Phytochemical analysis of chilli leaves in response to Chilli mite, *Polyphagotarsonemus latus* (Banks) infestation

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ABSTRACT

Chilli (*Capsicum annuum* L.) (Family: Solanaceae) is one of the important spice crops of India and is being widely cultivated throughout warm temperate, tropical and subtropical countries. The broad mite, *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae), is a serious pest of chilli crop. During study it was observed that, NPK content of chilli leaves showed a significant decline with increase in *P. latus* infestation. With increase in *P. latus* population from 8.91 to 22.08 mites/leaf, nitrogen, phosphorus and potassium content in chilli leaves dropped from 6.62 to 1.12% dry weight, 0.48 to 0.14% dry weight and 4.75 to 1.50% dry weight, respectively. Phenolic compounds exhibited a marked increase from 0.35 to 0.54% dry weight with increase in mite infestation. Total phenols were 0.35 % dry weight in least susceptible hybrid (9/CHIhyb-10) as compared to 0.54 % dry weight in highly susceptible hybrid (10/CHIhyb-7). In uninfested leaves, total sugar content was 1.34 mg/g fresh weight which decreased significantly to 0.50 mg/g fresh weight with increase in *P. latus* infestation (22.08 mites/leaf). The reducing sugar content was found to be significantly increased from 0.24 mg/g fresh weight in uninfested leaf to 0.48 mg/g fresh weight in highly susceptible hybrid (10/CHIhyb-7). Mites use carbohydrates as feeding stimulants and promote ovipositions. These nutrients needed to synthesize body tissue and serve as energy sources. A significant effect of mite incidence depicted a progressive decline in non-reducing sugars in accordance with increase in *P. latus* infestation as compared to uninfested plant.

Keywords: chilli hybrids, NPK, *Polyphagotarsonemus latus*, phenol, sugar content

Introduction

Chilli (*Capsicum annuum* L.) is an important commercial vegetable cum spice crop grown in southern India. India is the largest producer and consumer of chilli in the world. Chillies constitute about 20 per cent of Indian spice exports in quantity and about 14 per cent in value. It is known for its pungency due to presence of capsaicin (8-methyl-N-vanillyl-6-enamide), aroma, spicy taste, oleoresin, red pigment, minerals and rich sources of vitamin A, B, C, E. It's grown in almost all the state throughout the country. Andhra Pradesh is the largest producer of chilli in India and contributes about 30% to the total area; followed by Karnataka (20%), Maharashtra (15%), Orissa (9%), Tamil Nadu (8%) while other states contributing nearly 18% to the total area under chilli. India produces about 13.03 lakh tonnes of chilli grown in an area of 7.24 lakh hectares whereas Karnataka produces about 1.28 lakh tonnes in an area of 1.14 lakh hectares (Anon., 2011).

This crop is known to harbour more than 293 insects and mite pests of which, aphids, *Myzus persicae* Sulzer, thrips, *Scirtothrips dorsalis* Hood and the mites, *Polyphagotarsonemus latus* (Banks) are the major constraints for higher yields (Sujay and Giraddi, 2016). These sucking pests attack the crop at seedling stage itself and continue until last harvest. *P. latus*, a member of the family Tarsonemidae commonly known as yellow mite, broad mite *etc*. is an important mite pest of chilli with wide host range. It is known to cause leaf curling, infested leaves bend and turn to coppery and purple colour. The mite injects toxic saliva causing twisted, hardened and distorted growth in the terminal buds, leaf curling, elongation of leaf petiole, buds drop immaturely, plants become stunted in growth which causes significant reduction in yield in the range of 30-90% (Jangra et al., 2023) that lead to huge economic losses. However, in extreme cases, the complete failure of the crop occurs. Apart from quantitative losses, qualitative losses due to mite pests also occur. To date, the data on the relationship between mite feeding and changes in the mineral composition of leaf tissue are scarce and more studies are needed to shed light on this area.

Material and methods

The studies were carried out in Acarology laboratory, screen house of Department of Zoology, CCS Haryana Agricultural University, Hisar and Research Farm Area, Department of Vegetable Sciences, CCS Haryana Agricultural University, Hisar (Haryana). Field experiments were conducted following standard agronomical practices under randomized block design with three replications.

