Original Research Article

Morpho-Molecular Characterization and Seasonality analysis of *Greenidea psidii* van der Goot on Guava in Terai zone of West Bengal.

ABSTRACT

Greenidea psidii, Guava aphid comes under the subfamily Greenideinae within the Aphididae and was first discovered in the World in 1916 on guava. In terai region of West Bengal these species are regarded as potential pest of guava (*Psidium guajava*). Therefore, quick and precise species identification is immediately needed for early detection and risk analysis followed by integrated management of the aphid pests. During morpho- taxonomy study we found that apterous aphids have long sized siphunculi, alongwith long setae present on the body. Moreover, the identity of the aphid species was confirmed through DNA barcoding of the mitochondrial cytochrome c oxidase sub unit I (COX-I) gene, another analytical tool for quick and precise identification. The COX-I sequence was successfully blasted for similarity search and uploaded in NCBI software (Accession No. OR085959). The seasonal fluctuation studies reveals that the maximum population of 21.85 aphids/ twigs and 17.45 aphids/ twigs were observed on 10^{th} SW during 2021 and on 11^{th} SW during 2022, respectively.

Keywords: Guava aphid, Identification, Terai region, Seasonal incidence, *Greenidea psidii.*

1. INTRODUCTION

Guava (*Psidium guajava* L; Myrtaceae) is one of the most vital commercial fruit crops in India. It is the fourth most valuable fruit after mango, banana and citrus. The production of guava affected by different insect pest. Among them, aphids are one of most important pests in India. Aphids are important phloem-feeding insect group of hemipterans, which comprises around 5000 species within three families, Adelgidae, Phylloxeridae and Aphididae (24 subfamilies). In addition, many species are serious pests for agriculture and forestry. Consequently, aphids have been considered superior model organisms for environmental and evolutionary studies (Baumann, 2005).

Greenidea psidii van der Goot comes under the subfamily Greenideinae within the Aphididae and includes approximately 45 species (Pérez Hidalgo et al., 2009). *Greenidea psidii* was first discovered in the World in 1916 on guava and its comparative, *Psidium cattleianum* Sabine in Brazil (Noemberg- Lazzari et al., 2006). In terai region of West Bengal these species are regarded as potential pests. *Greenidea psidii* are highly polyphagous in

nature and mainly distributed from east to west Himalayas (Singh, G *et al.,* 2017). Identification of the aphid specimens up to species level is needed to establish the diversity of the species, phylogenetic patterns and the evolutionary relationships (Platnick, 2014) as well as finally it helps for the management of the pests through sustainable way. Morphological identification of aphids is usually done based on different morpho-taxonomic keys. DNA barcoding is an analytic method for species identification. It aids in the identification of species in functional settings, the detection of morphologically cryptic species and the revealing of host-specific lineages (Miller and Foottit 2009). DNA barcoding has been used in a various range of taxa, including aphids (Hebert *et al*. 2003; and Chen *et al*. 2013; Wang *et al*. 2013). In this study, we test the effectiveness of DNA barcoding for species identification in Greenideinae. The standard molecular barcode, cytochrome *c* oxidase subunit I (COX I) was used for this subfamily.

The learning of association between aphids and its environment provides necessary information for interpreting spatial dynamics, scheming efficient sampling programmes for population judgment and helps in area wise pest management and the development of population models (Croft and Hoyt, 1983). The objective of the present study was to determine the aphid species of guava (*Psidium guajava*) in the Terai region of West Bengal, India through morpho-molecular characterization and their seasonality analysis, which aid in the development of appropriate management strategies timely.

2. MATERIALS AND METHODS

2.1 Sampling and Specimen Collection:

The aphid specimen was collected from the Guava orchard of Horticultural Farm, Uttar Banga Krishi Viswavidyalaya, Pundibari, West Bengal, India. During collection the aphids along with plant materials like tender leaves and apical twig portion were collected in plastic zipper bag. Afterwards, some aphids were transferred into the small vials containing 70% ethanol with a corresponding level and brought to the laboratory for taxonomic studies. The data on host plants, locality (GPS), date and other relevant information were noted.

