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RESEARCH ARTICLE

Seasonal distribution and damage potential of *Raoiella indica* (Hirst) (Acari: Tenuipalpidae) on areca palms of Kerala, India

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ABSTRACT: Tenuipalpid mites represent an important group of Acari characterized by polyphagous habit on a wide variety of crops. Among the injurious species of tenuipalpids, *Raoiella indica* Hirst (Trombidiformes: Tenuipalpidae), the Red Palm Mite (RPM), is a serious pest on a variety of plants. In the present study, infestation and distribution of the species on *Areca catechu* L. (Arecales: Arecaceae), an important plantation crop of Kerala (India), were studied. The leaf sampling was carried out once in a month in Kozhikode and Malappuram districts of North Kerala between 2011 and 2013. Both uninfested and infested leaf samples were subjected to observation under a stereo zoom microscope (MVNSZ-450) to collect data on the population density of the mite. Feeding impact of the RPM on the areca palm was assessed through quantitative estimation of the total contents of nitrogen, phenolic compounds and chlorophyll, and free proline. Results of population studies revealed peak population density of RPM during April-May months. A positive correlation was recorded between mite population and temperature and a negative correlation between mite population and relative humidity. The percentage losses of 'total nitrogen' and 'total chlorophyll contents were estimated as 51-53% and 59-60% respectively in RPM infested leaves. The percentage increases recorded in the proline and phenolic compound contents of mite infested leaf samples were 182-198% and 90-92%, respectively, thereby revealing the stress induced by the RPM to areca palms. Data obtained on the changes induced by RPM on chlorophyll, proline, phenolic compound and total nitrogen contents were found significant at 0.05 level, when subjected to statistical analysis following t-test, thereby establishing the pest status of RPM on *A. catechu*.

Keywords: *Areca catechu*, proline, *Raoiella indica*, total chlorophyll, total nitrogen, total phenolic compound.

INTRODUCTION

Mites and ticks constitute an important and most diverse group of arachnids and they are noticeably less known than the other groups of arthropods such as spiders. The present work is focused on an important species of phytophagous Acari, *Raoiella indica* Hirst (Trombidiformes: Tenuipalpidae) which feeds voraciously by sucking the cell sap of the host plant, *Areca catechu*. The Red Palm Mite (RPM), *R. indica* causes severe damage to plants belonging to the family Arecaceae, especially to coconut (*Cocos nucifera* L.), and also to bananas, *Musa acuminata* (Musaceae) and some other plant families (Flechtmann and Etienne, 2004, 2005; Etienne and Flechtmann, 2006). It is the first mite species in which feeding was observed through stomata of its host plants (Ochoa et al., 2011). Significantly, higher population densities of RPM have been recorded on areca palm, with peak population in April/May in Karnataka, India (Yadavbabu and Manjunatha, 2007). This study was in line with the present study. However, despite of the high incidence and wide distribution trend of RPM on varied economic crops of Kerala, only very scanty information (on only population dynamics) was available on this mite in South India (Taylor et al., 2011). Hence the present study has been carried out to procure data on seasonal population density and the severity of damage induced by *R. indica* on an important economic crop of Kerala, viz. *A. catechu* by making qualitative and quantitative assessment of damage potential induced by *R. indica* in Kozhikode and Malappuram districts of Kerala between 2011 and 2013.

Goal of the present study was to determine the seasonal population density of *Raoiella indica* on areca palms of two districts of North Kerala. And also the present work was taken up to understand the severity of damage induced by *R. indica* on host plant *Areca catechu* by estimating biochemical components such as chlorophyll, nitrogen, phenol and proline.

MATERIAL AND METHODS

Mite rearing in the laboratory

The mites were cultured in the laboratory condition for observation on the feeding habits of mites. Rearing of different life stages of mites were maintained on the host plant leaves in the laboratory condition and the leaves upon damage were replaced with fresh ones. Each culture set was maintained with two duplicates to confirm the observation and each culture set consisted of 1-3 mature leaf discs, kept in Petri dishes lined with a moistened cotton pads to maintain the water content of leaves up to maximum number of days. Along with experimental set up, stock cultures of the mites were also maintained in the laboratory on the areca leaf lets collected from the field to ensure constant supply of life stages.

The seasonal population density of *Raoiella indica* on *Areca catechu*

Field sampling of mite infested leaves was carried out from areca plantations in North Kerala where palms belonging to six to seven years of age were cultivated in separate gardens. Sampling was conducted for a three year period, from 2011 to 2013, from plants cultivated in 10 localities of Kozhikode and Malappuram districts of North Kerala. During the study period, field surveys were undertaken once in a month in order to procure temporal data on mite infestation. Total of 10 areca gardens were used for sampling of areca leaves. In each garden, young palms, not taller than 3 meters were selected randomly and three fronds (leaf of the palm) were sampled (bottom, middle and top). On each frond, five leaflets were removed from the region close to the rachis. Leaflets were transported to the laboratory for subsequent microscopic observation, to record the presence of *R. indica* and its life stages. The number of different life stages of the mite present on individual leaflets of bottom, middle and top fronds was counted separately under a stereo zoom microscope at fortnightly intervals. The mites were carefully mounted on a slide and these slide mounted specimens were identified under the high power of a Carl Zeis research microscope with the help of keys, literatures and then it was confirmed with the help of an expert. No other phytophagous inhabitants could be noticed during field sampling. The injurious status of the mite was analyzed through repeated field studies by collecting mite infested and uninfested (control) leaves and recording damage symptoms such as chlorotic spots, yellowing, greyish brown patches etc. The injurious status of the mite infestation was determined by analyzing how much area of the leaf lamina was infested by the mites. The leaves with more than half of the laminar surface showing visible symptoms of mite infestation were categorized as severely infested.

Qualitative assessment of damage potential

Qualitative assessment of damage potential of *R. indica* was made by conducting regular observation on the feeding activities of the various life stages and recording the damage symptoms induced on the host plant during progressive feeding. Qualitative assessment of feeding was made simultaneously through repeated field cum laboratory studies. Field collected mite infested and uninfested samples of host leaves were subjected to microscopic examination in order to record the nature of incidence, severity of infestation, population density, distribution pattern, damage symptoms induced on the leaves etc. Results of field studies were confirmed through simultaneous microscopic observation of leaves maintained in stock cultures in the laboratory. Observation on individual leaf was continued to record the qualitative difference between the experimental and control leaves and also to gather knowledge on the damage potential of the mite. Renewal of damaged/decayed leaf discs was made in every 2 weeks and the observations were continued with fresh leaves.

