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AT Chaudhary
Ph.D. Scholar, Department of Entomology, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, Gujarat, India

HV Pandya
Associate Professor, Department of Entomology, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, Gujarat, India

Correspondence
AT Chaudhary
Ph.D. Scholar, Department of Entomology, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, Gujarat, India

Biochemical basis of resistance against thrips (*Scirtothrips dorsalis* Hood) infesting chilli (*Capsicum annum* L.)

AT Chaudhary and HV Pandya

Abstract
Investigations were carried out on "Biochemical basis of resistance against thrips (*Scirtothrips dorsalis* Hood) infesting chilli [*Capsicum annum* L.]" at Instructional Farm, ASPEE College of Horticulture and Forestry, Regional Horticultural Research Station, Navsari Agricultural University, Navsari during 2018-19.

The results revealed that, maximum moisture content (90.86 %) was recorded in chilli variety GVC-101 which harboured moderate thrips population (4.25/3 leaves). The lowest ash content (33.45%) was recorded in GAVC-112 which in turn had moderate thrips population (4.70/3 leaves). Significantly minimum total soluble sugar content (3.60 %), minimum reducing sugar content (1.02 %), maximum non-reducing sugar (2.58 %), maximum total phenol (0.62 ppm), minimum nitrogen (0.15 %), minimum protein (0.90 %), maximum chlorophyll A (5.21 mg/g), maximum chlorophyll B (10.53 mg/g) and maximum chlorophyll total (15.74 mg/g) were recorded in GVC-111 which in turn had moderate thrips population (3.34/3 leaves).

Keywords: Thrips, *Scirtothrips dorsalis* Hood, chilli, *Capsicum annum* L.

Introduction
Chilli (*Capsicum annum* L.) is a member of solanaceae family which represents a diverse plant group. The name is derived from Latin word "Capsa" that means "hallow pod". There are various biotic and abiotic factors responsible for reducing in yield of chilli. The insect pests being the major which in over 25 insects have been recorded attacking leaves and fruits of chilli in India, of which thrips (*Scirtothrips dorsalis* Hood), aphid (*Aphis gossypii* Glover) and mite (*Polyphagotarsonemus latus* Banks) are the considerable and important pests [1]. In Gujarat, thrips, aphid, cutworm, whitefly and mites have been reported to infest the chilli crop. Thrips is one of the most serious pests causing about 60.5 to 74.3 per cent yield loss of green chilli and considered as an important enemy of chillies. Thrips are also responsible for transmission of leaf curl disease locally known as "*kokadva*".

Although, insecticidal applications bring down the pest damage, it leads to problem of pesticide residues in fruits [4]. Pesticide residues in spices, especially in chillies are major barrier against export to developed countries. Similarly, indiscriminate use of insecticides has led to insecticide resistance, pest resurgence, environmental pollution and upsetting the natural ecosystem [5]. Abundant literature is available on life history, feeding habit and control measures of thrips however, work on biochemical basis of resistance against thrips infesting chilli is not much available hence, attempts were made to have comprehensive information on biochemical basis of resistance against thrips.

Materials and Methods
Present investigation on "Biochemical basis of resistance against thrips (*Scirtothrips dorsalis* Hood) infesting chilli [*Capsicum annum* L.]" was carried out at Instructional Farm, Regional Horticultural Research Station, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari during late *kharif* 2018-19. Experimental materials for the present investigation consisting of varieties of chilli were obtained from the Main Vegetables Research Station, Anand Agricultural University, Anand and Spices Research Station Jagudan, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar. For the purpose, eight varieties were grown in randomized block design replicated thrice. The varieties under

test were kept unsprayed throughout the crop period and all other recommended agronomical practices were followed for raising the chilli crop.

When thrips crossed ETL (3 nymphs or adults/leaf), leaves of chilli varieties were brought to Soil Chemistry Laboratory and Post-Harvest Technology Laboratory, ASPEE College of Horticulture and Forestry, NAU, Navsari. Chilli leaves samples were kept in marked brown paper bags having wax coated inner side and brought to the laboratory for studying biochemical variations of chilli with respect to the damage by thrips.

Methods of recording observations

The observations on biochemical parameters were recorded from the selected chilli varieties for studying resistance to thrips during 2018-19. The details of biochemical parameters are given here under.

