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Distribution and molecular characterization of *Lasiodiplodia theobromae* and *Aceria guerreronis* associated with coconut

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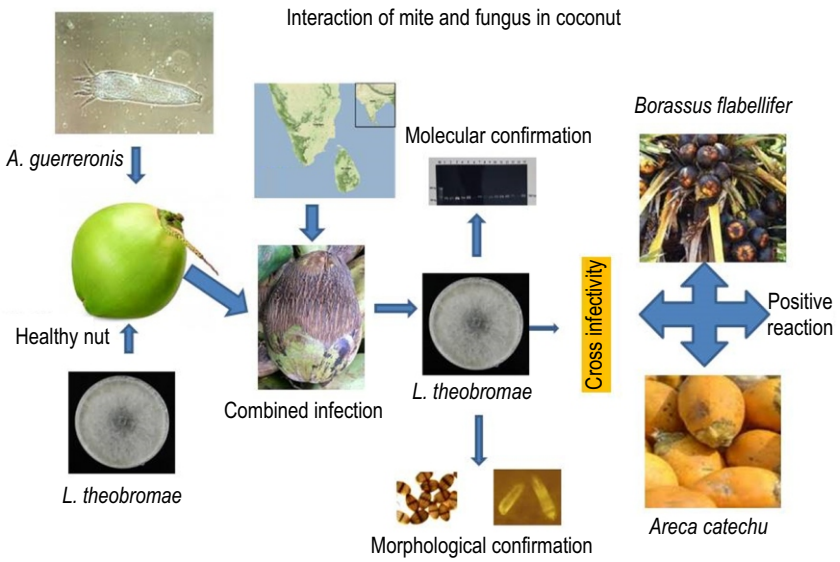
Abstract

Aim: A survey was conducted in major coconut-growing areas of Tamil Nadu in order to determine the presence of a fungus *Aceria guerreronis,* and a mite *Lasiodiplodia theobromae* and their interactive effects. The objective of the study was to ascertain the interaction, pathogenicity and cross infectivity reaction to infection.

Methodology: Pathogenicity studies demonstrated the intensity of pest incidence, both individually and in combination, through morphological and molecular studies. Interestingly, interactions in a few crops were confirmed through PCR amplification.

Results: The survey revealed a significant positive correlation between mite and fungus infestations and GPS location (χ^2 = 2114.22, df = 1; P < 0.001). Molecular studies identified product sizes of 347 bp for *Lasiodiplodia theobromae* KKLT01 and 500 bp for *Aceria guerreronis* KKAG01. Cross-infectivity tests demonstrated that toddy palm and arecanut experienced the same effects as coconut, which was confirmed through PCR amplification.

Interpretation: The present study shows that apart from mite infestation, the fungus also play a major role in increasing infestation and the interaction was positively correlated for cross infectivity. The results of the present study will help to restructure the existing management strategy.



Key words: *Aceria guerreronis,* Coconut, Infestation, *Lasiodiplodia theobromae,* Morpho-molecular variations

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Introduction

Cocos nucifera L. is a member of family Arecaceae, and it is an important plantation crop in India. The coconut palm is widely grown in the Southern states of India, including Tamil Nadu and Kerala (Ramjegathesh *et al.,* 2019; Kappil *et al.,* 2021). Coconut production is threatened by several factors, with plant diseases among the major contributors (Salvatore *et al.,* 2020; Santos *et al.,* 2020). In 1998, Lakshmanan and Jagadeesan (2004) reported that malformation and cracking of coconuts are widely reported in the state of Tamil Nadu, which could be due to the interactive effects of eriophyid mite (*Aceria guerreronis*) infestation with the fungus *Lasiodiplodia theobromae* based on the expression of symptoms, however, they failed to prove through molecular approaches for confirmation of mite infestation and cross infectivity studies. They also reported that the nut rot of coconuts caused a 25% yield loss in old palms during severe incidences due to leaf blight. A recent survey (Srinivasan *et al.,* 2018) reported that around 70 % of the coconut palms are still affected by similar symptoms and have resulted in a massive yield loss. However, studies on the incidence of these two pests, their interactions, and their effects on a range of hosts have not been conducted.

A few reports cited for the fungal infestation in leaves and nuts of coconut palm (Dheepa *et al.,* 2018; Ramjegathesh *et al.,* 2019) have been molecularly proven and characterized. Hence, the fungus can cause severe infestation in the leaves and nuts of coconut. *Lasiodiplodia theobromae,* is a fungal pathogen that was described for the first time around 1890 by Saccardo, and is known to infect several crops, like cocoa, citrus, coconuts and mango. (Crous *et al.,* 2016; Saeed *et al.,* 2017; Mutia *et al.,* 2021). It is a cosmopolitan in distribution with a wide range of hosts, such as saprobe endophytes or destructive plant pathogens with symptoms of leaf spots, necrosis, gummosis and even the death of many host plants (Dissanayake *et al.,* 2016; Mehl, *et al.,* 2017). A 20–80% yield loss in various crops due to the fungus alone has been reported (Dheepa *et al.,* 2018). Though it is a cosmopolitan pathogen and has a wider host range, the researchers thought that there was a possibility for secondary infection and that the fungus may enter through the wounds created by the mite. Similarly, *Botryodiplodia theobromae* enter the twigs through wounds created by the feeding of the capsid insects *Sahlbergella singularis* and *Distantiella theobroma.* This can avoid the resurgence and percent incidence in coconut plantations.