2.2 Morphological Studies:

The collected aphid specimen was identified under a ZEISS (AXIOLAB 5) microscope in the Taxonomy laboratory of Uttar Banga Krishi Viswavidyalaya. Pundibari, West Bengal. Four to five apterous individuals of the sample were slide mounted by following the protocol of Brown & De Boise (2006). The taxonomic key of Blackman and Eastop (1994 and 2000) was followed for identification and by comparing with recognized specimens and the innovative morphological descriptions.

2.3 Molecular Identification:

The total genomic DNA was extracted from each aphid specimen following non-destructive method (Rowley *et al*., 2007). For DNA extraction, DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany) was used following manufacturer's protocol.

The extracted DNA was used for PCR amplification of the cytochrome oxidase subunit I (COI) region using universal forward primer LCO 1490 (5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′) and reverse primer HCO 2198 (5′- TAA ACT TCA GGG TGA CCA AAA AAT CA-3′). The cycling conditions followed for PCR were an initial denaturation step for 5 minutes at 94° C, followed by 30 cycles consisting of 45 Sec at 94° C, annealing for 45 Sec at 47° C and final extension step for 1 minute at 72°C; followed by final extension for 10 minutes at 72°C. The PCR were performed using a C1000^{TM} thermal cycler.

Polymerase chain reaction products were purified using Qiagen PCR purification kit and then directly sequenced. The amplified PCR product was sequenced by outsourcing of sample. The NCBI Blast software (Meier *et al*. 2006) was used to find the closest species match for each query. Afterwards the sequence was uploaded in NCBI Gen Bank to get the accession number.

2.4 Seasonality Study:

The seasonal incidence data of aphid was recorded at weekly interval basis in the Guava orchard of Horticultural Farm, Uttar Banga Krishi Viswavidyalaya, Pundibari, West Bengal, India in the year of 2021-2022. Aphid population was counted from five tender twigs of the randomly selected 20 guava plants. Meteorological data on temperature, relative humidity and rainfall for the period of experimentation were collected from the Meteorological Observatory Unit of the University located approximately at 300-meter distance from the guava orchard. Correlation and regression analysis was studied between the aphid population and weather parameters to find out the impact of weather on the aphid dynamics.

3. RESULT AND DISCUSSION:

3.1 Morpho Taxonomy:

The collected apterous aphid specimen was identified as *Greenidea psidii* with the taxonomic key of Blackman and Eastop (1994 and 2000). The aphids were reddish brown in colour and had long siphunculi covered with numerous long hairs. Siphunculi were dark brown in colour, apex and base portion were much darker as compared to middle portion but these were less dark as compared to dorsal abdominal sclerotization. The base portion of siphunculi had reticulation and abdominal sclerotization also found. The cauda was helmet shape with so many hairs. Head structure was undeveloped with no presence of spicules and rhinaria found on the antennae 3rd segment. It was observed that *G. psidii* was one of the fast-moving aphid species as compared to the other species. Therefore, *G. psidii* can be distinguished easily from other aphid species based on this morphological character.

Greenidea psidii has been reported in India, Bangladesh, Nepal, China, Taiwan, Japan, Indonesia, Philippines, Costa Rica and California (Beardsley, 1993; Gill, 1998; Halbert, 2004; Sugimoto 2008).

Comment [mm1]: Add dendrogram from phenetic tree

3.2 Molecular Taxonomy:

The COX-I sequence of the aphid specimen was successfully uploaded in NCBI software. The sequence showed 99 -100% similarity after BLAST and identified the species as *Greenidea psidii*.