Moisture

Procedure

Two grams of fresh sample of leaves was taken in crucible with its weight and put in oven for drying at 60-80^{0}C for overnight. After oven drying, it was removed and again weighed and moisture content was calculated by the following formula:-

$$\text{Moisture \%} = \frac{\text{(Wt. of sample - crucible) - (oven dried wt. of sample - crucible)}}{2} \times 100$$

Ash

Ash content was worked out by using the procedure suggested by Bhatnagar (2005) [2].

Procedure

The sample was weighted accurately equivalent to 2g of sample material (moisture free basis) in tarred porcelain of 75ml capacity. It was heated at about 300ºC for 2 hours. Again, it was ignited at 500ºC for 2 hours in an electric muffle furnace provided with temperature control. Now, it was cooled to room temperature in a desiccator over unhydrous calcium chloride and weight of ash. The percentage of total ash was calculated by using as following formula.

$$\text{Ash content (\%)} = \frac{\text{Weight of ash}}{\text{Weight of moisture free sample}} \times 100$$

Total Soluble Sugar

Through Anthrone method this was prepared as described by Trevelyan & Harrison (1952) [10]

Procedure

The sample of 100 mg was taken in a boiling tube. It was hydrolyzed by keeping it in a boiling water bath for three hours with 5ml of 2.5 N HCL and was cooled at room temperature. Neutralization was done with solid sodium carbohydrate until the effervescence ceases. After making up volume to 100ml centrifugation was done. Supernatant was collected and 1ml aliquots were taken for analysis. Standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8, and 1ml of the working standard. '0' was served as blank. 1ml volume was made in all tubes including the sample tubes by adding distilled water. Thereafter, 4ml anthron reagent was added. It was heated for 8 minute in a boiling water bath. After rapid cooling, its observation was taken by reading green to dark green colour at 630 nm. Standard graph was plotted by taking concentration of the standard on the X-axis versus absorbance on the Y-axis. Amount of carbohydrates present in the sample tube was calculated from the graph.

$$\text{Amount of carbohydrate present in sample (\%)} = \frac{\text{Sugar value from graph } (\mu g)}{\text{Aliquot sample used (1ml)}} \times \frac{\text{Total volume of extract (ml)}}{\text{Weight of sample (mg)}} \times 100$$

Reducing Sugars

Procedure

The sample of 100mg was weighed and the sugars were extracted with 80% alcohol twice (5ml each time). Supernatant were collected and evaporated on water bath. Thereafter, 10ml water was added. Aliquots of 0.2 ml of alcohol free extract were taken in separate test tubes. Pipette aliquots of 0.2, 0.4, 0.6, 0.8 and 1ml was taken as working standard solution and were taken into a series of test tubes. The volume was made up in both the samples and standard tubes up to 2ml with distilled water. Thereafter, 2ml distilled water was taken in a separate tube to serve as a blank. 1ml of alkaline copper reagent was added to each tube. The tubes were placed in boiling water for 10 minutes and cooled down. One ml of arsenomolybdic acid reagent was added to all the tubes. The volume was made up in each tube to 10ml with water. The absorbance was measured at 620 nm after 10 min

Non-Reducing Sugar

Procedure:

Non-reducing sugar = Total Sugar – Reducing sugar (Franscistt *et al*., 1971) [3]

Total phenol

Procedure

One gram fresh sample were crushed into 0.3 N HCl prepared in ethanol. Finally, 25 ml volumes were made and kept for shaking for one hour. The extraction was filtered with the help of Whatman filter paper No. 40. Filtrate obtained was evaporated to dryness on water bath and final volume adjusted to 250 ml with hot distilled water in volumetric flask. For total phenol content, 1 ml of this aliquot was taken in the test tube. To this, 1 ml each of Folinciocalteau reagent diluted 1:2 and 1 ml of 35 per cent sodium carbonate were added. After 1 hr 2 ml water were added to adjust final volume. The intensity of colour developed was recorded at 650 nm against a reagent blank. Preparation of a standard curve was done using different concentrations of catechol.

Calculation

From the standard curve, concentration of phenols in the test sample was found and expressed as mg phenols/100g material.