A. guerreronis was initially discovered in Guerrero, Mexico, in 1965. In India, it was discovered in the Ernakulam region of Kerala in 1998. *Borassus flabellifer* (toddy palm), *Cocos nucifera* (coconut), *Lytocaryum weddellianum* and *Syagrus romanzoffiana* (queen palm) have been described as an eriophyid hosts (Navia *et al.,* 2015). Previous studies were conducted to study the infectivity, pathogenicity, and yield loss of the mite and the fungus individually, but they failed to study the interaction and yield loss. To resolve the uncertainty, an advanced study was conducted to prove the pathogenicity of mite and fungus that it would help to restrict resurgence of pest. The results of the study will assist the farmers in selecting varieties (green long, orange dwarf) and in monitoring the palms without mite damage, along with management strategies. In view of the above, this study was conducted to document the current distribution, identification, pathogenicity, scientific association of the fungus (*L. theobromae*) and the mite (*A. guerreronis*) in Southern India and their infestations. Difference in infectivity on different host plants was also evaluated.

Materials and Methods

Survey for the prevalence of the pests: The incidence of *L. theobromae* and eriophyid mites on coconut nuts was observed during survey in various coconut-growing areas areas *Viz.*, Trichy, Dindigul, Ramanathapuram, Erode, Pudukottai, Coimbatore, Karur, Salem, Tirunelveli, Kanyakumari, Kanchipuram, Salem, Tuticorin, Theni, Cuddalore, Sivagangai, Madurai, Chitambaram, Namakkal, Tanjore, Thiruvallur, Virudhunagar, Dharmapuri, Krishnagiri, Vizhupuram, Thiruvarur, Thiruvannamalai, Vellore, Perampalur of Tamil Nadu, South India, during 2020–21. Longitude and Latitude values were used to map exact sites using 'R Studio' software with the data packages: "ggplot" and "ggmap" (RStudio, 2020). In each site, 200 palms (10–30 years old) infested with eriophyid mites were randomly selected. Each selected tree was either climbed or a prismatic binocular was used to count the total number of nuts from a tree and record the presence of fungus and mites. The data recorded were expressed as: nuts with mite only, nuts with mite and fungus and nuts with fungus only. Mite infestation was recorded by the presence of typical damage symptoms (mite scars) on the nuts. The mite population was counted as 'number cm^{-2} of tissue area' from underneath the perianth. The fungus infestation was recorded based on the symptoms (lesion development) on the surface of the nut, and the severity was measured using a 4-point scale (0–3), where 0 = no lesion in the nut; 1 = 25% nut area affected; 2 = 26–50% nut area affected and 3=>50 % nut area affected (Lakshmanan and Jagadeesan, 2004).

Isolation and purification of fungal pathogen: Three-month-old nuts (cv. local) showing malformation and cracking symptoms were obtained from a Coconut Breeding Farm at Tamil Nadu Agricultural University, Coimbatore (one of the survey sites). Infected sections from the edge of the lesion were collected and surface-disinfected in 0.5% NaCl for 3 min placed on Potato Dextrose Agar (PDA) in 9 cm Petri plates, and incubated at 25±3ºC for 7 days (Phipps and Porter, 1998). Stock cultures of the pathogen were obtained by the hyphal tip transfer procedure (Marcenaro and Valkonen, 2016; Sudha *et al.,* 2021a,b) and maintained in tube slants of PDA at 10ºC. On the basis of the infestation recorded during a survey, a virulent single isolate was selected for further molecular characterization, pathogenicity studies, cross infectivity and to identify the resistant sources.