Quantitative assessment of damage potential

Quantitative assessment of damage potential of RPM on leaves of *A. catechu* was carried out through biochemical analysis of various parameters such as chlorophyll a, chlorophyll b, total chlorophyll, phenolic compound, free proline and total nitrogen contents. For rating the damage, field sampling was done in April-May months, in this period the active number of mites inhabited on the single leaf let were 50 -70 and these number of mites were quite similar in all infested sample leaves during this period.

Estimation of Chlorophyll loss

The chlorophyll contents of mite infested and uninfested (control) leaf samples were estimated following the method of Arnon (1949). The exuviae, eggs, life stages and fecal matter of mites were carefully removed with the help of camel hair brush under a stereomicroscope from the infested leaves before subjecting these for chlorophyll analysis. Chlorophyll was extracted from 2g of the infested and uninfested leaf sample using 20 ml of 80% acetone. The supernatant was transferred to a volumetric flask after centrifugation at 5000 rpm for 5minutes. The extraction was repeated until the residue became colorless. The absorbance of the solution was recorded 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.

Chlorophyll **a** (μg/ml) = [12.69 (A_{663}-A_{750})-2.69 (A_{645}-A_{750})/ Dry weight] x Volume

Chlorophyll **b** (μg/ml) = [22.9 (A_{645}-A_{750})-4.68 (A_{663}-A_{750})/ Dry weight] x Volume

Total chlorophyll (μg/ml) = [20.12 (A_{645}-A_{750}) + 8.02 (A_{663}-A_{750})/ Dry weight of Sample] x Volume

A = Absorbence

Estimation of Nitrogen

Estimation of total nitrogen content present in mite infested and uninfested leaf sample was made by following the method of Kjeldahl (1883) from Interfield Laboratories Pvt. Ltd., Cochin, Kerala.

The amount of nitrogen was calculated as follows:

Nitrogen (%) = (A-B) X C/D X E/(FXG) X 100

A = Titer value for digested sample, ml

B = Titer value for blank, ml

C = Nitrogen equivalent of ammonium sulphate, mg

D = Titer value for ammonium sulphate, ml

E = Volume of digested sample, ml

F = Volume of sample taken for distillation, ml

G = Sample weight, mg

Estimation of Proline

Free proline contents present in the mite infested and uninfested samples were estimated following the method of Bates et al. (1973). Other intrusive materials were also presumably removed by absorption to the protein sulphosalicylic acid complex. The extracted proline was made to react with ninhydrin in acidic conditions (pH 1) to form the chromophore (pink color) and read at 520 nm. Two hundred (200 mg) milligrams of mite infested and uninfested fresh leaf samples were weighed separately and homogenized in 10 ml of 3% (w/v) aqueous sulfosalicylic acid using a clear glass mortar and pestle. The homogenate was filtered through Whatman No. 2 filter paper. From the filtrate, 2 ml aliquot was taken and mixed with 2ml glacial acetic acid and 2ml acid ninhydrin. The tubes with mixture were heated in a boiling water bath for 1 hour and then the reaction was terminated by placing the tubes in an ice -bath. For color development, 4.0 ml of toluene was added to the reaction mixture and stirred well for 20-30 seconds. Then, the pink colored toluene layer was separated and brought to room temperature. The color intensity of the solution was measured at 520 nm using toluene as reagent blank in a Spectrophotometer. L. Proline was used as the standard. The amount of proline in the test sample was calculated from the standard curve (mg/g tissue).

Estimation of Phenolic Compounds

The response of plants to mite attack in terms of concentration of total phenolic compound content of each extract was determined, following Folin-Ciocalteu's colorimetric method, based on oxidation-reduction reaction method (Waterhouse, 2003). The sample (0.5 g) was homogenized in 10 times volume of 80% ethanol. The homogenate was centrifuged at 10,000 rpm for 20 minutes. The extraction was repeated with 80% ethanol. The supernatants were pooled and evaporated to dryness. The residue was then dissolved in a known volume of distilled water. Different aliquots were pipetted out and the volume in each tube was made up to 3.0ml with distilled water. Folin-Ciocalteau's Reagent (0.5 ml) was added and the tubes were placed in a boiling water bath for exactly one minute. The tubes were cooled and the absorbance was read at 650 nm in a spectrophotometer against a reagent.

RESULTS

Results of the present study clearly revealed the seasonal incidence and injurious status of *R. indica* on areca palms cultivated the Kozhikode and Malappuram districts of North Kerala. Population buildup of the mite was found initiated during April to May months of the years 2011-2013, in the field in which a rise in temperature was experienced. The mite population attained the peak level in April i.e. it approximately 100-120 active and inactive stages of mites per leaflet and subsequently showed a decline from the last week of May and this declining trend was continued till December. During the study, the lowest population density of the mite could be recorded during the months of October, November and December. The population density of the mite was high during the first week of March and continued up to the first week of May with the peak formation in April-May. A decline in population could be observed from June onwards. The number of mites was found to vary with respect to the leaflets of individual leaf frond. High degree of mite incidence was noted on the bottom frond leaflet when compared to the top and middle frond leaflets (Tables 9, 10). Very low population density of the mite could be recorded during the monsoon and winter seasons. In all examined cases, infestation of RPM was found confined to the lower surface of leaflets and often the number of mites recovered from a single leaflet ranged from 70-100 during the months of April and May (Tables 9, 10). All the life stages of the mite were predominantly red in color, while the adult females often exhibited dark colored areas on the body (Fig. 1 A). Feeding activity of large numbers of the various life stages of the mite imparted the development of localized yellow coloration on the leaf lamina (Figs 1 B-D). On progressive feeding, these yellow patches coalesced to form bronze colored areas, thereby leading to the drying up of leaves.

During heavy infestation (more than half of the leaf lamina showing chlorotic spots, yellow patches and greyish brown areas etc.), simultaneous and repeated feeding resulted in the formation of irregular greyish brown patches on the leaf surface. The symptoms could be easily perceived by the bronzed appearance of the leaves. Results of quantitative studies clearly established that infestation by *R. indica* on areca palms could induce the drastic alterations in chlorophyll a, b and total chlorophyll, proline, phenolics and total nitrogen. The amount of chlorophyll presents in the mite infested and uninfested areca leaf samples on estimation revealed a drastic decline in both 'a', 'b' and total chlorophyll pigments. The mean amounts of chlorophyll 'a', 'b' and total chlorophyll in the infested leaf samples recorded during the study were 0.47±0.01, 0.72±0.01 and 1.180±0.009 were mg/gm tissue respectively (Table 1). This showed that the mite infested leaf samples had a loss of 62.21±0.63% of Chlorophyll 'a' pigment when compared to the uninfested leaves of areca. The amount of chlorophyll 'b' pigments recorded in the mite infested and uninfested areca leaves were 1.71±0.02 and 0.72±0.01 mg respectively which showed a loss of 57.67±0.73% chlorophyll 'b' owing to infestation by RPM. The percent loss of total chlorophyll in mite infested leaf samples recorded during the study were 59.884±0.375% tissue, respectively (Figs 2-4).