Comment [mm2]: Not only blast. Can u add dendrogram from phylogenetic tree, include others isolat with highly similarity index from blast

Sequence confirmation details:

Species Name	Common Name	Host	Accession Number (NCBI)
Greenidea psidii	Guava Aphid	*Pisidium guajava*	OR085959

3.3 Seasonal Incidence:

The Guava aphid, *Greenidea psidii* was noted to be one of the most important insect pests of guava plant in northern Bengal. The infestation of *Greenidea psidii* on guava started from 4th SW in both the year 2021 and 2022. Maximum population of 21.85 aphids/ tender twigs and 17.45 aphids/ tender twigs cm was observed on 10th SW during 2021 and on 11th SW during 2022, respectively (Fig. 1 and 2). Thereafter gradual decline in the population of aphid was evident and the population were become zero. Then again, the population occurs on 43rd SW and 46th SW during 2021 and 2022 and again the population decline thereafter. Severity of aphid incidence was higher in the first year as compared to the second year. The abundance of guava aphid population during March, owing to the coincidence of emergence

of new shoots after the winter months, which is in line with the observation of Salas *et al*. (2011). There was no aphid population in guava plant from May onwards presumably due to maturity of the leaves and decline of the nutritional content in the food materials.

Aphid population was negatively correlated with maximum temperature and minimum temperature, maximum relative humidity, minimum relative humidity and with rainfall in both the year 2021 and 2022 (Table 1). While with bright sunshine hours it showed positive correlation in both the year. This study reveals that high temperature and rainfall had detrimental role on the guava aphid population. So, it may be proclaimed that life cycle of *G. psidii* is adapted to complete before the onset of summer and rainy season to avoid the high temperature and rainfall.

The multiple regression analysis of the aphid population with weather parameters has been worked out and the results have been presented in Table 2. Inspection of the results bare that the abiotic parameters had significant influence on the population fluctuation of aphid during both the years and collectively all the abiotic parameters were responsible for 62.5% and 52.7% variation in the aphid population during 2021 and 2022, respectively.

Comment [mm3]: Add discussion. Please explain the linkages between morphology identification and molecular identification

4. CONCLUSION:

The knowledge on occurrence and seasonality of *Greenidea psidii* will be helpful to formulate the integrated management schedule. It is also noted that no proper information on seasonality of Greenidea psidii was not found in Indian context while literature survey. Therefore, this information on seasonality will be helpful or supportive. Besides further research work is needed to evaluate the natural enemy complex of the aphid species for their conservation and multiplication. The taxonomic as well as molecular characterisation was followed for species identification might assists to recognise the phenotypic plasticity as well as cryptic species.

REFERENCE:

Comment [mm4]: Add references using limited 5 years ago

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Table 1: Correlation coefficients between Guava aphids with weather parameters during 2021-22

Year	Insect	Temperature (°C)	Relative humidity	Rainfall	Sunshine

				(%)			
		Max.	Min.	Max.	Min.		
2021	Aphid	-.058	-.388**	-.505**	-.588**	-.310*	.148
2022	Aphid	-.028	-.294*	-.449**	-.572**	-.245	.303*

**** Correlation is significant at the 0.01 level (2-tailed).**

*** Correlation is significant at the 0.05 level (2-tailed).**

Table 2: Multiple regression analysis of aphid with weather parameters

Year	Multiple regression equation	R^2	Adjusted R^2
2021	$Y= 40.258 + .346X_1 + .140X_2 - .052X_3 - .555X_4 + 0.079X_5 - 2.566X_6$	0.625	0.575
2022	$Y= 30.044 + .250X_1 + .217X_2 - .056X_3 - .439X_4 + 0.050X_5 + 1.529X_6$	0.527	0.463

X1= Maximum temperature, X2= Minimum temperature, X3= Maximum relative humidity, X4= Minimum relative humidity, X5= Rainfall, X6= Sunshine Hour.