Pathogenicity and morphological characterization of fungal pathogen and mite: To ascertain the mite and fungal

pathogenicity, virulent isolate was mass-grown in potato dextrose broth at 25±2°C for two weeks. Spore-containing mycelial mat was removed from flasks, combined, and filtered through three layers of muslin cloth. The spore concentration in the KKLT01 suspension was adjusted to $5x10^5$ cfu ml^{-1}. Three-month-old, healthy nuts were collected in the laboratory, along with bunches (10–15), and the surfaces were treated with 95% ethyl alcohol for surface disinfection. The inoculation techniques as injection of 1 ml of fungal pathogen isolate KKLT01 spore suspension (5×10^5 spores) under the perianth after eriophyid mite-infested coconut tissue (10–15 per microscopic field) from 3-month-old nuts, placing tissue with eriophyid mites alone, injection of spore suspension following a single, mild puncture. Nuts were exposed to pure PDA plugs devoid of any fungi or mites as a negative control. They were incubated at 25±3°C in the laboratory. Fifty nuts were incubated under glasshouse conditions for each treatment. As previously stated, disease severity was evaluated on a 0–3 scale every two days for 30 days. The pathogenicity was proved from inoculated nuts, infected with mites and fungi, DNA was extracted following the CTAB method described by Schneider *et al.* (2012), and the species was validated using specific primers.

Molecular characterization of fungal pathogen: Following the Cetyltrimethyl Ammonium Bromide (CTAB) method (Watanabe *et al.,* 2010), total DNA was extracted from the culture of fungal pathogen. Primers specific to each species were carefully designed and selected for PCR analysis, targeting the ribosomal DNA internal transcribed spacer (ITS) region. Primers Lt347-F (AACGTACCTCTGTTGCTTTGGC) and Lt347-R (TACTACGCTTGAGGGCTGAACA) was used for fungal pathogen specificity.

Molecular characterization of fungal pathogen

DNA extraction and sequencing: This method was described by Chetverikov *et al.* (2021 and 2022) and Watanabe *et al.* (2010). For DNA extraction, mite specimens were crushed with a fine pin in 3 µl distilled water on a cavity well microscope slide. To identify the mite primers were designed to amplify a 444 base pair-long fragment from the internal transcribed spacer (ITS) region of the mite (http://primer3.ut.ee/). An *A. guerreronis* ITS sequence (Genbank accession number DQ060619) was used as a template to obtain forward: (AgM_F; 5'-ATGGTCCTTCAATGGCTACG-3') and reverse (AgL_R: 5'-CATG[1]CAAGACGAAACTCGAA-3') primers. The percentage of sequence similarity with the ITS region of *A. guerreronis* was recorded (accession number DQ060624.1) (Chetverikov *et al.,* 2022).

Infectivity of fungal pathogen and eriophyid mite on different crops: Various plant parts of thirty-three crops grown around coconut gardens were selected to assess the infectivity of KKLT01 and KKAG01 in combination. As per the method described in pathogenicity studies the plant parts were inoculated and incubated. To study the infectivity by morphological method, the population of the mite was counted as 'number/ cm^2 of tissue area' from the underneath of perianth. The fungal infestation was recorded based on the method described earlier in survey for the prevalence of the pests. Five replications were used for each plant species. Further, the results of the morphological infectivity molecular confirmation were carried out based on the methods of Watanabe *et al.* (2010).

Statistical analyses: Relationship between the mite and fungus was performed using a generalized linear model (GLM) with binomial distributions through a logit link function. All GLM analyses were carried out with statistical software R using 'glm' package. Disease indexes (PDI) were compared among treatments using an LMM. Using an unstructured covariance matrix structure, the LMM modelled the correlation of PDI among repeated measurements during the post-inoculation period as a random effect. Data on 'nuts with mites only (%)' and 'nuts with fungus only (%)' were selected as two explanatory numerical variables and 'nuts with the mite and fungus (%)' as a dependent variable. In each relationship analysis, latitude and longitude values were selected as a single explanatory numerical variable.

Results and Discussion

A survey was conducted to study the prevalence of *L. theobromae* and eriophyid mites during 2020-21 (Fig. 1). Out of 24211 nuts sampled, the symptoms of mite alone were observed on 12054 nuts, while symptoms due to fungal pathogen were observed on 508 nuts (Table 1). About, 49.78% of the nuts (12054) displayed the symptoms of both mite and fungus. The incidence of fungus and mite was highest in Kanyakumari district (74.73%) and lowest in Vellore district (26.87%). Infestation of mite alone was maximum in Madurai district (77.43%), lowest in Kanyakumari district (27.97 %), while the fungal incidence was maximum in Cuddalore district (3.68%) and lowest in Vellore district (0.99%) (Table 1). Nuts with both mite and fungal infestation were positively correlated (χ^2= 2114.22, df = 1; P<0.001; Fig. 2), while correlation was not significant with nuts with fungal and mite infestation separately (χ^2= 0.68, df = 1; P=0.409; Fig. 3). Both latitude and longitude values were negatively correlated for all types of infestation (Latitude: χ^2 = 397.38, df = 1; P<0.001, χ^2 = 8.23, df = 1; P=0.004, χ^2 = 331.36, df = 1; P<0.001, respectively; Longitude: (χ^2= 74.70, df = 1; P<0.001, χ^2= 12.46, df = 1; P<0.001, χ^2= 55.03, df = 1; P<0.001, respectively).