Similar to chlorophyll 'a' and 'b' pigments, RPM infestation was also found to lead to loss in nitrogen content. The mite infestation was found to induce 51.92±0.72% loss of total nitrogen content in areca leaves (Table 2, Fig. 5). The amount of proline in the mite infested samples showed an increase than that of uninfested leaves (Table 3, Fig. 7). The mean proline content of mite infested leaf sample recorded during the study was about 3 times than that of the uninfested sample which could be accounted to 190.8%. Similar to proline content, the phenolic compound content of the areca leaves also showed an increase owing to infestation by the RPM. The uninfested and infested areca leaves contained 1.40±0.01 and 2.65±0.02 mg of phenolics/g tissue, respectively (Table 4,

Table 1. Quantitative difference in chlorophyll pigments induced by the feeding activity of *Raoiella indica* on *Areca catechu*.

Chlorophyll	S. No.	Chlorophyll (mg/g 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

Fig. 6). The per cent increase in phenolic compound content due to *R. indica* was recorded as 91.18±1.77%. These data when subjected to statistical analysis (t-test) were found significant at 0.05 levels (Tables 5-8).

DISCUSSION

Areca palms are widely cultivated throughout Kerala and the present observation forms the first report on the incidence of Red Palm Mite on areca nut in Kozhikode and Malappuram districts of North Kerala (Prabheena and Ramani, 2014). The results of the present study enabled to record infestation by *R. indica* as one of the major problems in the areca nut plantations of North Kerala. Temperature is a main abiotic factor for poikilothermic insects (Parmesan, 2006) and changes in surrounding temperature regimes would certainly influence the development rates, voltinism and survival of insects and subsequently act upon size, density and genetic composition of populations, as well as the extent of host plant exploitation (Bale et al., 2002). In the present study also, temperature and relative humidity of the habitat were found to exert a profound influence in determining the population size of the red palm mite.

Rainfall generally exerts a negative impact on the population density of the insect and mite pests on various crops through mechanical action. A lowest rate of incidence was recorded for the spider mite pest, *Oligonychus ilicis* in rainy season (Pallini et al., 1992). Similarly, a decrease in the phytoseiid predatory mite population was recorded in citrus plantation as a function of rainfall (Reis et al., 2000). Results of the present study helped to confirm the above findings by recording a decline in RPM population in areca plantations under the influence of rainfall in Kerala, as the number of mites encountered during this

Table 2. Quantitative difference in nitrogen contents induced by the feeding activity of *Raoiella indica* on *Areca catechu*.

S.No.	Nitrogen (mg/g tissue)		Nitrogen loss in mg	% loss
	Uninfested	Infested		
1	22.90	12.20	10.70	46.72
2	22.00	13.90	8.10	36.82
3	24.30	9.50	14.80	60.91
4	22.30	11.77	10.53	47.22
5	25.70	10.90	14.80	57.59
6	24.70	9.50	15.20	61.54
7	26.60	11.70	14.90	56.02
8	22.10	11.31	10.79	48.82
9	19.40	8.90	10.50	54.12
10	23.14	11.69	11.45	49.48
MEAN±SEM	23.31±0.20	11.14±0.14	12.18±0.24	51.92±0.72

Table 3. Quantitative difference in proline contents induced by the feeding activity of *R. indica* on *Areca catechu*.

S. No.	Free proline (mg/g tissue)		Raise in Proline	% Raise in Proline
	Uninfested	Infested		
1	0.483	0.986	0.503	104.14
2	0.576	1.407	0.831	144.27
3	0.345	1.048	0.703	203.77
4	0.379	1.310	0.931	245.65
5	0.428	1.441	1.013	236.68
6	0.462	1.172	0.71	153.68
7	0.317	1.124	0.807	254.57
8	0.297	1.324	1.027	345.79
9	0.359	0.959	0.6	167.13
10	0.510	1.290	0.78	152.94
Mean±SEM	0.416±0.01	1.21±0.02	0.791±0.02	190.8±7.10

Table 4. Quantitative difference in phenol content induced by the feeding activity of *Raoiella indica* on *Areca catechu*.

S. No.	Phenolic compounds (mg/g tissue)		Raise in Phenolics	% Raise in Phenolics
	Uninfested	Infested		
1	1.446	2.768	1.322	91.42
2	1.353	2.772	1.419	104.88
3	1.195	2.348	1.153	103.00
4	1.465	2.605	1.141	77.82
5	1.473	2.336	0.863	58.59
6	1.295	2.754	1.459	112.66
7	1.349	2.625	1.276	94.59
8	1.281	2.601	1.320	103.04
9	1.488	2.490	1.002	67.34
10	1.614	3.192	1.578	97.77
Mean±SEM	1.40±0.01	2.65±0.02	1.25±0.02	91.18±1.77

Table 5. Statistical analysis using t-test – Chlorophyll.

		Levene's Test for Equality of Variances		t-test for Equality means						
		F	Sig	t	df	Sig. (2-tailed	Mean Diffe-rence	Std. Error Diffe-rence	95% Confidence Interval of the df Lower	Upper
Chlorophyll a	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 6. Statistical analysis using t-test – Nitrogen.

		Levene's Test for Equality of Variances		t-test for Equality means						
		F	Sig	T	df	Sig. (2-tai-led	Mean Differen-ce	Std. Error Diffe-rence	95% Confidence Interval of the df Lower	Upper
Nitrogen	Equal Varriance assumed	.936	.346	14.993	18	.000	12.17700	.81221	10.4706	13.883
	Equal Varriance not assumed			14.993	16.344	.000	12.17700	.81221	10.4581	13.896

Table 7. Statistical analysis using t-test – Proline.