Seeds of chilli (*Capcicum annum* L.) hybrids were grown under natural conditions from June, 2013 to November, 2013. The field was divided into plots of 3m × 2.7 m with spacing 60 cm ×45 cm. Plots were made as per requirement of experiment design.

Phytochemical analysis

Chilli hybrids tested, least susceptible (9/CHIhyb-8, 9/CHIhyb-10), moderately susceptible (10/CHIhyb-10, 10/CHIhyb-12) and highly susceptible (10/CHIhyb-6, 10/CHIhyb-7) for phytochemical estimation at harvest. For above mentioned hybrids, various phytochemical parameters *viz*., total sugars, reducing and non-reducing sugars, phenolic compounds and mineral contents (Nitrogen, Phosphorous and Potassium) were estimated in all the three replications. Each replicate consisted of ten randomly selected plants following standard methodology.

For estimation of these parameters dried leaf samples were used. Samples were air dried at room temperature for 3 to 4 days in the laboratory before shifting to oven at 60°C to take the dry weights. The samples were weighed thrice to record the constant dry weight. After that, they were ground in a Micro-Willey grinding mill (0.2 mm sieve) for analysis of various other parameters. The detailed procedures are described as under:

Estimation of Minerals

Nitrogen, Phosphorous and Potassium content in chilli leaves were estimated to ascertain the effect of mite feeding on these parameters.

Digestion of the sample

To estimate minerals, digestion of sample was carried out using the method of Fiske and Subba Row (1925).

Nitrogen

Leaf-nitrogen content was estimated using the method of Lindner (1944).

Phosphorus

Phosphorus was estimated by the method of Jackson (1973).

Potassium

Potassium in the digested sample was estimated in the above acid digest with a Micro Flame Photometer (Elico CL 361, India) by direct reading (Richards, 1954).

Estimation of Total Phenols

The total phenolic content of sample was estimated by the method of Swains and Hillis (1959).

Estimation of Sugars

After the mites had fed on the chilli leaves the sugar (carbohydrate) content of the leaves was determined to see the effect of mite feeding at different durations.

Extraction of Sugars

Fine powdered samples (500 mg) were taken in 100 ml volumetric flask and 30 ml of 80 per cent methanol was added to each flask. The replicated samples prepared in volumetric flasks were kept in water bath (maintained at 60°C) and heated for one hour. The extracts were allowed to cool and then filtered by Whatman filter paper no.1. Clear supernatants were obtained, collected in 100 ml beakers and volumes made to 30 ml with 80 per cent methanol. Contents were mixed well and used for the determination of total, reducing and non-reducing sugars. Blank was prepared in the above manner but without sample.

Total Sugars

Total sugars were estimated according to the method of Dubois *et al.* (1956).
The percent reduction in total sugars in infested plants was worked out by following formula:

$$\text{Reduction in total sugars} = \frac{\text{Total sugars in uninfested plant - Total sugars in infested plant}}{\text{Total sugars in uninfested plant}} \times 100$$

Reducing Sugars

Reducing sugars were estimated by the method of Nelson (1944) and Somogyi (1945).

Non- Reducing Sugars

The amount of non-reducing sugars was calculated from the difference between total sugars and reducing sugars.

$$\text{Non reducing sugars} = \text{Total sugars} - \text{Reducing sugars}$$

Statistical Analysis

Field experiments were laid in randomized block design with three replicates. From each replicate observation was recorded from ten plants.

The Software 'OPSTAT', developed at the Computer Centre, College of Basic Sciences and Humanities, CCS Haryana Agricultural University, Hisar was used for the analysis.

The experiment on phytochemical analysis was also conducted and analyzed using Randomized Block Design (RBD). Critical difference (CD) was calculated to know the variation in sugar, nitrogen, phosphorous, potassium and phenol content in least, moderate and highly susceptible hybrids.

