Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(2): 722-725
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Received: 06-01-2020
Accepted: 10-02-2020

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First report of rugose spiraling whitefly, *Aleurodicus rugioperculatus* martin (Hemiptera: Aleyrodidae) on coconut in Gujarat, India

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Abstract
Rugose Spiraling Whitefly (RSW), *Aleurodicus rugioperculatus* Martin firstly reported from Tamil Nadu state in India and recently, this invasive pest has entered in the coconut growing areas in Gujarat state. A survey was conducted for the identification of this invasive pest infesting coconut in Saurashtra region of Gujarat during August, 2019. During the survey, coconut fields were infested by this invasive pest in the villages of Mangrol and Veraval of Saurashtra region. The infestation due to the pest was observed very severe in all the palms present in the field and lower leaves were mostly infested as compared to the middle and upper leaves. The population of RSW was observed on both tall and dwarf palms, but it was severe in dwarf and hybrid coconut palms. Nymphs and adults of RSW were collected from all the infested fields and brought to the Biocontrol Research Laboratory, Department of Entomology, Junagadh Agricultural University, Junagadh for further morphological and molecular identification. The pest is identified as Rugose Spiraling Whitefly (RSW), *Aleurodicus rugioperculatus* Martin on the basis of spiral pattern of egg laying, morphology and molecular characterization and it is the first confirmed report of occurrence of invasive pest in coconut fields of Gujarat, India.

Keywords: *Aleurodicus rugioperculatus*, coconut, Gujarat, invasive, spiraling whitefly

1. Introduction
Whiteflies belong to the order Hemiptera and comprise a single superfamily, Aleyrodoidea, within the suborder Sternorrhyncha. The whitefly genus *Aleurodicus* Douglas encompasses 35 species, of which only the spiralling whitefly *Aleurodicus disperses* Russel was so far known to occur in India [1]. This pest was found in oil palm mainly in the states of Andhra Pradesh and Karnataka in India with severe incidence and infestation. This dangerous invasive pest was firstly reported on coconut (*Cocos nucifera* L.) at Pollachi, Tamil Nadu in India during August, 2016 [2]. *A. rugioperculatus* was originally described from Belize [3] and belongs to the niveus species-group of *Aleurodicus* [4]. It is naturally distributed in Belize, Guatemala, Mexico [4] and subsequently, it has spread to 22 other countries in Central and South America, including Florida, USA. It invaded Florida in the United States in 2009 and Guatemala [5] and since then its range expanded considerably within the United States [6]. The RSW is highly polyphagous with 118 hosts belonging to 43 plant families including economically important crops in the United States [6]. India is the only country in the Oriental region where the whitefly has been introduced. Rugose whitefly feeding causes stress to the host plant by removing water and nutrients and also by producing honeydew, which covers the lower leaves and results in the growth of sooty mold. Although sooty mold is not a plant disease, its presence on the upper surface of the leaf can potentially reduce photosynthesis of the plant [1].
Due to the invasive nature and fast dispersal behaviour of RSW, a survey work was carried out in the coconut growing areas by the scientists of Department of Entomology, Junagadh Agricultural University to study the occurrence of this pest during August, 2019 in Gujarat state. The visible observations and further identification during the survey lead to confirmation of the occurrence of rugose spiraling whitefly, *A. rugioperculatus* on coconut in Gujarat state.

2. Materials and Methods
An intensive continue survey work for monitoring of rugose spiraling whitefly in coconut was carried out in Junagadh and Veraval district of Saurashtra region of Gujarat. During monitoring programme, distinguished damage symptoms was observed and this pest was firstly recorded in coconut from Husenabad village (21.1171° N, 70.1639° E) of Mangrol

Taluka of Junagadh district of Saurashtra region of Gujarat state. The infested palms showed the typical characteristic like eggs on the underside of leaves in a concentric circular or spiral pattern and cover it with white waxy matter on leaves and fruits. Due to the heavy occurrence, the palms were fully covered by RSW and glistening liquid substance (honeydew) was deposited on leaves and turned blackish. During further survey, the pest was also observed from Mankhetra (21.1521° N, 70.1303° E), Farangata (21.2039° N, 70.0701° E) villages of Mangrol taluka and Supasi (20.9839° N, 70.3175° E), Gadu (21.0538° N, 70.2910° E) villages of Veraval district. As the occurrence of RSW was confirmed from different locations, the samples were collected and brought to the Biocontrol Research Laboratory, Department of Entomology, Junagadh Agricultural University, Junagadh for detailed observations.