		Levene's Test for Equality of Variances		t-test for Equality means						
		F	Sig	t	df	Sig. (2-tai-led	Mean Differen-ce	Std. Error Diffe-rence	95% Confidence Interval of the df Lower	Upper
Proline	Equal Varriance assumed	7.037	.016	-12.77	18	.000	-.79050	.06187	-.92048	-.66052
	Equal Varriance not assumed			-12.77	13.636	.000	-.79050	.06187	-.92352	-.65748

Table 8. Statistical analysis using t-test – Phenol.

		Levene's Test for Equality of Variances		t-test for Equality means						
		F	Sig	t	df	Sig. (2-tailed	Mean Difference	Std. Error Difference	95% Confidence Interval of the df Lower	Upper
Phenol	Equal Varriance assumed	1.944	.180	-14.303	18	.000	-1.25320	.08762	-1.4373	-1.0692
	Equal Varriance not assumed			-14.303	13.198	.000	-1.25320	.08762	-1.4422	-1.06420

Table 9. Sesonal population density of *Raoiella indica* on areca palms of Kozhikode and Malappuram districts in 2011-2012.

Month of sampling 2011 April – 2012 March	Average Temp (°C)/ Average RH (%)	Mean number of different life stages of *Raoiella indica* mites/leaf let								
		Bottom frond			Middle frond			Top frond		
		Egg	Immature stages	Adult	Egg	Immature stages	Adult	Egg	Immature stages	Adult
April	29/70	48	28	26	49.8	30	28.8	54	33	27.6
May	34/65	33	18	9	15.6	11.4	12	31.8	8.34	11.4
June	29/75	12.6	12	8.4	9.6	12	12.6	12	12.9	10.8
July	27/80	3	2.4	3	2.4	1.5	3	3.9	3.6	4.2
August	27/85	6	1.2	.6	.3	.3	.6	1.2	.6	1.5
September	28/80	1	2	1	3	1	1	1	1	1
October	27/85	2	1	2	1	.5	1	1	2	1
November	26/85	.5	1	1	.5	1	.5	.5	1	1
December	25/85	2	2	5	1	2.5	2	1.5	3	2.5
January	29/80	16	12	12	8	8	6	12	10	4
February	28/75	14	20	20	20	18	16	18	12	10
March	29/75	33	18.6	13.5	30	20.4	18.9	21.6	12	11.4

season was comparatively low, 5-10 active life stages of the mites per leaflet.

The population density of the RPM could be observed as the maximum during April-May and then it followed a declining trend to reach moderate and scanty levels during November-March and June-October periods respectively. This is in accordance with the earlier reports (Yadavbabu and Manjunatha, 2007) on *R. indica* which showed the peak population of the mite from March - to the first week of May and then a decline in population from June onwards. Feeding activity of the RPM was found to induce development of localized yellow patches on the leaflets of areca palm. Stunted growth and withering of leaves in RPM infested palms were already report ed in South Indian conditions (Puttarudraiah and Channabasavanna, 1956).

Apart from the physical damage, the feeding activity of *R. indica* was found to induce alterations in the biochemical constituents of the host plant, *A. catechu*. Among plants, chlorophyll pigments are responsible for absorbing light energy from the sun and therefore, chlorophyll content

Table 10. Sesonal population density of *Raoiella indica* on areca palms of Kozhikode and Malappuram districts in 2012-2013.

Month of sampling 2012 April – 2013 March	Average Temp (°C)/Average RH (%)	Mean number of different life stages of *Raoiella indica* mites/leaf let								
		Bottom frond			Middle frond			Top frond		
		Egg	Immature stages	Adult	Egg	Immature stages	Adult	Egg	Immature stages	Adult
April	30/75	58.8	39.9	29.1	57.9	34.2	32.4	60.9	33.3	32.7
May	34/65	36	22.5	12.6	33.3	24.6	15	34.5	19.2	15.6
June	28/70	16.5	15	14.7	15.9	13.5	17.1	14.4	15.6	13.2
July	27/75	5.1	3.6	5.4	6.3	4.5	8.1	4.8	6.6	4.5
August	27/80	2.7	3.3	2.1	3	2.1	2.4	8.4	2.4	2.1
September	30/75	4	3	1	2	2	1	1	2	1
October	28/80	1.5	2	2.5	1.5	2	2.5	1	3	1
November	27/85	1	1	2.5	1	1.5	1	1	1.5	2
December	25/85	2	3	3	1.5	3	2	2	2.5	4
January	27/80	12	15	8	10	8	8	10	14	6
February	29/80	20	16	12	14	12	10	18	10	10
March	29/78	37.8	24	20.4	30.3	28.5	23.4	30.6	28.5	17.7

is considered as a key experimental parameter in plant biology and agronomy (Lamb et al., 2012). Feeding activity of RPM caused significant reduction in the chlorophyll contents of their host plants thereby supporting earlier findings (Ghoshal, 2013; Prabheena and Ramani, 2013).

Nitrogen is one of the vital elements in plants and which plays a key role in chlorophyll production and forms part of the various proteins that have major roles in many metabolic processes associated with plant growth (Sinfield et al., 2010). Leaves of areca plants infested with *R. indica* disclosed significant reduction in the total nitrogen content. Similar results were reported by earlier workers (Ghoshal et al., 2005) also by accounting the percent loss of nitrogen in *Corchorus capsularis* L. (Malvaceae) owing to feeding by *Polyphagotarsonemus latus*.

The amino acid, proline is believed to be a compatible solute and the accumulation of proline was noticed in plants, produced when unfavourable conditions prevailed (Aspinall and Paleg, 1981). During the microbial infection, the proline contents of certain plants become raised many folds in sensitive and resistant cultivators (Raj et al., 1983; Gupta, 2001). In the present investigation, the amount of proline was found increased significantly on the host plant of RPM thereby supporting the earlier records on the impact of mite feeding on tomato (Kielkiewicz, 2005) and bean (Farouk and Osman, 2012). Phenolic compound plays an important role in plant defence mechanism and an increased concentration of phenolics was observed in plants infested with fungi (Senthil et al., 2010). In the present study, results of phenol estimation in mite infested and control leaves of various host plants clearly revealed the elevation of phenol concentration in the mite infested leaves.

The results of the study disclosed that the temperature and relative humidity of the habitat exert a major role in determining the population size of the *R. indica*. The heavy loss of biochemical components such as chlorophyll contents and nitrogen as evidenced by the present study revealed the potential of *R. indica* to affect adversely the general health of the host plant *A. catechu*, thereby reduction in the growth rate of the host plant. This study enabled to record *R. indica* as one of the major mite species infesting areca nut plantations of Kerala, and which warrants the urgent attention to formulate appropriate regulatory measures to be adopted to check the population rise of this notorious pest and safeguard the areca plantations of Kerala in future.