The prevalence of mites and fungi was widespread in all the coconut areas surveyed. The highest rates of infested nuts were found in Kanyakumari and the lowest in Vellore district. Interestingly, the climate of these areas is harsh, and it is consistent with the findings of Shah *et al.* (2009), which elaborates that disease incidence were peak in cooler region compared to hotter areas. Similarly, Venugopal and Mohan (2010) confirmed the association between relative humidity, the lowest temperature, and the frequency of rainy days. The mite and fungus outbreaks began in April and reached their peak during October. The results were supported by meteorological data, which showed that the Kanyakumari and Vellore districts have an average temperature of 23°C and 31.3°C, respectively

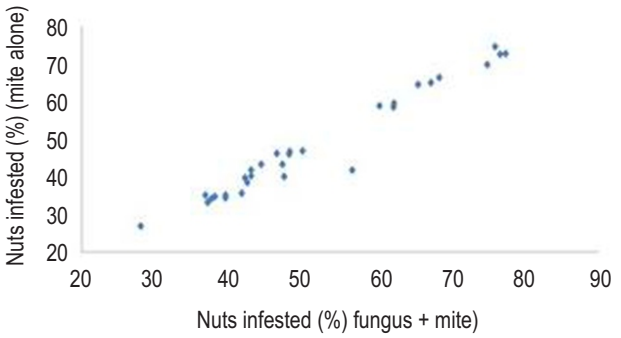


Fig. 1: Pearson correlation between nuts infested with both fungus and mites with nuts infested with mite alone. Each blue dot represents data from different survey sites.

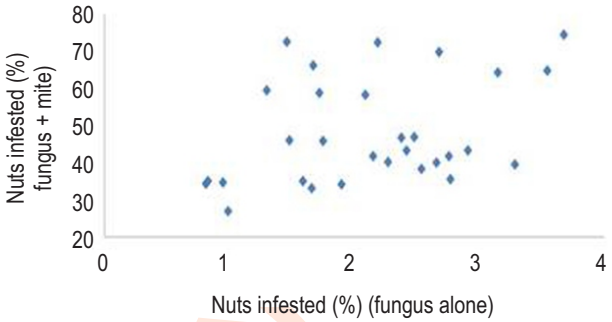
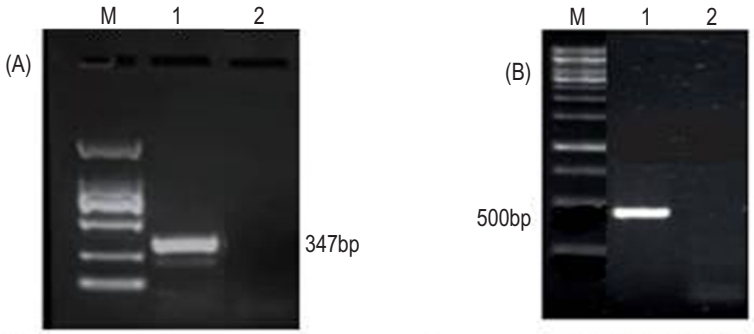


Fig. 2: Pearson correlation between nuts infested with fungus alone with nuts infested with both fungus and mites. Each blue dot represents data from different survey sites.

Fig. 3: Survey on the prevalence of *L. theobromae* KKL T01 and eriophyid mites (*A. guerreronis*) in coconuts palms. (a) Eriophyid mite alone; (b) Eriophyid mite + *L. theobromae* KKL T01 and (c) *L. theobromae* KKL T01 alone.



Lane M: Marker; Lane 1: *Lasiodiplodia theobromae;* Lane 2: Negative control (PDA plug)

Lane M: Marker; Lane 1: *Aceria guerreronis;* Lane 2: Negative control (without mite)

Fig. 4: Evaluation of primer specificity using specific primers Lt347-F and Lt347-R for *Lasiodiplodia theobromae* (A); Molecular characterization of *Aceria guerreronis* using primers AgM-F and AgL-R (B).

(Tamil Nadu's Statistical Handbook).

The fungus produced a light grey-pattern, fluffy, and aerial mycelium on PDA media peculiar to *L. theobromae.* The culture became dark coloured in advanced stages. Mycelium was immersed or superficial, branched, septate and brown. Early, the hyphae were thin and sub-hyaline whereas they became thick, closely septate, and brown in colour with time (3.7–11.5 µm in width). Pycnidial primordia appeared as tiny, dark brown structure with slightly raised spots, first on mycelium close to the inoculum.

