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Studies on Feeding Characteristics of *Oligonychus biharensis* (Hirst) (Acari: Tetranychidae) infesting Cassava

Sangeetha G. Kaimal and N. Ramani

Division of Acarology, Department of Zoology, University of Calicut, (Kerala)

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ABSTRACT : Feeding characteristics and damage induced by the spider mite, *Oligonychus biharensis* (Hirst) on the detached leaves of cassava *Manihot esculenta* L. were evaluated in the laboratory at 30 + 2°C and 70 + 5% RH. Cultivation of mite-infested (M^+) and mite-free (M^-) host plants was done by constructing block design plots and replicated. Live cultures of different stages of mites were also maintained in the laboratory using leaf flotation technique. The results showed that *O. biharensis* colonized on both surfaces of the leaf lamina of mature leaves and the petioles showed high population densities. Initial symptoms of damage were numerous white spots at the points of feeding on the leaf surface. Prolonged feeding encompassed the formation of dark brown patches, crinkling and subsequent drying and defoliation of affected leaves. Attack by these mites affected the growth and vigour of cassava plants. Analysis of damage symptoms revealed extensive bleaching and chlorosis of the leaves. Protein profile of *M. esculenta* leaves revealed 5 prominent bands each, both in M^+ and M^- host samples. However, M^- samples invariably showed higher protein concentration than the M^+ samples. Per cent loss in chlorophyll '*a*' and '*b*' recorded 86.40 ± 1.6 % and 81.03 ± 1.2 % respectively. Chlorophyll loss was significant at 1% levels. Increase in total phenol content of M^+ plants ranged from 11.67 ± 0.17 mg phenol/gm plant material.

Keywords : Spider mite, *Oligonychus biharensis*, cassava, chlorotic spots, phenol.

INTRODUCTION

The spider mite *O. biharensis* is a sporadic pest of vegetable crops and many other economically important plants (Jeppson *et al.*, 1975; Gupta, 1985; Sangeetha and Ramani, 2011). This spider mite is well known for its extraordinary ability to colonise vegetable plants, to replenish all the available nutrients and to cause serious injuries to its host plants (Chen *et al.*, 2005; Ji *et al.*, 2005). The mites occur invariably on both the leaf surfaces preferably near the petioles of mature leaves causing crinkling, drying and defoliation of affected leaves. This has raised their importance as pests in terms of the degree of damage induced. The host plant, *M. esculenta* (cassava) stands out in the country as the most important source of energy especially for the low income calorie-deficient population and a major horticultural export commodity earning foreign exchange in addition to its use as animal feed and industrial usage (Hillocks *et al.*, 2002). Field observations showed that these plants are highly susceptible to attack by *O. biharensis* as the species emerged as the prominent faunal element on them. Whereas a large body of work is available on the breeding biology of these mites, much less is known about the feeding injury induced by *O. biharensis*. In the present study, the feeding characteristics and damage induced by *O. biharensis* on cassava is addressed in a series of laboratory experiments.

MATERIAL AND METHODS

Outdoor culturing of mites

Live cultures of *O. biharensis* were maintained on cassava in the field to observe closely the mode of infestation, progressive damage symptoms induced on the host plant and also to make quantitative estimation of damage potential of the concerned species. To achieve this objective, two mite treatments (*i*) M^-, mite free plants and (*ii*) M^+, plants artificially infested with mites, were included in a randomized block design plots (3 m × 3 m) which was replicated four times within a season. Cultivation of host plants was done by planting stem cuttings of cassava in enriched soils prepared for the study. The plots were irrigated regularly and the plants were made mite-free by spraying a broad-spectrum insecticide thiodicarb to eliminate mite pests and predators (Reddall *et al.*, 2004). Artificial infestations of M^+ plants were done by stapling mite-infested leaf bits grown in the glass house 60 days after planting. The plots were covered with fine nets to ensure protection from pest attack and to reduce the risk of cross infestation between M^+ and M^- plots.