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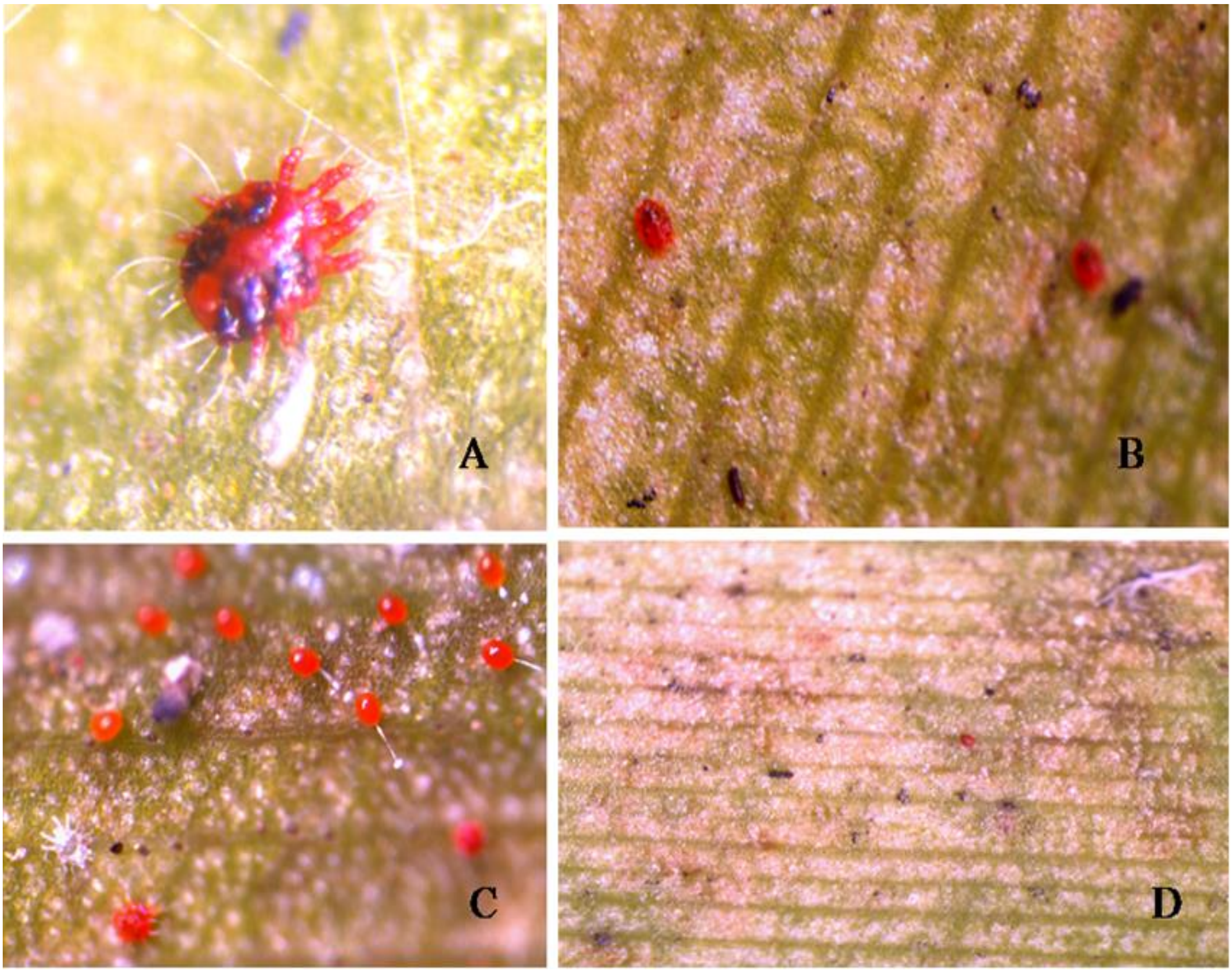

Figure 1. A) Adult female of *Raoiella indica* on areca leaf, B) Infested leaf lamina with *R. indica*, C) Eggs and larva of *R. indica*, D) Heavily infested leaf.

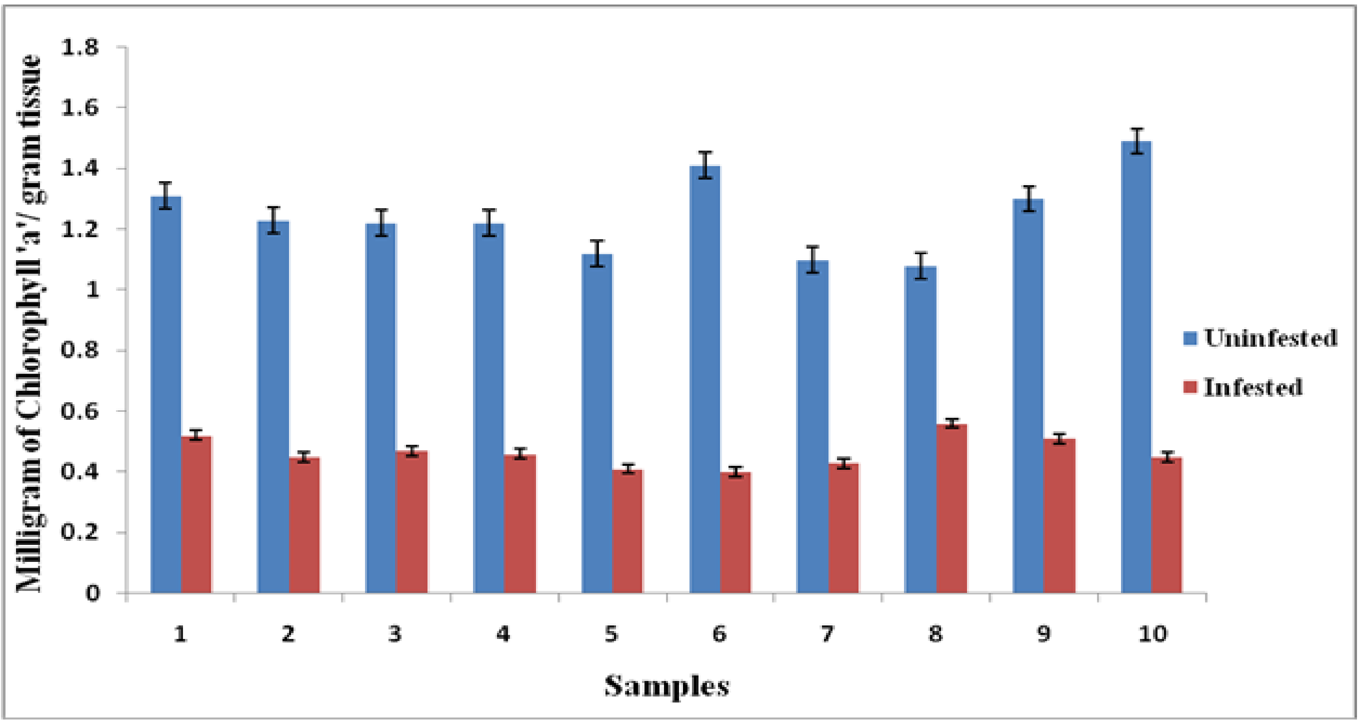

Figure 2. Amount of chlorophyll 'a' in *Raoiella indica* infested and uninfested *Areca catechu* leaf sample.

