808  | 1.036 | 46600 |
| 9.       | 663  | 3523 | 2860 | .812  | 1.044 | 47100 |
| 10.      | 665  | 3550 | 2885 | .813  | 1.072 | 48100 |
| Mean     | 654  | 3409 | 2755 | .808  | 1.031 | 46930 |
| SEM      | 1.747 | 9.863 | 9.440 | 0.001 | 0.017 | 145.453 |

| Infested |       |      |      |       |         |  |
| 1.       | 422  | 1064 | 642  | .603  | 0.119  | 24000 |
| 2.       | 382  | 721  | 339  | .470  | 0.019  | 6000  |
| 3.       | 584  | 969  | 385  | .397  | 0.012  | 7000  |

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| 4. | 114 | 170 | 56 | 0.329 | 0.006 | 12000 |
| 5. | 106 | 202 | 96 | 0.475 | 0.014 | 1000 |
| 6. | 372 | 721 | 349 | 0.484 | 0.016 | 8000 |
| 7. | 594 | 969 | 375 | 0.387 | 0.009 | 10000 |
| 8. | 114 | 170 | 56 | 0.329 | 0.007 | 7000 |
| 9. | 609 | 1264 | 655 | 0.518 | 0.057 | 16700 |
| 10. | 278 | 521 | 243 | 0.466 | 0.036 | 14300 |
| **Mean** | **358** | **677** | **320** | **0.446** | **0.030** | **10600** |
| ± SEM | ± 20.100 | ± 39.900 | ± 21.599 | ± 0.009 | ± 0.004 | ± 648.742 |

**FIGURES**

Fig.1: A- Adult female Raoiella indica on areca leaf, B- Infested leaf lamina with R. indica, C- Eggs & larva of R. indica, D – Heavily infested leaf
Fig. 2: Amount of chlorophyll ‘a’ in the R. indica infested and uninfested leaf samples of A. Catechu.

Fig. 3: Amount of chlorophyll ‘b’ in the R. indica infested and uninfested leaf samples of A. Catechu.
Fig. 4. Quantitative loss in total chlorophyll pigments induced by the feeding activity of R. indica on A. catechu.

Fig. 5. Quantitative loss in Photosynthetic parameters induced by the feeding activity of R. indica on A. catechu.