Indoor culturing of mites

Live cultures of different stages of the mites were maintained in the laboratory at 30 ± 2°C and 70 ± 5% relative humidity on fresh leaves of cassava, collected from the

plots at an interval of 2 days or at the time of need. Mite culturing was carried out following the leaf flotation technique (Sangeetha and Ramani, 2007). Each culture set consisted of 2-4 leaves, kept in petri dishes lined with moistened cotton pads and was treated as an experimental unit. Stock cultures of the mites were also maintained in the laboratory in the same manner so as to ensure constant supply of life stages.

Chlorophyll estimation

The concentrations of chlorophyll (*a* and *b* separately) was determined using the method described by Ekanayake and Adeleke (1996). Fully expanded 2-3 main stem leaves 5 nodes below the terminal taken from two M^+ and M^- plants per plot were used for extraction. This area marked the region showing highest mite density within the M^+ plant. 2gms each of M^+ and M^- leaf tissue was crushed using mortar and pestle and 20ml of 80% acetone was added to it to allow the tissue to be thoroughly homogenized. The supernatant was decanted through filter paper into a 100 ml volumetric flask. The extraction procedure was repeated by the addition of 20 ml acetone to the residue. Additional acetone was added to wash off the chlorophyll and the solution was made upto 100 ml mark in the volumetric flask. The resulting solution was thoroughly mixed and 5 ml pipetted into a 50 ml volumetric flask and made up the volume with 80% acetone. The absorbance of the extract from M^+ and M^- plants was measured at 645, 663 and 652 nm wavelengths for chlorophyll *a*, *b* and total chlorophyll respectively against 80% acetone as blank. Concentrations of chlorophyll (mg /g fresh leaf weight) were calculated as follows:

Chlorophyll $a = (20.2 \times D\,645) \times (50/1000) \times (100/5) \times (1/2)$,

Chlorophyll $b = (8.02 \times D\,663) \times (50/1000) \times (100/5) \times (1/2)$,

where D = absorbance.

The data were statistically analyzed using students *t* test with SPSS (Version 12). Values are expressed as Mean ± SEM. '*n*' indicate the number of trials.

Estimation of Total Phenol content

The response of plants to mite attack in terms of concentration of total phenols content of each extract was determined using methods previously described by Singleton *et al.* (1999). 1gm each of M^+ and M^- fresh leaf sample was ground in a pre-cooled mortar into fine paste with 10 ml of 80% ethanol. The homogenate was centrifuged at 10,000 rpm for 20 min, the supernatant was saved, and to the residue 5 ml of 80% ethanol was added for re-extraction. Pooled supernatant was evaporated to dryness in a hot water bath. Dried residue was dissolved in 5 ml of distilled water. To 1 ml of the above solution, 2 ml of distilled water and 0.5 ml of Folin-Ciocalteau reagent were added and incubated for 3 minutes. Added 2 ml of 20% Sodium bi-carbonate solution (freshly prepared), mixed thoroughly and placed in boiling water bath for 1 minute. Cooled the reaction mixture and the absorbance value was read at 650 nm using a Shimadzu UV-VIS spectrophotometer (Model UV - 1601). Final concentration of phenol present in each test sample was calculated by plotting a standard graph prepared using different concentrations of standard Tannic acid. Values are expressed in terms of mg phenols/ 100g material.

Estimation of Protein Profile

Protein profiles of the M^+ and M^- leaf samples of *M. esculenta* were prepared by SDS Poly Acryl amide Gel Electrophoresis (Gaal *et al.*, 1980). 1 gm each of M^+ and M^- leaf tissue sample in duplicate were weighed separately and ground into a fine paste in ice cold condition using 5ml of Tris extraction buffer and fine sand powder. In order to control the phenolic oxidation, β - mercaptoethanol (200 µl) was used during the preparation. The fine paste obtained after grinding was transferred to centrifuge tube (8ml) and spun at 10,000 rpm at 4°C using a refrigerated centrifuge (Plast Crafts Model - Rota 4R - V/F M) for 30 minutes. The supernatant was collected using micropipette in a separate micro-centrifuge tube and stored at –28°C. 50 µl of sample was loaded in the wells of the apparatus (Genei Mini Model Electrophoresis Unit) assembled for running at a voltage set at 80V. The gel was stained in coomassie brilliant blue and molecular weights of the bands were determined using Quantity One software.