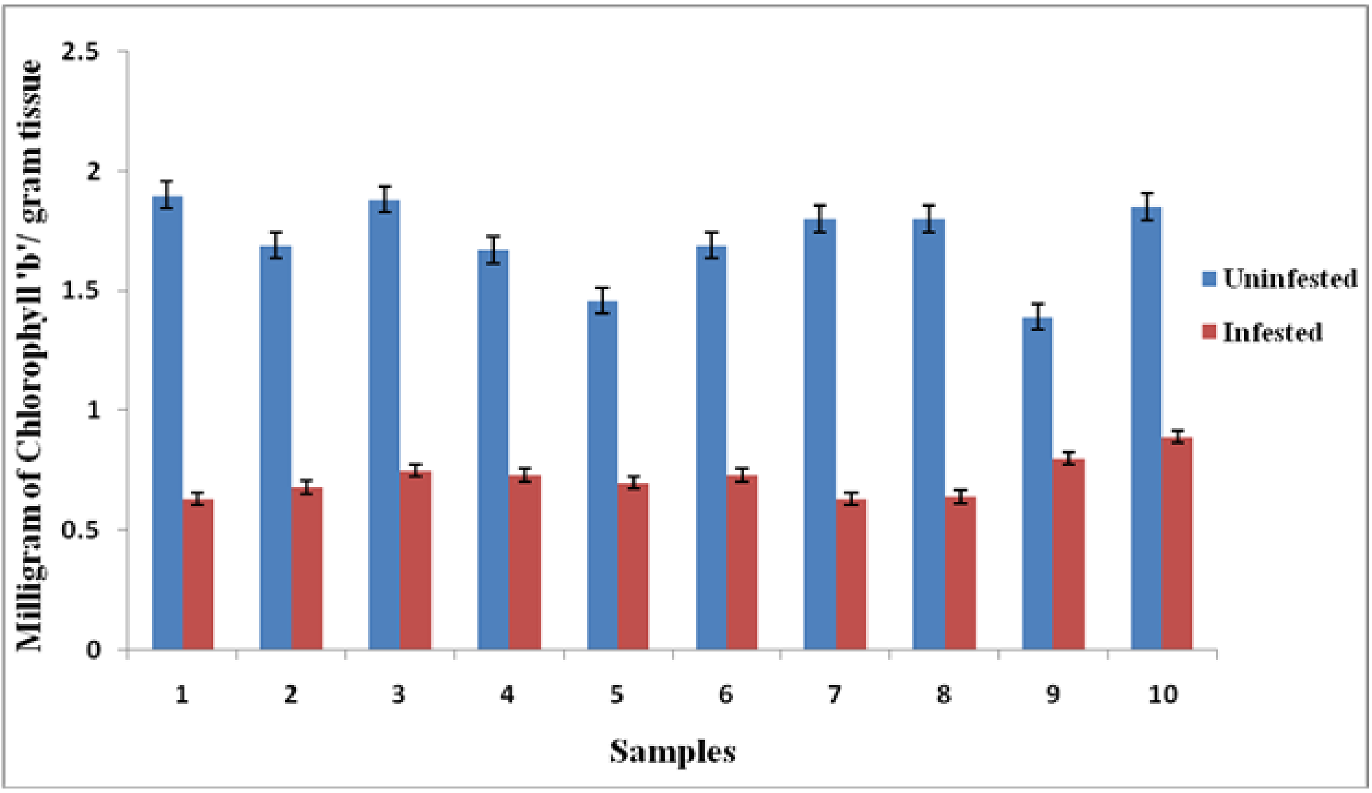

Figure 3. Amount of chlorophyll 'b' in *Raoiella indica* infested and uninfested *Areca catechu* leaf sample.