Thereafter, dark brown flask shaped, ostiolate pycnidia appeared in 7-day-old cultures. The ostiole was circular and oriented at the apex of an elongated neck through which pycnidiospores extruded. Pycnidia varied in size, 125-180 µm x 80-145µm. Initially the pycnidiospores were hyaline globose to

Table 1: Levels of mite incidence (%), mite + fungus incidence and fungus incidence (%) alone, mean of number of nuts/tree and total number of nuts

Districts	Latitude Longitude	Mean no. of nuts/ tree	Total no. of nuts	Nuts with mite scars alone	% mite infested nuts	Nuts with mite and fungus	% mite and fungus infested nuts	Nuts with fungus alone	% fungus infested nuts
Trichy	10.9549N 78.4439E	37.6	756	298	39.42	265	35.05	12	1.59
Dindigul	10.4897N 77.7544E	54.7	1098	842	76.68	798	72.68	24	2.19
Ramanathapuram	9.7438N 79.0185E	58.5	1198	745	62.19	701	58.51	25	2.09
Erode	11.0797N 77.1758E	22.3	454	214	47.14	197	43.39	11	2.42
Pudukottai	10.1486N 78.8015E	41.6	997	621	62.29	594	59.58	13	1.30
Coimbatore- I	11.0122N 76.9354E	45.8	1009	756	74.93	706	69.97	27	2.68
Coimbatore- II	11.1465N 76.9445E	29.9	605	302	49.92	284	46.94	15	2.48
Karur	10.7202N 77.8900E	51.3	1020	698	68.43	678	66.47	17	1.67
Salem- I	11.6940N 77.9710E	43.7	998	375	37.58	342	34.27	19	1.90
Tirunelveli	8.9564N 77.3152E	40.3	842	312	37.05	279	33.14	14	1.66
Kanyakumari	8.2456N 77.3156E	56.4	1112	845	75.99	831	74.73	41	3.68
Kanchipuram	12.6819N 79.9888E	33.6	706	303	42.92	284	40.23	16	2.27
Salem - II	11.5867N 78.7583E	31.9	687	286	41.63	245	35.66	19	2.77
Tuticorin	8.7063N 77.8550E	28.7	601	285	47.42	241	40.10	16	2.66
Theni	10.1239N 77.5475E	34.6	756	364	48.15	354	46.83	18	2.38
Cuddalore	11.5159N 79.3269E	22.5	406	245	60.34	239	58.87	07	1.72
Sivagangai	9.8260N 78.2576E	18.6	354	150	42.37	136	38.42	9	2.54
Madurai	10.0313N 78.3382E	47.9	978	554	56.65	409	41.82	21	2.15
Chitambaram	11.3921N 79.7147E	45.9	1028	796	77.43	749	72.86	15	1.46
Namakkal	11.3593N 78.0892E	35.8	845	321	37.99	294	34.79	8	0.95
Tanjore	10.9233N 79.2864E	44.2	865	341	39.42	297	34.34	7	0.81
Thiruvallur	13.3339N 80.1943E	43.9	842	309	36.70	296	35.15	7	0.83
Virudhunagar	9.3580N 77.9156E	36.2	810	376	46.42	374	46.17	12	1.48
Dharmapuri	12.3712N 78.2159E	27.8	549	243	44.26	238	43.35	16	2.91
Krishnagiri	12.7409N 77.8253E	28.9	608	256	42.11	241	39.64	20	3.29
Vizhupuram	11.6342N 78.8785E	34.8	796	342	42.96	333	41.83	22	2.76
Thiruvarur	10.6649N	44.8	826	541	65.50	533	64.53	26	3.15

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	79.4507E								
Thiruvannamalai	12.2529N	42.9	816	549	67.28	531	65.07	29	3.55
	79.4160E								
Vellore	12.6825N	46.7	908	254	27.97	244	26.87	9	0.99
	78.6167E								
Perampalur	11.2286N	30.8	741	356	48.04	341	46.02	13	1.75
	79.0207E								
Total			24211	12879		12054		508	

Table 2: Morphological expression of pathogens

Treatments	Total no. of nuts	Symptom expressed in no. of nuts	Days after inoculation	Percentage of expression	Days taken for complete expression of symptom in all 50 nuts
Mite alone	50	35	15	70	20
Fungus alone	50	29	12	58	15
Mite + Fungus	50	38	7	76	12

oblong and unicellular however, became brown and 1-septate with age and measured 20.5-30.0 µm x 11.0-13.5 µm. The results of both field survey and laboratory experiments suggested an interactive effect of the presence of mites and fungus on symptoms of cracking and malformation of nuts, while individual pest effects were symptomatically different (Fig 4). This indicates that the fungus *L. theobromae* is able to infect the nuts faster if there is a predisposing factor, *i.e.,* mite scars. Due to shorter life cycle of mites, the population multiplies numerous times in a short span of time (8–10 days). Symptoms of infestation usually occur 3–4 weeks after the first colonization. The degree of deformity varies according to mite and fungal infestation.