2.1 Molecular identification

The molecular characterization RSW was carried out at SLS Research Pvt. Ltd., Surat. DNA from RSW was isolated and its quality was evaluated on 1.0% Agarose Gel, a single band of high-molecular weight DNA has been observed. Fragment of gene was amplified by PCR. A single discrete PCR amplicon band was observed when resolved on Agarose Gel. The PCR amplicon was purified by column purification to remove contaminants. DNA sequencing reaction of PCR amplicon was carried out with primer HCOI and LCOI using BDT v3.1 cycle sequencing kit on ABI 3730xl Genetic Analyzer. The gene sequence was used to carry out BLAST with the database of NCBI Genbank database. Based on maximum identity score, first ten sequences were selected and aligned using multiple alignment software programs.

3. Results and Discussion

Recently, an infestation of yet another invasive species, *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae), commonly known as the rugose spiraling whitefly (RSW), was firstly observed on coconut palm (*Cocos nucifera* L.) from coastal belt of Mangrol in Junagadh district, Gujarat during month of September. The typical characteristics like eggs covered with wax were laid by females in a circular or spiral fashion usually on the abaxial surface of leaves. The same pattern was also observed on the nuts and the developed fruits of the coconut (Fig. 1). Nymphs and adults were congregated on the leaf surfaces and prolific feeding resulted in profuse honeydew that covered the undergrowth of plants which in turn became black due to the development of sooty mould. All the stages of RSW were carefully observed during the survey for the confirm identification of this pest.

3.1 Egg stage

Eggs are smooth, elliptical, whitish to yellow, translucent and laid by the females on underside of the leaves and fruits in spiral pattern which was covered with the waxy matter (Fig. 3A).

3.2 Nymphs

Rugose spiralling whitefly has 5 developmental stages. The first instar known as the crawler stage (because it is the only mobile immature stage) hatches out of the egg, and looks for a place to begin feeding with its needle-like mouth parts and sucks plant sap. Crawlers molt into immature stages that are immobile, oval and flat initially but become more convex with the progression of its life cycle [7]. During the survey nymphs were observed and they are light to golden yellow in colour in a concentrate manner fully covered with the waxy material and wax filaments are thin which may become denser after time lapse (Fig. 3B).

3.3 Puparium

Puparia are covered with wooly wax with a group in the colonies under the leaves of the coconut. Puparia of *A. rugioperculatus* are characterized by an apically acute lingual that is exerted and slightly short of the posterior margin of the pupa and a quadrate operculum with wrinkled or 'rugose' texture [1].

3.4 Adults

Adults are congregated with covering whole leaves and found to suck the sap under surface of leaves/leaflets (Fig. 3C). They are larger in size (about three times) as compared to commonly found whiteflies. Honey dew excretion in large quantity leads due to the feeding of the pest under surface of the leaves and premature leaves are dried because of heavy infestation. The lack of photosynthesis process, symptoms like sooty mold are observed (Fig. 2). Although taxonomic identification is required for species confirmation, rugose spiraling whitefly adults can be distinguished by their large size and the presence of a pair of irregular light brown bands across the wings [5].