Nitrogen

Procedure

Chilli leaves (20 g) crushed was transferred in 800ml distillation flask from Kjeldahl flask. The sample was moistened with distilled water. $KMnO_4$ solution 0.32 per cent (100 ml) was added in the flask, in which few glass beads and

2ml paraffin liquid was added. Boric acid 4 per cent containing mixed indicator was measured up to 25ml in a 250ml beaker and placed it under the receiver tube. Receiver tube end was dipped in the boric acid. After that 100ml of 2.5 per cent NaOH solution was added and immediately fitted it up in the distillation apparatus. Heater was turned on and distillation continued until about 150ml of distillate was collected. The heater was switched off to avoid back suction after removal of the beaker containing distillate. Then blank observation was taken. Thereafter, titration of distillate was done with standard 0.05N H_2SO_4 up to pink colour (end point).

$$N(\%) = \frac{(\text{HCL in sample in ml}) - (\text{HCL in blank in ml}) \times \text{normality of acid} \times 14.01 \times 1}{\text{Weight of sample (mg)}}$$

Protein

Procedure

Estimation of protein content in chilli leaves was worked out by Macro Kjeldahl's method as described by Association of Official Agricultural Chemists (AOAC) (Bhatnagar, 2005) [2]. Protein content (%) was worked out by the following formula. Per cent protein = Per cent Kjeldahl's N x Conversion factor (6.25)

Chlorophyll A, Chlorophyll B and Total Chlorophyll

Procedure

One gram of finely cut and well mixed representative sample of leaf or fruit tissue was taken in a clean mortar. Grinding of the tissue to a fine pulp was done with addition of 20ml of 80% acetone was done. After the centrifugation of this mixture at 5,000 rpm for 5 minutes the supernatant was transferred to a 100ml volumetric flask. Grinding of the residue with 20ml of 80% acetone. Thereafter it was centrifuged and the supernatant was transfer to the same volumetric flask. Repetition of this procedure was done until the residue became colour less. Washing of the mortar and pestle thoroughly with 80% acetone and collection of the clear washings in the volumetric flask was done. The volume was made up to 100 ml with 80% acetone. Observation was taken by reading the absorbance of the solution at 645 nm (chlorophyll a), 663 nm (chlorophyll b) and 652 nm (total chlorophyll) against the solvent (80% acetone) blank.

Results and Discussion

The results (Table 1) revealed that maximum moisture (90.86 %) were recorded in GVC-101 which had thrips population (4.25/3 leaves) whereas minimum moisture (79.22 %) were recorded in AVNPC-131 indicated thrips population (5.16/3 leaves). The lowest ash (33.45 %) in GAVC-112 which moderate thrips population (4.70/3 leaves) whereas GCH-3 had highest ash (50.09 %) and had maximum thrips (6.07/3 leaves). Significantly minimum total soluble sugar (3.60 %) were recorded in GVC-111 had moderate thrips population (3.34/3 leaves). The total soluble sugar were significantly maximum in GCH-3 (4.50 %) had highest thrips population (6.07 thrips/3 leaves). Significantly minimum reducing sugar content (1.02 %) were recorded in GVC-111 had moderate thrips (3.34/3 leaves). The reducing sugar content were significantly maximum in GCH-3 (2.04 %) had highest thrips population (6.07/3 leaves). GVC-111 had maximum non-reducing sugar content (2.58 %) and moderate thrips population (3.34/3 leaves) whereas minimum non-reducing sugar (2.46 %) were recorded in GCH-3 which had maximum thrips population (6.07/3 leaves). Maximum total phenol (0.62 ppm) were recorded in GVC-111 where moderate thrips population (3.34/3 leaves) were observed minimum total phenol (0.42 ppm) were recorded in GCH-3 had maximum thrips population (6.07/3 leaves). Minimum nitrogen (0.15%) in GVC-111 which had moderate thrips population (3.34/3 leaves) whereas, maximum nitrogen (0.22 %) were recorded in GCH-3 which had maximum thrips population (6.07/3 leaves). Minimum protein (0.90 %) was recorded in GVC-111 which had moderate thrips population (3.34/3 leaves) whereas maximum protein (1.36 %) were recorded in GCH-3 which had maximum thrips population (6.07/3 leaves). GVC-111 recorded maximum chlorophyll A (5.21 mg/g) had moderate thrips population (3.34/3 leaves) whereas minimum chlorophyll A (3.42 mg/g) were recorded in GCH-3 had maximum thrips population (6.07/3 leaves). GVC-111 recorded maximum chlorophyll B (10.53 mg/g) had moderate thrips population (3.34/3 leaves) whereas minimum chlorophyll B (5.79 mg/g) were recorded in GCH-3 had maximum thrips population (6.07/3 leaves). GVC-111 recorded maximum total chlorophyll (15.74 mg/g) had moderate thrips population (3.34/3 leaves) whereas minimum total chlorophyll (9.21 mg/g) were recorded in GCH-3 had maximum thrips population (6.07/3 leaves).

All the biochemical parameters showed non-significant correlation with thrips population (Table 2).