The infested nuts were further proved by molecular characterization for their pathogenicity. On the basis of the generation of pycnidiospores, a virulent isolate known as *L. theobromae* KKLT01 was selected (isolated from Kanyakumari district), and tested for pathogenicity. The degree of sequence similarity between different ITS regions was evaluated. Based on the comparison of the sequences, it was determined that a portion of the internal transcribed spacer region (ITS) of *L. theobromae* was unique. Lane 1, amplified for *L. theobromae* KKLT01 A at 374 bp of primers were tested, specific for one species: Lt347-F/R for *L. theobromae*. As a negative control, DNA from Lane 2, was used which had been infected with PDA plugs (Fig. 5). PCR analysis showed that the primers AgM F, AgL R produced a single band of 500 bp for *A. guerreronis* KKAG01 isolated from Kanyakumari district. As a negative control, DNA from Lane 2, was used as a negative control (without mite) (Fig. 5).

Nuts inoculated with eriophyid mite alone produced typical symptoms of nut cracks with a corky appearance on 15th day. No symptoms of any fungal diseases were present. On 12th day of inoculation, there was an initiation of brown lesions from the perianth region. Hence, inoculation of fungus alone exhibited brown lesions without crack or malformation. On the 7th day of inoculation of both mite and fungus, the symptoms of cracks along with malformation and hardness were observed. It took 12 days for the symptoms to be visible in all 50 nuts. The first visible symptoms on nuts was due the inoculation of neither mite nor fungus alone, and both took 15, 12 and 7 days, respectively (Table 2; Fig. 6.). The results proved that within 7 days of inoculation, the nuts infested with mite and fungus could produce symptoms, but it may not possible individually by mite and fungus. Johnson *et al.* (2014) described that species of *Lasiodiplodia* have been differentiated based on phylogenetic analysis and morphological characteristics.

The fungus can show aseptate paraphyses when young, however, these paraphyses may develop septa as they mature (Abdollahzadeh *et al.,* 2010). Ramjegathesh *et al.* (2019) described that pycnidium of *L. theobromae* isolated from coconut produced pycnidiospores and the spores were initially hyaline, elliptical and aseptate. After 5 days of incubation, brown lesions appeared on the nuts, and DNA recovered from the infected tissue was amplified by PCR. The results showed that the PCR experiment conducted with the primers Lt 347-F/R, AgM F, AgL R produced predicted bands of 347 and 500 base pairs for each nut sample (Fig. 7). Lane 2, with PDA was used as a negative control for KKLT01, on the other hand, did not produce any bands that were amplified (Fig. 7 A). Consequently, the findings of this study revealed that amplification using PCR is an effective method for proving pathogenicity. In contrast, Lane 2, which had been injected with aphids and acted as a negative control for KKAG01, did not exhibit any amplification of bands (Fig. 7B). The identification of *L. theobromae* through amplifying of ITS regions with forward and reverse primer pairs was reported by Ramjegathesh *et al.* (2019). The morphology combined with

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Fig. 5: Pathogenicity study and Symptom expression (a) Eriophyid mite alone; (b) Eriophyid mite + *L. theobromae*; (c) *L. theobromae* alone.

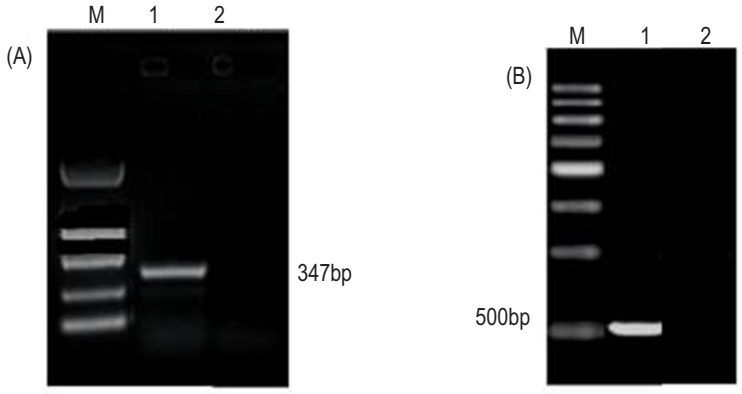

Fig. 6: Koch's Postulates: Pathogenicity based on molecular confirmation. (A). Lane M. marker; Lane 1. *Lasiodiplodia theobromae,* lane 2, Negative control (sterile water); (B). Lane M. marker; Lane 1. *Aceria guerreronis,* lane 2, Negative control (without mite)