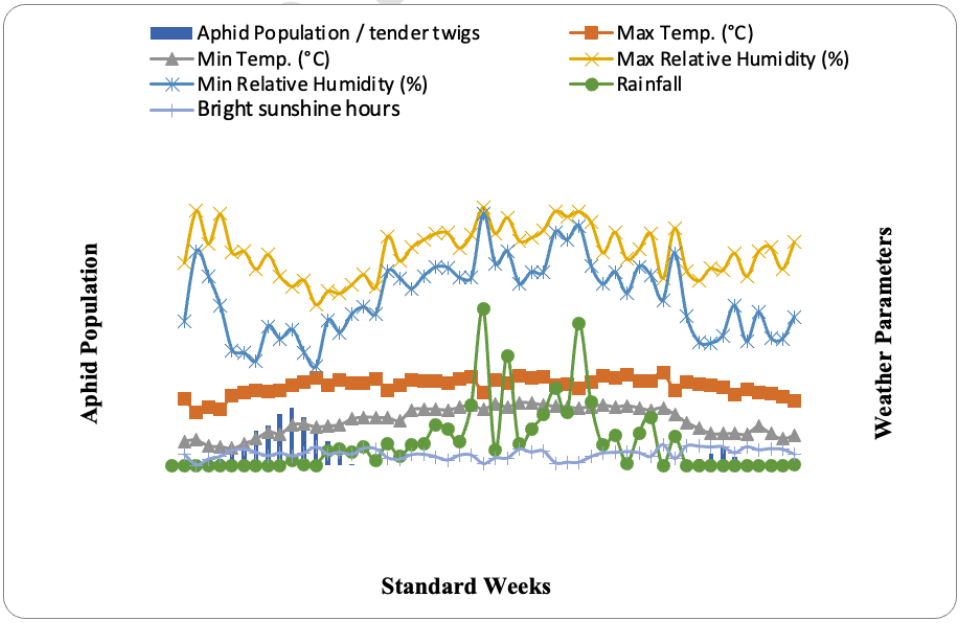

Fig 1: Incidence pattern of guava aphid in relation to meteorological parameters during 2021.

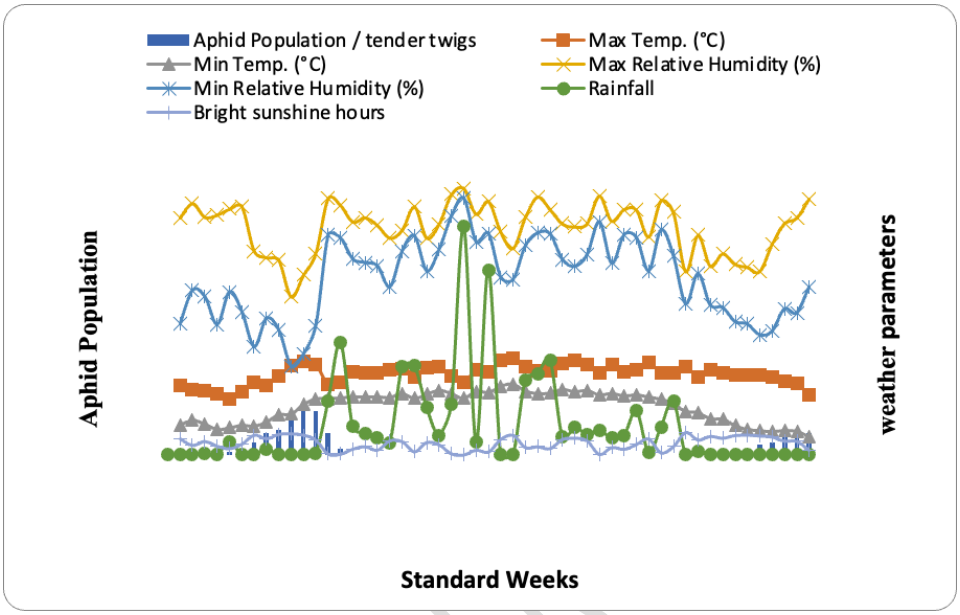

Fig 2: Incidence pattern of guava aphid in relation to meteorological parameters during 2022