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IMPACT OF BIOCHEMICAL CONSTITUENTS OF HOST PLANTS ON THE RUGOSE SPIRALING WHITEFLY, *ALEURODICUS RUGIOPERCULATUS* MARTIN INFESTATION LEVELS IN ANDHRA PRADESH

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ABSTRACT: The rugose spiraling whitefly (RSW), *Aleurodicus rugioperculatus* Martin is an invasive pest of polyphagous in nature and attacks a wide range of host plants. RSW has become a consequential pest and identified in the several regions of India, including Godavari districts of Andhra Pradesh. Since, it is a new species, lack of information necessitated immediate attention of scientific community to focus on detailed studies of the pest. In this scenario, a study has been conducted to know the impact of biochemical constituents of host plants in relation to RSW infestation. Results revealed that, a lower level of total phenol content resulted highest RSW infestation in oil palm in which other constituents *viz.,* moisture, chlorophyll, proteins and total sugars were in higher levels among the host plants studied, coconut, guava, banana and cocoa. Lowest RSW infestation was observed in cocoa leaves in which higher level of total phenols and lower levels of moisture, chlorophyll, proteins and total sugars were recorded. The correlation coefficients of RSW infestation were strong and negative with total phenols whereas positive and significant with respect to moisture, chlorophyll, proteins, total sugars; non significant with fiber and lignin.

Key words: Invasive pest, Rugose spiraling whitefly, Biochemical and *Aleurodicus*

INTRODUCTION

Invasive species compete with native species and cause biotic upsets which is the vital reason for crop loss and can adversely affect food security. In past, this type of invasions of exotic pests such as Coffee berry borer (*Hypothenemus hampei*) on coffee (1990 in Wayanad, Kerala), coconut eriophid mite (*Aceria gurrreronis*) on coconut (1997 in Ernakulam, Kerala), Papaya mealy bug (*Paracoccus marginatus*) on Papaya (2007 in Coimbatore, Tamil Nadu) (SATHYANARAYANA and SATYAGOPAL, 2013) and South American tomato pinworm, *Tuta absoluta* on tomato (2014 in Pune, Maharastra) (SINGH and PANCHBHAIYA, 2018) in India affected economy of these crops and caused dreadful situations among the farmers. Recent menace, rugose spiraling whitefly (RSW), *A. rugioperculatus* Martin (Hemiptera: Aleyrodidae) has entered India (KRISHNA RAO and RAO, 2019) and reported on coconut palm (*Cocos nucifera* L.) during August-September months in Pollachi, Tamil Nadu (SUNDARARAJ *et al.,* 2016). However, this pest on coconut was first reported in Andhra Pradesh during the December, 2016 (RAO *et al.,* 2018). Later the pest assumed significance in Andhra Pradesh and within a short period of time has extended over a wide area in other states. RSW is mainly a phloem feeder and excretes large quantities of honeydew causing the growth of sooty mold which disrupts normal leaf physiology (FRANCIS *et al.,* 2016).

The documented information on the effect of host plant biochemical constituents on *A. rugioperculatus* is not available and this was the first study carried out in coastal districts of Andhra Pradesh where horticultural crops including coconut and oil palm cultivation is in large scale. The biochemical constituents present in different quantities to each other in host plants have been reported to exert profound influence on growth, development, survival and reproduction of insects in various ways (PAINTER, 1958). Various biochemical characters of the plants play an important role by providing resistance against number of insect pests (HALDER and SRINIVASAN, 2011). Biochemical composition of the host plants especially coconut, oil palm, guava, banana and cocoa has got a profound influence on the infestation of the pest. Therefore, present study was undertaken to know the role of the biochemical constituents of host plants on infestation of *A. rugioperculatus*.

MATERIALS AND METHODS

The present study was conducted at College of Horticulture, Venkataramannagudem, West Godavari district, Andhra Pradesh, 2018-19. The leaf samples were collected from five major host plants *viz.,* coconut, oil palm, guava, banana and cocoa, and analyzed for biochemical constituents *viz.,* moisture (%), chlorophyll (SPAD units), proteins (g/100 g), phenols (mg/g) and total sugars (%), fiber (%) and lignin (%) contents by following suitable scientific methods.