Results

As presented in Table 1 an average number of mites 8.19, 11.21, 16.86, 18.66, 21.18 and 22.08 mites/leaf were recorded on the ventral surface of leaf of chilli hybrids; 9/CHIhyb-10, 9/CHIhyb-8, 10/CHIhyb-10, 10/CHIhyb-12, 10/CHIhyb-6 and10/CHIhyb-7, respectively.

Effect of Mite Infestation on the Phytochemical Content

The results of the effect of *P. latus* infestation on the Phytochemical contents (Nitrogen, Phosphorus and Potassium) of chilli leaves selected from least susceptible, moderately susceptible and highly susceptible hybrids accessed at the time of harvest are presented and summarized in Table 2 & 3.

Effect of Mite Infestation on nitrogen content

As presented in Table 2, nitrogen content of chilli leaves showed a significant decline with increase in *P. latus* infestation. Hybrids (9/CHIhyb-10, 9/CHIhyb-8) with less mite count (8.19, 11.21 mites/leaf) recorded significantly higher nitrogen content (6.62, 5.50 % dry weight) as compared to other hybrids (CD= 0.47; p= 0.05). Both the hybrids were categorized as least susceptible hybrids. In moderately susceptible hybrids (10/CHIhyb-10, 10/CHIhyb-12) and highly susceptible hybrids (10/CHIhyb-7, 10/CHIhyb-6), nitrogen content decreased (3.93, 2.37, 1.31, 1.12 % dry weight) significantly with increase in *P. latus* population (16.86, 18.65, 21.18, 22.08 mites/leaf), however the latter two hybrids (highly susceptible) were statistically at par with each other.

Effect of Mite Infestation on phosphorous content

The data pertaining to variations in phosphorus content in varying degree of mite damaged leaves is presented in Table 2. With increase in *P. latus* population from 8.19 to 22.08 mites/leaf, phosphorus content in leaves dropped from 0.48 to 0.14 % dry weight. In least susceptible hybrid (9/CHIhyb-10), significant increase in phosphorus content was recorded as compared to moderately and highly susceptible hybrids (CD= 0.10; p= 0.05). Among the moderately susceptible hybrids (10/CHIhyb-10, 10/CHIhyb-12), decrease in phosphorous content was non-significant but values differed significantly with phosphorous content in highly susceptible hybrid, 10/CHI Hyb-7. Similar trend was witnessed in highly susceptible hybrids (10/CHIhyb-6 and 10/CHIhyb-7) as these hybrids were statistically comparable with each other.

Effect of Mite Infestation on potassium content

The effect of mite infestation on potassium content of chilli leaves is presented in Table 2. The significant decline in potassium content was recorded as mite infestation increased (CD= 0.87; p= 0.05). The mean potassium content was recorded as 4.75% dry weight when the average mite population was 8.19 mites/leaf. It was significantly higher than the potassium content observed in other hybrids. In hybrids, 9/CHIhyb-8, 10/CHIhyb-10, 10/CHIhyb-12 and 10/CHIhyb-6, potassium content dropped to 3.12, 2.62, 2.50 and 2.06% dry weight, respectively with mite count of 11.21, 16.86, 18.65 and 21.18 mites/leaf. The latter three hybrids were at par with each other with respect to variations in potassium content. In highly susceptible hybrid, 10/CHIhyb-7 lowest potassium content (1.50 % dry weight) was recorded which was comparable with potassium content in other highly susceptible hybrid, 10/CHIhyb-6. However, it was significantly less than the potassium content observed in other hybrids.

Effect of Mite Infestation on phenol content

Changes in the phenol content of chilli leaves showed reverse trend as was seen in other phytochemicals (nitrogen, phosphorous, potassium). Phenolic compounds exhibited a marked increase with increase in mite infestation (Table 2). Total phenols were 0.35% dry weight in least susceptible hybrid (9/CHIhyb-10) showing mite count of 8.19 mites per leaf. It significantly increased to 0.54 % dry weight in highly susceptible hybrid, 10/CHIhyb-7 showing mite count of 22.08 mites/leaf (CD= 0.01; p= 0.05). In other hybrids, total phenols were 0.39, 0.46, 0.48 and 0.53% dry weight in 9/CHIhyb-8, 9/CHIhyb-10, 10/CHIhyb-12 and 10/CHIhyb-6, respectively. The difference between total phenols was significant in each case.