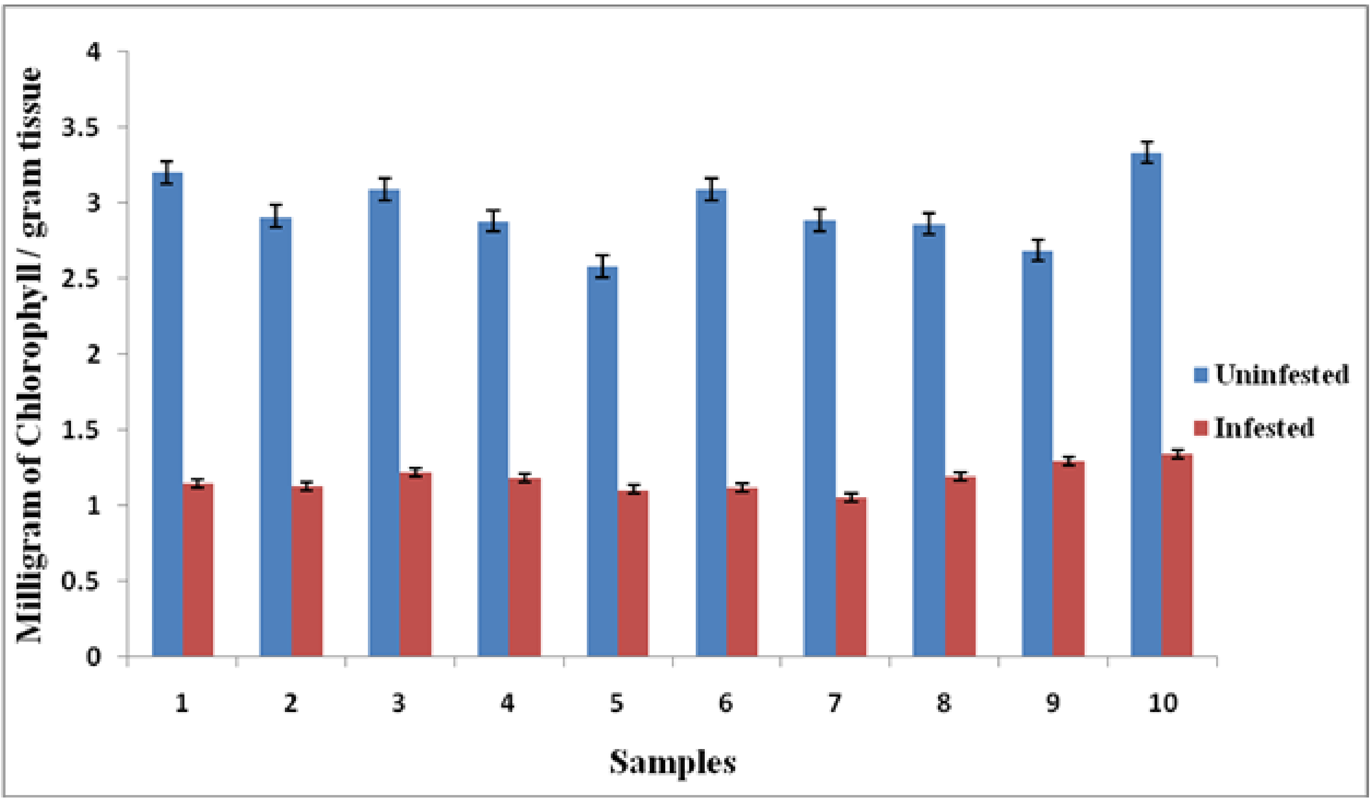

Figure 4. Amount of total chlorophyll in *Raoiella indica* infested and uninfested *Areca catechu* leaf sample.

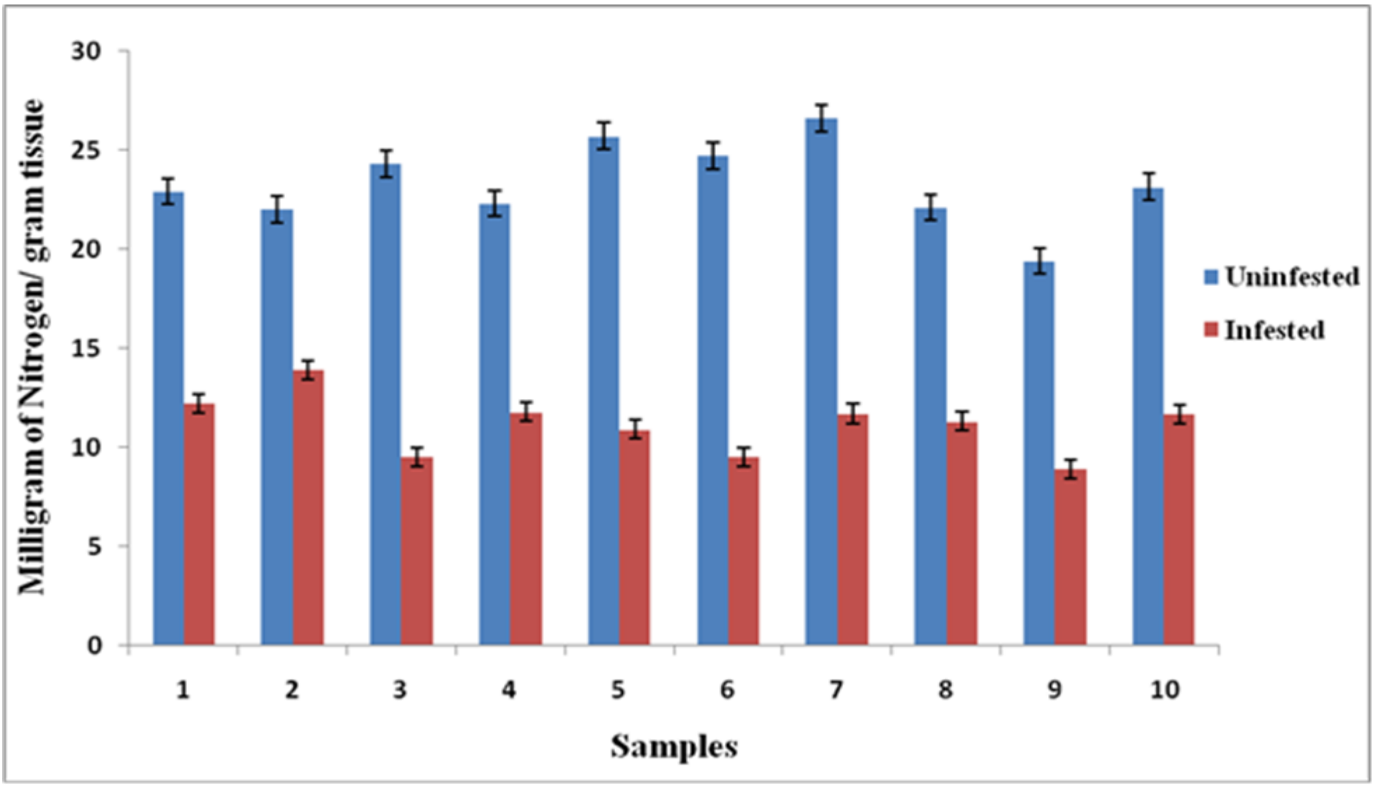

Figure 5. Amount of nitrogen in *Raoiella indica* infested and uninfested *Areca catechu* leaf sample.