Moisture content in the fresh leaves of host plants was determined on per cent basis by using Shimadzu MOC63u infrared moisture analyzer. Chlorophyll content in the leaves was estimated by using the instrument SPAD chlorophyll meter.

Protein content of leaves was estimated by Lowry's method (1951). Fresh leaf sample of 0.5 g was extracted in sodium phosphate buffer (pH 6.8) and centrifuged at 5000 rpm for 10 min. An aliquot of 0.2 ml of sample extract was taken in a test tube and the volume was made up to 1.0 ml with distilled water. To the sample, 5 ml of reaction mixture solution C (mixed 50 ml of reagent 'A' with 1 ml of reagent 'B'; reagent 'A' containing 2% sodium carbonate in 0.1N sodium hydroxide, reagent 'B' containing 0.5 per cent copper sulphate in 1% sodium potassium tartrate) was added and incubated at room temperature for 10 min. Finally, 0.5 ml of Folin Ciocalteu's reagent was added, mixed well and incubated in dark chamber for further 30 min at room temperature. The absorbance was measured at 660 nm against blank on spectrophotometer (systronic 117). The amount of protein was calculated using a standard graph prepared from bovine serum albumin (200 $\mu g\ ml^{-1}$).

Total phenol content was estimated by using Folin-Ciocalteau reagent method (SADASIVAM and MANICKAM, 2009). Leaf sample of 0.5 to 1.0 g was weighed and it was grounded with a mortar and a pestle with 10 time volume of 80% ethanol, and later it was centrifuged and homogenated at 10,000 rpm for 20 min and the supernatant was saved. The residue was re-extracted for five times with 80% ethanol, in a centrifuge and the supernatants were pooled. The supernatant was evaporated to dryness. The residue was dissolved in a known volume of distilled water (5 ml). Different aliquots (0.2 to 2.0 ml) are pipetted out into test tubes. Volume was made up in each tube to 3ml with water and 0.5 ml of FCR was added.

After 3 minutes, 2 ml of 20% sodium carbonate (Na_2CO_3) solution was added to each test tube, mixed thoroughly and the test tubes were placed in the boiling water exactly for one min. After cooling, it was measured at an absorbance of 650 nm against a reagent blank. A standard curve was prepared by using different concentrations of catechol. The standard curve was found out with a concentration of phenols in the test sample expressed as mg of phenols/100 g material.

The total sugar was determined by using the method given by LANE and EYNON (1923). Ten grams of leaf sample was taken, ground well and transferred to 250 ml volumetric flask. The sample solution was filtered through Whatman filter paper and the residue was discarded. A quantity of 100 ml of filtrate of the above leaf sample was taken into a separate 250 ml conical flask to which 5ml of 50% HCl was added and mixed well. The contents so obtained were kept overnight at room temperature. The acid content in the filtrate was then neutralized with drops of NaOH using a few drops of phenolphthalein as an indicator till the pink colour persisted for at least 15 seconds. Total sugars were then estimated by taking this solution in a burette and titrating against standard Fehling's mixture of A and B (1:1) while it is boiling; till brick red colour end point is obtained.

$$\text{Total sugars (\%)} = \frac{\text{Factor} \times \text{Volume made up}}{\text{Titre value} \times \text{Weight of the sample}} \times 100$$

The crude fiber content of leaves was estimated using the method outlined by the SADASIVAM and MANICKAM (2009). Two grams of dried leaf sample was boiled with 20 ml of sulphuric acid (H_2SO_4) for 30 minutes with bumping chips. The solution was filtered through muslin cloth and residue was washed thoroughly with boiling water until washing was acid free. The residue obtained was boiled with 800 ml sodium hydroxide (NaOH) for 30 minutes. The solution thus obtained was filtered through muslin cloth and the residue was washed with 25 ml of boiling H_2SO_4, water and 25 ml of alcohol. The residue obtained after clean washing was transferred to a pre weighed washing dish (W1). Then the residue was dried in a dessicator for two hours and weighed (W2). Then it was ignited in muffle furnace for 30 minutes at 600 ^{0}C. The tray was cooled and weighed (W3).