Effect of mite infestation on sugar content

The results of the effect of *P. latus* infestation on the sugar (carbohydrate) contents of chilli leaves selected from least susceptible, moderately susceptible and highly susceptible hybrids accessed at the time of harvest are presented and summarized in Table 3.

In uninfested leaves, total sugar content was 1.34 mg/g fresh weight which decreased significantly with increase in *P. latus* infestation (CD = 0.005; p = 0.05). This decrease was 0.99 and 0.95 mg/ g fresh weight in least susceptible hybrids (9/CHIhyb-10 and 9/CHIhyb-8) which showed a mite count of 8.19 and 11.21 mites/leaf. In moderately susceptible hybrids

(10/CHIhyb-10 and 10/CHIhyb-12) as the population count was more (16.86 and 18.65 mites/leaf), significant decline was witnessed.

Total sugar content was lowest (0.50 and 0.54 mg/ g in highly susceptible hybrids (10/CHIhyb-7 and 10/CHIhyb-6) depicting effect of high mite count (21.18 and 22.08 mites/leaf) in these hybrids. In infested leaves, percent reduction in total sugar content was observed as 26.11, 29.10, 49.25, 52.23, 59.70 and 62.68 percent in 9/CHIhyb-10, 9/CHIhyb-8, 10/CHIhyb-10, 10/CHIhyb-12, 10/CHIhyb-6 and 10/CHIhyb-7 hybrids, respectively.

On the contrary, reducing sugars exhibited a marked increase with *P. latus* infestation (Table 3). The reducing sugar content was found to be significantly affected by mites population build up (CD=0.005; p=0.05). With increase in the number of *P. latus* (8.19, 11.21, 16.86, 18.65, 21.18 and 22.08 mites/leaf) in chilli hybrids; 9/CHIhyb–10, 9/CHIhyb–8, 10/CHIhyb–10, 10/CHIhyb–12, 10/CHIhyb-6 and 10/CHIhyb-7, reducing sugars also showed a significant corresponding increase (0.27, 0.29, 0.33, 0.43, 0.45 and 0.48mg/g fresh weight), respectively. In uninfested plants where mites number nil, reducing sugar was recorded to be 0.24 mg/g fresh weight.

The non reducing sugars exhibited a marked decrease when chilli hybrid leaves showing different levels of *P. latus* infestation were assessed. A significant effect of mite incidence (CD=0.01; p=0.05) (Table 3) depicted a progressive decline in non-reducing sugars in accordance with increase in *P. latus* infestation as compared to uninfested plant. The non reducing sugar content declined from 1.10 mg/ g in uninfested plants to 0.72, 0.66, 0.35, 0.21, 0.09 and 0.02 mg/ g after an infestation level of 0, 8.19, 11.21, 16.86, 18.65, 21.18 and 22.08 mites/leaf in 9/CHIhyb-10, 9/CHIhyb-8, 10/CHIhyb-10, 10/CHIhyb-12, 10/CHIhyb-6 and 10/CHIhyb-7 hybrids.

Discussion

Effect of phytochemicals on *Polyphagotarsonemus latus* population

Many factors like primary and secondary metabolites, plant morphology and phenology have been hypothesized to be important for host discrimination by phytophagous arthropods (Schoonhoven *et al.*, 1998). Often, it is the balance between positive and negative cues presented by phytochemicals that determines whether a plant is accepted or rejected by the herbivore (Krokos *et al*., 2002). It was reported that sap feeding pests are affected more by small changes in nitrogen status of host (Wensler, 1962). Changes induced in the concentration of plant metabolites affect the insects at the nutritional level. During present study, least susceptible chilli hybrids showed higher level of nitrogen (6.62% dry weight), phosphorus (0.48% dry weight) and potassium (4.75% dry weight) content as compared to highly susceptible hybrids (1.12, 0.14 and 1.5% dry weight, respectively).