Fig 1: Damage symptoms on coconut due to rugose spiraling whitefly

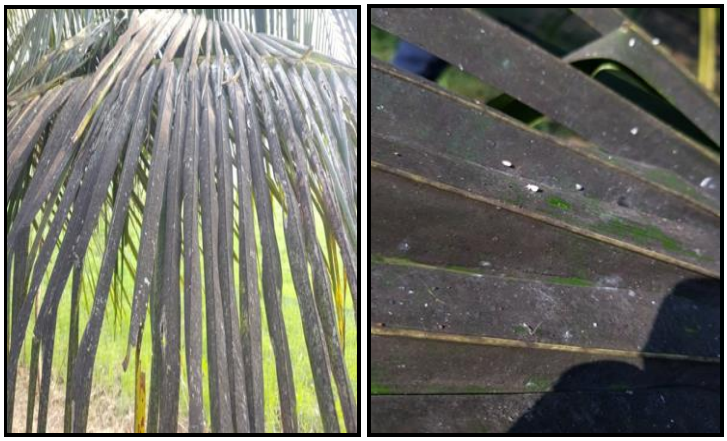

Fig 2: Development of sooty mould on coconut leaves

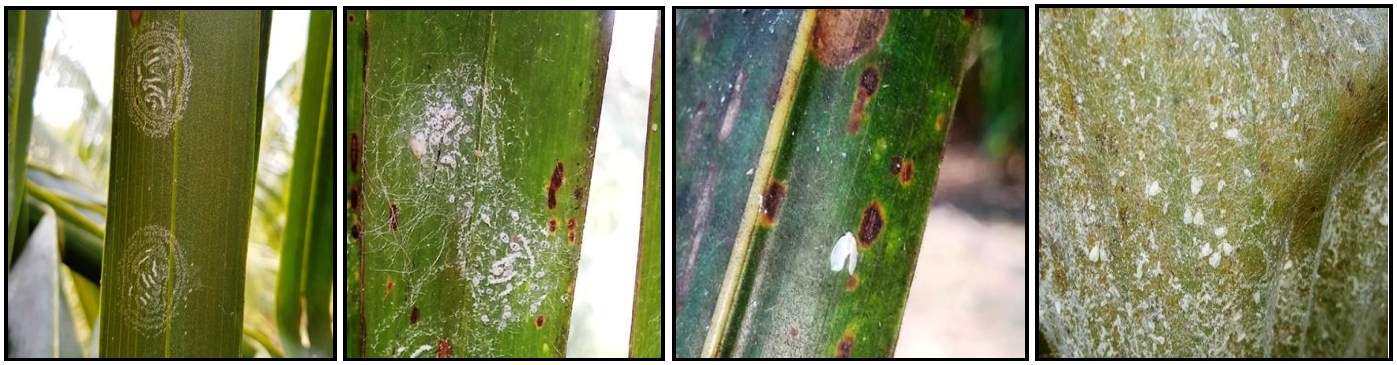

Fig 3: Different stages of RSW: A) Eggs B) Nymphs C) Adults

3.5 Molecular characterization

Fragments amplified from the HCOI and LCOI primer pairs aligned to their target reference sequence. The analysis results through search showed that the analyzed species belongs to *A. rugioperculatus*. The sequence obtained through molecular characterization showed that the population from Husenabad village (Mangrol) was 99% resemblance with the sequences of *A. rugioperculatus* having accession numbers, MK159741.1, MK159740.1, MK159739.1 and MK159738.1 from NCBI-Genbank.

Rugose spiraling whitefly, *A. rugioperculatus* gene sequence

GCACTTTAATTTAAAGTTGGTGGTTCCACAAAATCAT
AAAGATATTGGAATTTTATACCCTACCCCCGGGATC
AGAAGAGGATTTTATTGGTGCTTCTATAAGATTATTA
ATTCGAATAGAATTAAGAATATTAGGAAGTTATATA
AATAATGATCAGTTATATAATACATTAGTGACTTCAC
ATGCTTTTATTATAATTTTTTTTATAACTATACCTTTA
GTTATTGGTGGATTTGGAAATTGATTAGTTCCTTTAA
TAATTGGAGCTCCTGATATAGCTTTTCCTCGTATAAA
TAATTTAAGTTTTTGATTATTAATTCCTTCTTTGTTAT
TTTTAATTTTAAGAATATTAATTTCAAGAGGGGGGG
GAACTGGTTGAACAATTTATCCTCCTTTATCAACTCT
AATATTTCATAGAAGATGTTCTGTTGATTTAATAATT
TTTTCTTTACATTTAGCTGGTATTTCATCAATTCTTGG
TTCAATTAATTTTATTACAACAATTTTAAATATGCGG
TTAATTGGAATAAAATTAGAACAAATGTTTTTATTTG
TTTGATCAGTTTTAATTACTGTTTTTTTATTATTATTG
TCTTTACCTGTTTTAGCAGGAGCTATTACTATATTAT
TGATAGATCGAAATTTTAATAGAACTTTTTTTGATCC
TATGGGTGGAGGAGTGATTCCGTATTTTATATCAAC
ATTCCTTTTGATTTTTTGTTCTGCCCGCCAACTAAATT
CTGGGGGGGGGGGGAAAAACCACTCATCTTTTTATT
ATTTGTTTACCACACCACACATGATGTGGGGGGGAA
ACAAAACTCACATCTATTTTTTTGTTT

4. Conclusion

The invasive pest, *A. rugioperculatus* has already caused significant damage in the India. Currently, this pest has invaded mainly in coconut fields of Saurashtra region of Gujarat state. The morphological and molecular identification lead to confirmation of first ever report of the new invasive pest, rugose spiraling whitefly *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae) in coconut fields of Gujarat state, India. The current incidence of RSW in Gujarat is alarming due to its polyphagous nature and hence it has a great potential to extend its host range and spread to other coconut growing areas in the Gujarat state.

5. References

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