It can be inferred from the present investigation that chilli varieties with higher moisture (%), less ash (%), less total soluble sugar (%), less reducing sugar (%), less non-reducing sugar (%), more total phenol (ppm), less nitrogen (%), less protein (%) provided resistance against thrips infestation. On the other hand, chilli varieties which contained lower moisture (%), more ash (%), more total soluble sugar (%), more reducing sugar (%), less non-reducing sugar (%), less total phenol (ppm), more nitrogen content (%), more protein (ppm), less chlorophyll A (mg/g), less chlorophyll B (mg/g) and less total chlorophyll (mg/g) had higher susceptibility to thrips infestation.

In past, Muhammad *et al*. [6] revealed that the moisture content, nitrogen, protein and reducing sugars were positively and significantly correlated with thrips population. Megharaj *et al.* [7]. Reported the biochemical components like non-reducing sugars (r=-0.283) and total chlorophyll (r=-0.310) showed negative and significant association with thrips incidence. Reducing sugars (r=0.332) exhibited positive and significant correlation with thrips infestation. Rameash *et al.* [8]. Reported that chlorophyll and total phenol content showed negative significant association with thrips incidence in chilli. Samota *et al*. [9]. showed that the total sugar in fruits of different chilli varieties varied from 3.70 (Pant C-1) to 4.52 per cent (Pusa Jwala) and the total phenol varied from 0.44 (Pusa Jwala) to 0.67 per cent (Pant C-1). The total sugar content had a significant positive correlation (r=0.99) with the infestation of thrips on different chilli varieties however, total phenol content had significant negative correlation (r=-0.99) with thrips. Latha and Hunumanthraya [11]. Studied the biochemical characters *viz*., chlorophyll and phenol content which were significantly and negatively correlated with the population of thrips. In the present investigation, similar results based on relationship of thrips population and biochemical constituents of chilli varieties were observed which are in close confirmation with the past reports.

Table 1: Biochemical characters of chilli varieties in relation to thrips population

Varieties	Population of thrips	Biochemical characters										
		Moisture (%)	Ash (%)	Total soluble sugar (%)	Reducing sugar (%)	Non reducing sugar (%)	Total phenol (ppm)	Nitrogen (%)	Protein (%)	Chlorophyll (mg/g)		
										A	B	Total
GVC-101	4.25	90.86	36.19	3.86	1.31	2.55	0.60	0.16	1.03	4.70	9.18	13.88
GVC-111	3.34	90.43	34.54	3.60	1.02	2.58	0.67	0.15	0.90	5.21	10.53	15.74
GVC-121	2.89	90.63	39.61	4.37	1.89	2.48	0.45	0.20	1.29	3.68	6.47	10.14
GAVC-112	4.70	80.33	33.45	3.73	1.17	2.56	0.64	0.16	0.97	4.95	9.85	14.81
AVNPC-131	5.16	79.22	35.32	4.24	1.75	2.49	0.49	0.20	1.23	3.93	7.15	11.08
GAVC hybrid-1	3.80	79.39	36.57	3.98	1.46	2.52	0.56	0.18	1.10	4.44	8.50	12.94
GCH-1	5.61	80.29	34.70	4.12	1.60	2.52	0.53	0.18	1.16	4.19	7.82	12.01
GCH-3	6.07	80.87	50.09	4.50	2.04	2.46	0.42	0.22	1.36	3.42	5.79	9.21
S. Em ±	0.13	2.54	1.35	0.11	0.03	0.07	0.01	0.01	0.02	0.06	0.51	0.57
C D at 5%	0.39	7.69	4.10	0.32	0.09	NS	0.03	0.01	0.06	0.18	1.55	1.72
C V (%)	10.10	5.23	6.23	4.51	3.52	5.12	2.89	3.31	2.90	2.34	10.82	7.89

Table 2: Biochemical characters of chilli varieties and their relationship with thrips population

Population of thrips	Correlation coefficient (r)										
	Moisture (%)	Ash (%)	Total soluble sugar (%)	Reducing sugar (%)	Non reducing sugar (%)	Total phenol (ppm)	Nitrogen (%)	Protein (%)	Chlorophyll (mg/g)		
									A	B	Total
	-0.686	0.366	0.384	0.380	-0.355	-0.372	0.388	0.388	-0.381	-0.381	-0.381

Conclusion

The biochemical characters viz., moisture, non-reducing sugar, total phenol and chlorophyll were higher in resistant variety. However, ash, total soluble sugars, reducing sugar, nitrogen and protein were higher in susceptible variety as compared to resistant variety.

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