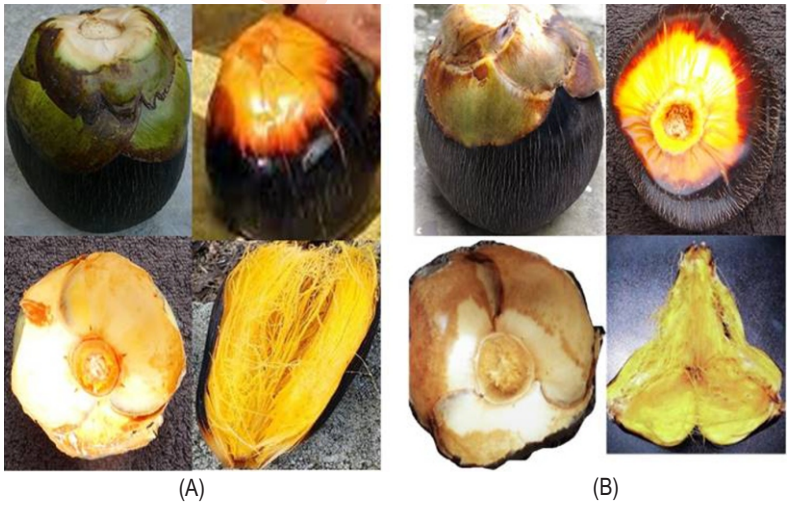

Fig. 7: Infectivity of *L. theobromae* and *A. guerreronis* in *Borassus flabellifer* (A) Healthy fruit; (B) Infected fruit.

Table 3: Infectivity of the pathogens on various crops

Common name	Disease incidence in percent* on 7 DAI	Presence of mite in nos.	Presence of hardiness and malformation
Mango	19.23	0.0	absent
Banana	21.57	0.0	absent
Papaya	16.58	0.0	absent
Sapota	0.0	0.0	absent
Avocado pear	0.0	0.0	absent
Apple	0.0	0.0	absent
Anona	0.0	0.0	absent
Grapes	0.0	0.0	absent
Pomegranate	18.56	0.0	absent
Guava	0.0	0.0	absent
Elephant- foot yam	18.35	0.0	absent
Carrot	0.0	0.0	absent
Beet root	0.0	0.0	absent
Turnip	0.0	0.0	absent
Knol-chol	0.0	0.0	absent
Cacao	0.0	0.0	absent
Jack fruit	0.0	0.0	absent
Cauliflower	0.0	0.0	absent
Brinjal	0.0	0.0	absent
Okra	0.0	0.0	absent
Tomato	0.0	0.0	absent
Onion	0.0	0.0	absent
Garlic	0.0	0.0	absent
Cluster bean	0.0	0.0	absent
Mandarin orange	0.0	0.0	absent
Pineapple	0.0	0.0	absent
Tapioca	0.0	0.0	absent
Sathukudi	16.32	0.0	absent
Lemon	17.24	0.0	absent
Sweet potato	0.0	0.0	absent
Water melon	0.0	0.0	absent
Arecanut /Betel nut	24.67	65.0	present
Palmyra palm or toddy palm	26.78	79.0	present

*Mean of three replications

molecular sequence analysis clearly define the taxonomy *L. theobromae.* The partial sequence of ITS regions confirmed that the fungus was *L. theobromae.*

Among the thirty-three plant species tested, *Borassus flabellifer* and *Areca catechu* displayed similar symptoms with those of coconuts within 3 days, when exposed to eriophyid mites and fungi (Fig 8). *Mangifera indica*, *Musa sapientum*, *Carica papaya, Punica granatum, Amorphophallus companulatus, Citrus lemettiodes* and *Citrus limonum* exhibited reddish brown lesions within 5 days of inoculation, but hardiness and malformation were not observed. Within 7–10 days, the tissues completely decayed and attained more than 15% incidence with the fungus alone, and no mite incidence was noted in the inoculated crops. (Table 3). The results of the plant species (*Borassus flabellifer* and *Areca catechu*) having both mite and fungus incidences were confirmed with molecular analysis for *L. theobromae* and eriophyid mite with their specific primers (Lt347-F/R and AgM-F, AgL-R) as described above. The primers exhibited bands at 347 and 500 bp for the fungus and mite.

The symptoms were not exhibited in *Abelmoschus esculentus* and *Anona squamosa* even after 30 days of post-treatment. The fungus, *L. theobromae,* is a weak parasite that requires some form of injury to plant parts for effective infection. For example, cashew inflorescence blight caused by *L. theobromae* requires injury from insect herbivores for infection (Adeniyi *et al.,* 2013; Correia *et al.,* 2016). Similar results were reported by Nath (2011) in bananas, where infection with the fungus was only successful following the pinprick method. The pathogen also causes coconut leaf blight, and this may act as reservoir for secondary infections in nuts (Dheepa *et al.,* 2018).