Application of organic manures such as FYM, vermicompost, crop residues enhanced the soil available nitrogen, phosphorus and potassium as compared to recommended dose of fertilizers (Narkhede *et al*., 2011). The groundnut plants that received organic manures recorded low nitrogen, higher level of phenols and tannins whereas the straight fertilized groundnut leaves recorded the high nitrogen, lower level of phenols and tannins (Rajasekhara Rao, 2002).

During present study, chilli plants showed response towards mite herbivory. There was marked increase in phenol content of *P. latus* infested leaves to deter them. Total phenols were 0.35% dry weight in least susceptible hybrid (9/CHIhyb-10) as compared to 0.54 % dry weight in highly susceptible hybrid (10/CHIhyb-7). The study of Mckey *et al.* (1978) also provides those high concentrations of polyphenolic compounds act as deterrent to herbivores. Earlier reports also showed that phytophagous mites induce defense reactions in their host plants. These reactions can occur after a few days of infestation even in susceptible plants. Higher level of total phenols following infestation with mites has been reported by many workers (Farouk and Osman, 2012; Kanika, 2013). Theerthagiri *et al.* (2007) reported higher level of total phenols following infestation with pests. These results also corroborate those of Senthil *et al*. (2010), who reported increased total phenols in fungus infected plants and claimed that they may play an important role in plant defense by increasing the physical and mechanical strength of the host cell wall.

Girish et al., (2019) studied genotypes for total phenol showed mite infested plants had significantly higher quantity of phenol (16.80 to 22.80 mg/g) in all the genotypes which were resistant or highly resistant to mite attack and these genotypes also recorded lower number of mites. In susceptible genotypes (with 10 to 18.50 mg/g phenols) this trend was not so evident. Thus, in resistant genotypes increase in the levels of total phenol observed with mite infestation, might have been induced to enhance the synthesis of phenolic precursors and their further oxidation into toxic quinones which prevented the further buildup of mite population as a hypersensitive reaction or induced resistance.

Impact of *Polyphagotarsonemus latus* Infestation on Sugar Content

Previous research has shown that most herbivorous insects and mites use carbohydrates as feeding stimulants and these nutrients needed to synthesize body tissue, serve as energy sources (Schoonhoven *et al*., 1998) and promote oviposition (Derridj *et al.*, 1989). Due to continuous feeding, *P. latus* modifies the levels of metabolic substances in the surrounding tissues. This is supported in the current work where the levels of total soluble carbohydrates (total sugars) of infested chilli leaves were significantly lower than those of healthy ones to as much as 0.50 mg/g. This effect of mite feeding on total soluble sugar content was also reported in chrysanthemum, bean and cucumber plants, soybean, grapevine (Sivritepe *et al*., 2009) and bean (Farouk and Osman, 2012). However, it has been noticed in the current study that the amount of reducing sugars showed an increase up to 0.48 mg/g over control in *P. latus* infested chilli leaves. Such effect might be due to decreased efficiency of conversion of monosaccharides (particularly glucose) into polysaccharides, leading to their accumulation (Tomczyk, 2001).

Many scientists have reported that the activity of phytophagous mites increased with increase in sugar content of host plants. A positive correlation between total sugars and *P. latus* population during the present study confirmed the earlier work (Hosamani, 2007). This also means that susceptible hybrids will have high sugar content than least susceptible hybrids. This contention was supported by Nawalgatti *et al.* (1993) who observed low sugar content in resistant chilli varieties. Hence, low sugar content was considered as one of the important factors in resistant varieties by Varadharajan and Veeravel (1996). However, contradictory opinions indicating the negative role of sugars with plant mite attack was reported by Goyal and Sadana (1983).