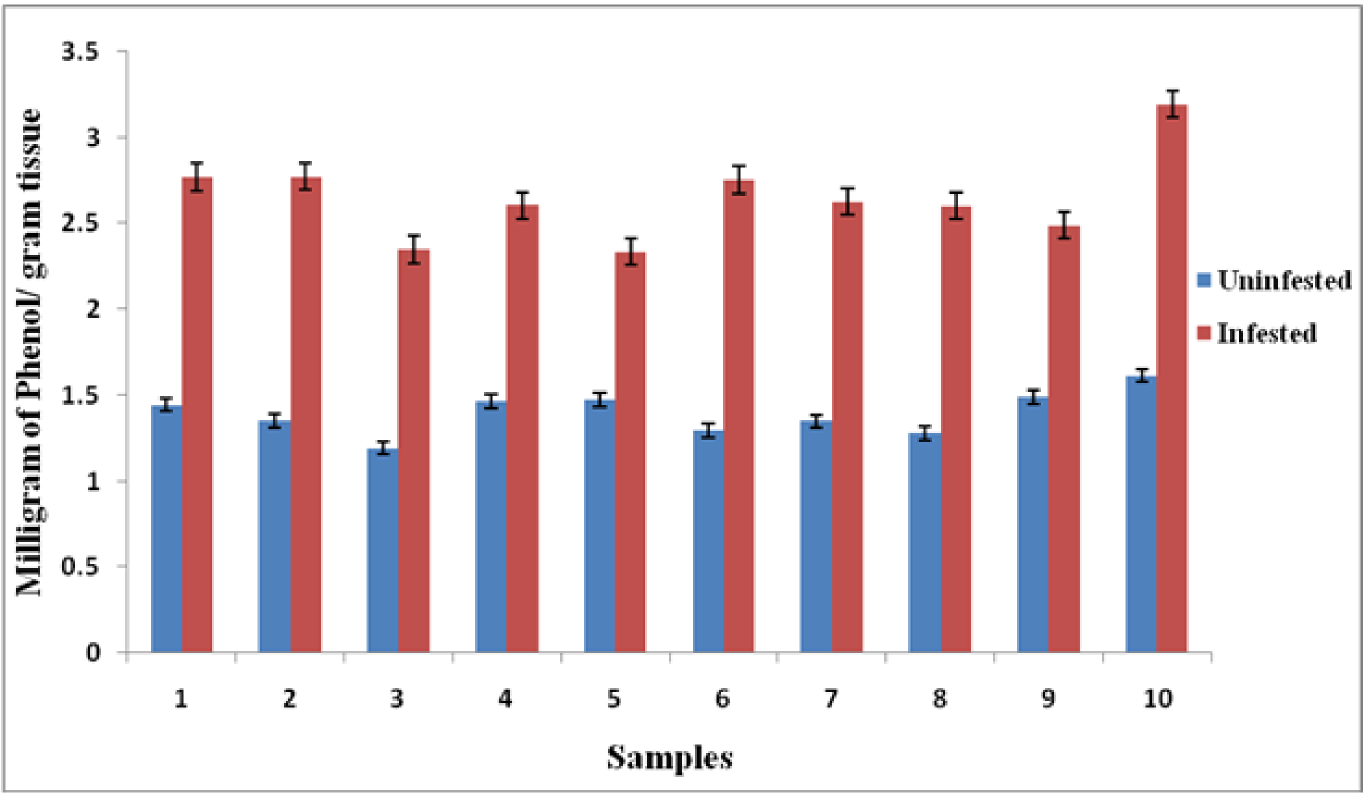

Figure 6. Amount of phenol in *Raoiella indica* infested and uninfested *Areca catechu* leaf sample.

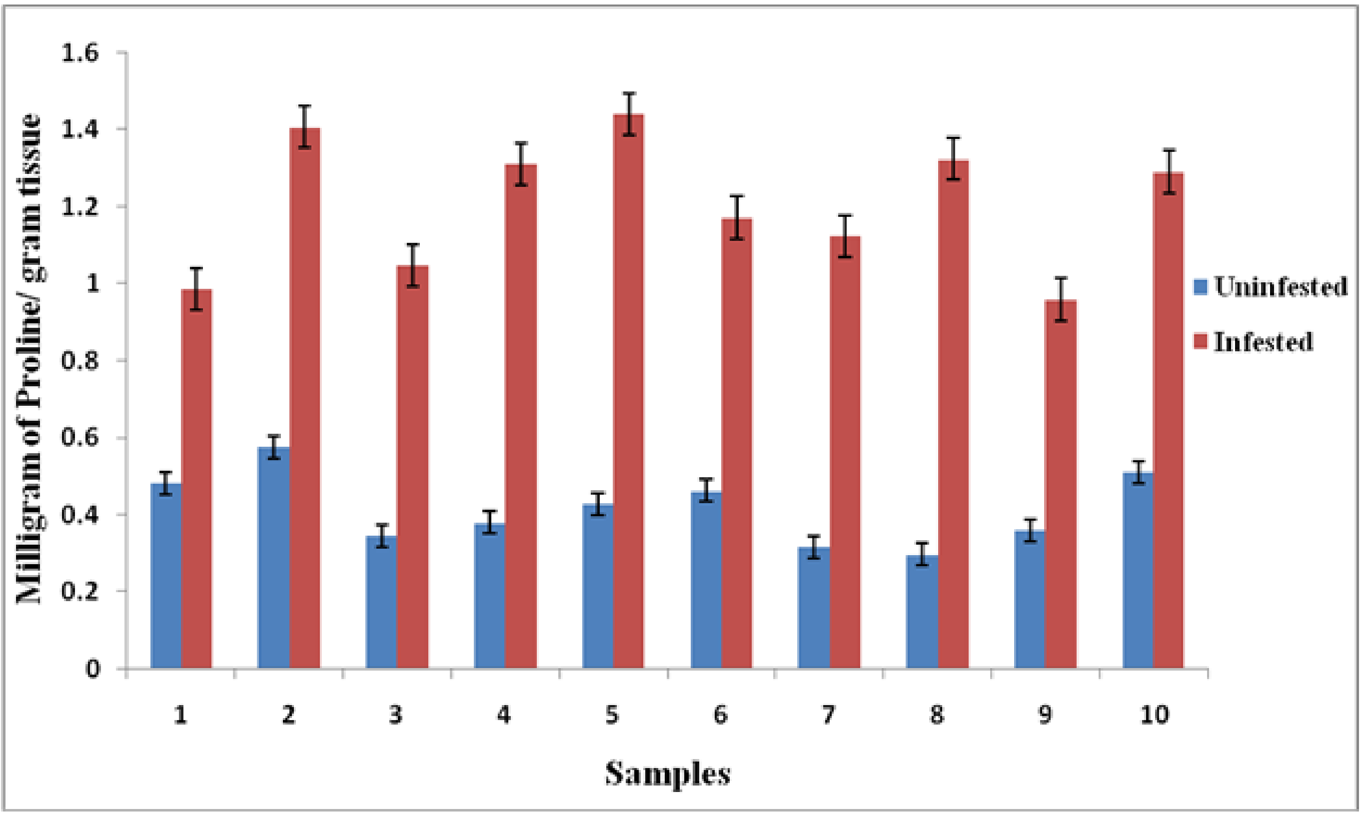

Figure 7. Amount of proline in *Raoiella indica* infested and uninfested *Areca catechu* leaf sample.

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