Loss of weight on ignition = (W2-W1)-(W3-W1)

$$\text{\% Crude fibre content} = \frac{\text{Loss in weight on ignition}}{\text{Weight of the sample}}$$

Lignin content was estimated through Kappa method given by HUSSAIN *et al.*, 2002. Data based on average of five observations. Leaf sample (0.5 gm) was added in 30 ml distilled water and ground to a fine paste by using a mortar and pestle. The disintegrated sample was transferred to 1000 ml conical flask and distilled water was added to make the total volume to 600 ml. 75 ml of potassium permanganate solution and 75 ml of sulphuric acid solution were mixed together and added immediately to the disintegrated Fiber sample. Thus, the total volume was made to 750 ml. The reaction was allowed to proceed at 250 ^{0}C temperature exactly for 10 minutes. Then 15 ml of potassium iodide solution was added and the free iodine was titrated with standard sodium thiosulphate solution using starch as an indicator. A blank titration was made using the same volume of water and reagents.

The Kappa Number was then calculated from the following equation:

$$\text{Kappa No.} = \frac{P \times f}{W}$$

Where,

P= 0.1N potassium permanganate (ml)

W= Weight of moisture free sample (g)

f= Factor for correction to 50 per cent permanganate consumption

Lignin content = Kappa No. × slope obtained from a graph between Kappa Number and Klason lignin

The results of Kappa Number determination by standard method are corrected to 50 per cent consumption of the permanganate added.

To know the rugose spiraling whitefly infestation per cent, five per cent sample palms or plants/ garden were selected randomly. Three sample leaflets per palm one each from the top, middle and lower whorls of the palm in case of coconut and oil palm whereas in case of guava, banana and cocoa; leaves were randomly selected from three levels of plant canopy from branches oriented in four directions *i.e.,* North, South, East and West. Total number of leaves and infested leaves were recorded and the per cent infestation was worked out.

$$\text{RSW infestation} = \frac{\text{No. of leaves infested with RSW per palm/plant}}{\text{Total no. of leaves per palm/plant}} \times 100$$

RESULTS AND DISCUSSION

The insects are attracted and deterred in one way or the other by the biochemical composition of the plants, which has been documented by findings of many earlier researchers (SINGH, 1983; WAR *et al.,* 2012). Many biochemical factors are known to be associated with insect resistance and it is obvious that the biochemical factors are more important than morphological and physiological factors in conferring non-preference and antibiosis (DAR *et al.,* 2017). Therefore, the estimation of biochemical constituents of host plant leaves is one of the most practical significance and efforts were also made to resolve the influence of biochemical characters on the infestation of RSW. Biochemical based defence is considered to be more effective as it directly affects insect growth and development.

The biochemical constituents from leaves of five crops *viz.,* coconut, oil palm, guava, banana and cocoa, were quantified and the results obtained were correlated with RSW infestation. Observations divulged that, infestation of *A. rugioperculatus* was highest in oil palm crop (83.92%) followed by banana (79.66%), coconut (76.84%), guava crop (72.90%) and low level of infestation were recorded in cocoa crop (24.74 %). The data on moisture content of host plants leaves revealed highly significant and positive correlation (r= 0.973) with the infestation of *A. rugioperculatus*. Among the crops studied the oil palm crop with highest moisture content (68.91 %) showed higher infestation (83.92%) whereas decreased infestation levels in other crops *i.e.,* 79.66%, 76.84%, 72.90% and 24.74% in banana, coconut, guava crop and cocoa, respectively were observed with decrease in moisture content of 62.73, 57.29, 59.35 and 36.08%, respectively (Table-1). Highest moisture content with high pest infestation indicated that moisture content of the leaves played a vital role for host plant preference which provided congenial conditions for pest survival, egg laying, feeding of early stages of pest with short life cycles and build up of population.

The chlorophyll content among host plants leaves showed significant variation which ranged from 28.62 to 64.18 SPAD values. Chlorophyll content in the leaves was found positively and significantly correlated (r= 0.912) with the infestation of RSW. Chlorophyll content observed in banana, coconut, guava crop and cocoa were 55.48, 51.12, 43.52, and 28.62%, respectively and RSW infestation was 79.66, 76.84, 72.90 and 24.74%, respectively (Table-1) indicating that, higher chlorophyll content favored the pest infestation and this might be a factor for feeding the pest population; higher the food resulting higher the pest sustainability and population buildup. The chlorophyll content in plant leaves is a powerful indicator of foliar nitrogen content which varies among plant tissues, being higher in structures such as flowers, pollen and leaves. Nitrogen content results plants with higher nutritional quality for herbivores, plant structures with high nitrogen levels may attract a greater diversity of insects. Hence the host having highest chlorophyll content *i.e.,* oil palm (64.18 SPAD value) was affected with highest infestation (83.92 %) of RSW.