Conclusion

At last, it was concluded that as increase in *P. latus* infestation nitrogen content of chilli leaves showed a significant decline. Hybrids (9/CHIhyb-10, 9/CHIhyb-8) with less mite count (8.19, 11.21 mites/leaf) recorded significantly higher nitrogen content (6.62, 5.50 %dry weight) as compared to other hybrids. With increase in *P. latus* population from 8.91 to 22.08 mites/leaf, phosphorus content in leaves dropped from 0.48 to 0.14 %dry weight. In least susceptible hybrid (9/CHIhyb-10), significantly higher phosphorus content (0.48 %dry weight) was recorded as compared to moderately and highly susceptible hybrids. In highly susceptible hybrid, 10/CHIhyb-7 lowest potassium content (1.50 %dry weight) was recorded which was comparable with potassium content in other highly susceptible hybrid, 10/CHIhyb-6. However, it was significantly less than the potassium content observed in other hybrids. Phenolic compounds exhibited a marked increase with increase in mite infestation. In uninfested leaves, total sugar content was 1.34 mg/g fresh weight which decreased significantly to 0.50 mg/g fresh weight with increase in *P. latus* infestation (22.08 mites/leaf). The reducing sugar content was found to be significantly increased from 0.24 mg/g fresh weight in uninfested leaf to 0.48 mg/g fresh weight in highly susceptible hybrid (10/CHIhyb-7). A significant effect of mite incidence depicted a progressive decline in non-reducing sugars in accordance with increase in *P. latus* infestation as compared to uninfested plant.

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Table 1: Population build-up of *Polyphagotarsonemus latus* on chilli hybrid

Observation periods	Mite population/leaf (Mean ± S.E.)			Mean
	Top leaf	Middle leaf	Bottom leaf	
9/CHIhyb-10	09.50±0.58	08.55±0.58	06.53±0.58	**8.19**
9/CHIhyb-8	13.67±1.03	12.33±1.01	07.63±0.49	**11.21**
10/CHIhyb-10	20.67±0.67	17.33±1.20	12.57±1.20	**16.86**
10/CHIhyb-12	22.33±0.60	19.33±1.20	14.33±1.86	**18.66**
10/CHIhyb-6	24.00±1.15	21.55±0.33	18.00±1.45	**21.18**
10/CHIhyb-7	25.09±0.59	22.59±0.14	18.55±0.29	**22.08**

Table 2: Effect of mite population on phytochemical contents of leaves in chilli hybrids

Hybrids	Number of mite/leaf	Nitrogen (% dry weight)	Phosphorus (% dry weight)	Potassium (% dry weight)	Phenol (% dry weight)
9/CHIhyb–10	8.19	6.62	0.48	4.75	0.35
9/CHIhyb–8	11.21	5.50	0.35^{a}	3.12^{a}	0.39
10/CHI Hyb-10	16.86	3.93	$0.26^{a,b}$	$2.62^{a,b}$	0.46
10/CHIhyb-12	18.66	2.37	$0.25^{a,b}$	2.50^{b}	0.48
10/CHIhyb– 6	21.18	1.31^{a}	$0.16^{b,c}$	$2.06^{b,c}$	0.53
10/CHIhyb– 7	22.08	1.12^{a}	0.14^{c}	1.50^{c}	0.54
SE(m)		0.15	0.02	0.28	0.00
C.D. (p=0.05)		**0.47**	**0.10**	**0.87**	**0.01**

Values with the same superscript do not differ significantly in column

Table 3: Effect of *Polyphagotarsonemus latus* population on sugar contents in chilli hybrid leaves

Hybrids	Number of mites/leaf	Total sugar (mg/g)	Reducing sugar (mg/g)	Non reducing sugars (mg/g)
Uninfested plant	0.00	1.34	0.24	1.10
9/CHIhyb-10	8.19	0.99 (26.11)	0.27	0.72
9/CHIhyb-8	11.21	0.95 (29.10)	0.29	0.66
10/CHI Hyb-10	16.86	0.68 (49.25)	0.33	0.35
10/CHIhyb-12	18.65	0.64 (52.23)	0.43	0.21
10/CHIhyb-6	21.18	0.54 (59.70)	0.45	0.09
10/CHIhyb-7	22.08	0.50 (62.68)	0.48	0.02
SE(m)		**0.00**	**0.00**	**0.00**
C.D. (p=0.05)		**0.005**	**0.005**	**0.01**

Figures in parentheses are percent reduction over control