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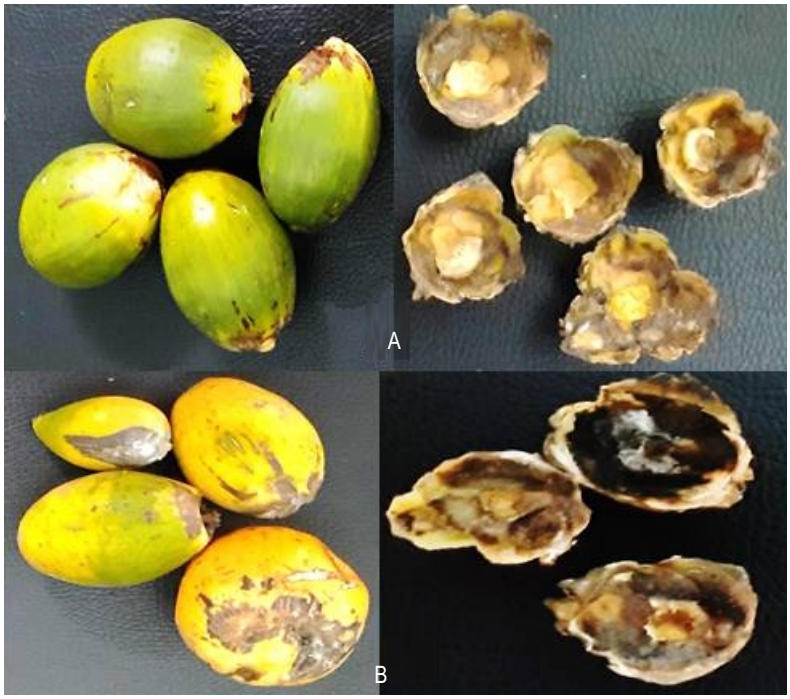

Fig. 8: Infectivity of *Lasiodiplodia theobromae* and *Aceria guerreronis* in *Areca catechu*. (A) Healthy nuts and (B) Infected nuts.

Olunloyo (1979) observed that cashew inflorescence blight incited by *L. theobromae* spread through insects, which develops wounds on the inflorescence axes to cause infection. However, there is no evidence that this mite transmits the fungal pathogen to coconut palm. Cruz *et al.* (2019) reported that *L. subglobosa, L. iraniensis* and *L. pseudotheobromae* were highly virulent, as they caused necrotic lesions and gummosis. While mites are known as vectors for some plant diseases such as Wheat streak mosaic virus (WSMV), wheat spot mosaic virus (WSPMV), ryegrass mosaic virus (RgMV), fig mosaic virus, cherry mottle leaf virus, current reversion disease, pigeon pea sterility mosaic disease and rose rosette diseases are all vectored by different species of eriophyid mites (Hadidi *et al.,* 2011; Vazquez-Iglesias *et al.,* 2020), Our results from laboratory studies do not support this hypothesis. Therefore, it is likely that the mite scars act as a predisposing factor for higher disease incidence in coconuts. There is no evidence for mite transmission of fungal pathogen in coconut, except for red gram and leaf blight of cashew.

Artificial fungus inoculation suggests a wide host range for the fungus. Only *Borassus flabellifer* and Areca catechu exhibited similar symptoms to those of coconuts among the 33 host plants tested. Infections were evident in 31 species after 15 days, whereas *Borassus flabellifer* and *Areca catechu* exhibited similar symptoms to those of coconuts. Symptoms of infection were not observed in two plant species even after 30 days of exposure. Abdollahzadeh *et al.* (2010) reported that *L. theobromae* has a wide host range found in tropical and subtropical regions (Javaid *et al.,* 2008; Coutinhoa *et al.,* 2017; de silva *et al.,* 2016; 2019; Dou *et al.,* 2017; Li *et al.,* 2018). The host range of *A. guerreronis* was also observed in palmyrah palm (*Borassus flabellifer*) in India (Ramaraju *et al.,* 2001). Other than coconut, an ornamental palm *Cocos weddelliana* was reported as a host plant of mite in Brazil, (Flechtmann, 1994). *A. guerreronis* insect pest affects the nuts of palmyrah palm, where the tree is an important source of toddy. The large number of plant species from where the fungus, *L. theobromae* has been recovered suggest that these hosts may potentially serve as inoculum sources and may pose a threat to coconut plantations (Johnson *et al.,* 2014; Li *et al.,* 2020; Dwiastuti and Aji, 2021). Therefore, it is important to monitor the intercrops and surrounding plants in the plantations, and the management of *A. guerreronis* mite, hence, focus should be on the serves as a predisposing factor for fungal infection (Chandrashekar *et al.,* 2020). Although, *L. theobromae* cause diseases in mango, papaya, citrus, etc., a 70–80% yield loss has been recorded earlier in tropical countries (Dheepa *et al.,* 2018)

The research findings concluded that invariably all coconut-growing locations of Tamil Nadu, was infected with *L. theobromae* and *Aceria guerreronis*. The pathogenicity, molecular characterisation proved the positive correlation of mite and fungus in the infected nuts and also confirmed the cross infectivity in palmyrah palm and arecanut. This alarming damage might be reduced by following integrated pest and disease management practices Hence, the coconut growers may concentrate on good sanitation of the farm with recommended

package of practices to avoid infestation.

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