Total sugar content in the leaves of oil palm, banana, coconut, guava and cocoa was recorded as 5.60, 3.60, 3.40, 3.20 & 0.90%, with infestation of 83.92, 79.66, 76.84, 72.90 and 24.74%, respectively (Table-1). Highest total sugars were found in the leaves of oil palm (5.60 %) showing highest RSW infestation (83.92%). The total sugars was found positively and significantly correlated (r= 0.890) with infestation of RSW in all the crops studied. Total sugars are major source for insect feeding as well as metabolic activities of the life stages; the present result strongly endorses the above fact.

The total protein content in the leaves of host plants ranged from 2.20 to 12.90 g/100g and the protein content was found significantly positively correlated (r= 0.885) with *A. rugioperculatus* infestation. Protein content in oil palm, coconut, guava, banana and cocoa leaves recorded as 12.90, 11.80, 9.80, 7.40 & 2.20%, respectively with RSW infestation of 83.92, 76.84, 72.90, 79.66 & 24.74%, respectively (Table-1). Total protein content of host plant plays a crucial role in insect development which is a key component in the enzymes required for developmental stages and metamorphosis. If the enzymatic activities and development is perfect, it hastens the population build up of the pest, ultimately leading to higher infestations and yield losses. Hence the host having highest protein content *i.e.,* oil palm (12.90 g/100g) was affected with highest infestation (83.92 %) of RSW.

Phenol content was found high in cocoa leaves (12.40 mg/g) whereas in guava, coconut, oil palm and banana leaves the phenol content was recorded as 6.81, 6.02, 2.68 and 0.31%, with RSW infestation of 72.90, 76.84, 83.92 & 79.66%, respectively (Table-1). The phenol content in the leaves was highly significantly negatively associated (r= -0.879) with pest infestation. Among plant constituents, it is the plant phenolics which plays a role in protecting plants from both insect and mammalian herbivory. Plants respond to insect feeding by increasing the synthesis of the phenolic toxins. Finally, the concentration of the toxic phenolic compounds in the plant is a key factor in deterrence and it is the accumulation of phenols in particular parts of the plant which represents a feeding barrier leading to the non preference of the host. Hence, the high phenol content in leaves of crop leads to less attack by the pest.

The fiber and lignin contents in host plant leaves ranged from 24.65 to 43.25 % and 6.37 to 37.40 %, respectively (Table-1). The fiber content in coconut, oil palm, banana and guava leaves was recorded as 43.25, 36.26, 29.02 & 26.45%, respectively and lowest *i.e.,* 24.65% was observed in cocoa leaves which is least affected by pest. The lignin content was observed as 37.40, 29.61, 24.92 &13.98% in coconut, oil palm, guava and banana leaves, respectively and in case of cocoa leaves it was least *i.e.,* 6.37% with RSW infestation of 76.84, 83.92, 72.90, 79.66 & 24.74%, respectively. With the pest infestation, the fiber (r= 0.564) and lignin (r= 0.712) were found non-significant and positively correlated.

Table-1: Status of biochemical components in host plant leaves and infestation levels of *A. rugioperculatus*

Host	Moisture content (%)	Chlorophyll content (SPAD Units)	Total protein content (g/100g)	Total phenol (mg/g)	Fibre content (%)	Lignin content (%)	Total sugars (%)	RSW infestation (%)
Coconut	57.29	51.12	11.80	6.02	43.25	37.40	3.40	76.84
Oil palm	68.91	64.18	12.90	2.68	36.26	29.61	5.60	83.92
Guava	59.35	43.52	9.80	6.81	26.45	24.92	3.20	72.90
Banana	62.73	55.48	7.40	0.31	29.02	13.98	3.60	79.66
Cocoa	36.07	28.62	2.20	12.40	24.65	6.37	0.90	24.74
C. D. (p=0.05)	1.893	5.803	1.206	0.674	1.937	1.823	0.34	3.518
SE(d)	0.885	2.714	0.564	0.315	0.906	0.853	0.159	1.645
Correlation coefficient	0.973**	0.912**	0.885**	-0.879*	0.564^{NS}	0.712^{NS}	0.890*	-

* - Significant at $p < 0.05$; ** - Highly significant $p < 0.01$; NS – Non-significant

From the study, it was observed that, the oil palm crop with highest moisture, chlorophyll and total sugars showed higher infestation whereas decreased infestation levels in banana, coconut, guava and cocoa were observed with decrease in these contents which is *vice versa* with total phenols. In comparison with the coconut, the decrease in lignin content in oil palm and banana might have resulted in increased RSW infestation. Whereas in guava, moderate levels of protein, fiber, lignin and RSW infestation was observed. But in case of cocoa, the lesser content of moisture, chlorophyll, total sugars, protein, fiber and lignin; and high total phenols resulted in lesser pest infestation. The biochemical characteristic of a plant affects the metabolism of insects inhibiting their population growth and hence ultimately reduces the damage caused by these insects. Population of pest varies from plant to plant and may be due to external or internal physiology of the plant. This is because plants have the ability to alter the behaviour of feeding insect (KARBAN and BALDWIN, 1997) through accumulation and excretion of toxic exudates. This elaborative array of phytochemicals may act as repellents, phagodeterrents and oviposition deterrents thus imparting the defensive mechanisms (HARIJAN *et al.,* 2017).

Biochemical factors of the host plant have been reported to play a vital role in resistance to various insect pests (PANDA and KHUSH, 1995) and relatively resistant genotypes contained higher amount of phenols inherently (DHALIWAL and DILAWARI, 1993) as these are often associated with the feeding deterrence, growth inhibition and in higher concentration could ward off insect pests because of the direct toxicity. There is a positive correlation between the nitrogen content in a plant and the leaf chlorophyll content (SCHADCHINA and DMITRIEVA, 1995), which probably explains why even non-flowering plants with higher LCI had a greater species richness and abundance of bark and wood-boring insects. Insects prefer plant tissues rich in nitrogen, since it is a limiting factor for development, and production of eggs by females (EUBANKS and STYRSKY, 2005; COELHO *et al*., 2009; MADRITCH and LINDROTH, 2015). These nitrogen-rich tissues can be used by females as preferred oviposition sites, since egg laying occurs on sites (leaves or stems) used by the larval phase (LARA, 1991).

SUNITHA *et al*. (2008) in cowpea and SINGH and SINGH (2014) in pigeon pea also reported a significant positive correlation of pod borer damage with moisture content. BOMMESHA *et al*. (2012) in pigeon pea reported a significant positive correlation between the incidence of leaf hopper and leaf roller with protein and chlorophyll content. In brinjal the low sugar content and higher total phenols in leaves offered a significant level of resistance to various biotic stresses (RAO and PANWAR, 2001; SOUNDARAJAN and BASKARAN, 2001; KHORSHEDUZZAMAN *et al*., 2010; KUMARI *et al*., 2014; PRASAD *et al*., 2014). DAR *et al*. (2014) also observed that crude fibre, ash, lignin had negative correlation and moisture content had positive correlation with *L. orbonalis* infestation in brinjal. Fiber and lignin contents were found non significantly correlated with pest infestation and the results were found contradictory to BARAD *et al*. (2016) in cowpea.

Thus moisture, chlorophyll, proteins, phenols and total sugars were found positively significantly correlated with RSW infestation except phenols whereas the constituents fiber and lignin contents were non-significantly correlated with the RSW infestation in host plants *viz.,* coconut, oil palm, guava, banana and cocoa. High phenol content in cocoa might have imparted resistance against pest whereas higher levels of moisture, chlorophyll, sugar and protein in oil palm helped to feed the insect and hence is susceptible. Thus present studies proved that biochemical factors played a major role in the resistance mechanism against the test insect. This study result may help in developing varieties which could provide resistance to sucking insect pests, if their biochemical constituents/ contents are improved through various breeding process.

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