RESULTS

Qualitative assessment of feeding injury

Studies on feeding activities of all life stages of *O. biharensis* on *M. esculenta*, damage symptoms and progress of infestation was made through repeated field cum laboratory studies.

Morphological responses of feeding

Feeding activity of O. biharensis: *O. biharensis* represented the category of 'leaf suckers' encountered on M. esculenta during the survey. It colonised the mature leaves and built large colonies. However, young or newly sprouted leaves of the host plant were left unfed by the mite. The adults, larval and nymphal stages equally engaged in active feeding by piercing their stylets set on protrusible stylophore that could be seen moving back and forth during feeding and sucking the tissue fluids out from the leaves. The colony structure of the mites as reflected in the current study were complex attained through construction of thinner complicated webbing on the leaf surface connecting the petioles and major veins of the leaf. Females initiated oviposition soon after web construction and laid golden brown eggs at random on the leaf surface. As feeding progressed, faecal matter was deposited as black globules

that spread on the leaf surface soon after ejection from the body. Such black patches could be seen scattered all over the leaf surface amidst a separate layer formed of webbing, egg cases, moulting skin, eggs and life stages, dust particles and damage symptoms. This coating imparted a cumulative effect on the retardation of photosynthesis by preventing the absorption of light by the residual chlorophyll left unfed by the mite. Formation of such a thick layer over the leaf surface could be explained on the basis of earlier reports by Sumangala and Haq (2000), Reddall *et al.* (2004) and Sangeetha and Ramani (2007b).

Damage symptoms induced by O. biharensis: Feeding injury to the host leaf was marked by the appearance of characteristic spots and later patches at the areas of suction of sap from the plant cells. Initial symptoms of damage were manifested in the form of numerous white spots at the points of feeding on the leaf surface. Continuous sucking by all stages of this mite from leaves and petioles caused fusion of these spots and formation of large chlorotic patches. Following this, a change in colour from white to yellowish brown patches could be observed. Severe infestation and prolonged feeding encompassed the formation of dark brown patches, crinkling and subsequent drying and defoliation of affected leaves. Attack by these mites was so severe that the whole plantation appeared to be crinkled due to water loss through the feeding punctures produced on the leaves. This had a negative impact on the growth and vigour of cassava plants. This observation is in support of the findings of Reddy and Baskaran (2006) at much lower infestation levels of *Tetranychus ludeni* on four varieties of egg plant.

Quantitative assessment of damage potential

Physiological responses from mite feeding

Estimation of Leaf Chlorophyll content: Chlorophyll content of the leaves is regarded as one of the parameters determining the photosynthetic efficiency of the plant (Lahai *et al.*, 2003). So chlorophyll content of M^+ leaves were compared with that of M^- leaves in order to quantify the extent of damage induced by the feeding activity of *O. biharensis.* Quantitative reduction in chlorophyll '*a*' and '*b*' was observed to be 7.56 + 0.04 and 6.70 + 0.10 mg/gm tissue respectively. Per cent loss in chlorophyll '*a*' and '*b*' was 86.40 + 1.6 % and 81.03 + 1.2 % respectively (Table 1). Results were found significant at 1% level. The leaf chlorophyll content thus decreased with increase in mite density and duration of feeding (Haq 1997; Sumangala and Haq 2000; Nachman and Zemek 2002; Park and Lee 2002 and Sangeetha and Ramani 2007b). In addition, feeding activity of the individuals induced heavy loss of water from the leaf tissue. The overall impact of the above processes had resulted in the total destruction of the photosynthetic machinery of the plant leading to its final collapse. These results have clearly established the potentiality of the leaf sucking forms in damaging the host plants (Iatrou *et al.* 1995; Ekanayake *et al.* 1996 & 1998; Bounfour *et al.* 2002; Lahai *et al.* 2003; Reddall *et al.* 2004; Sangeetha and Ramani 2007*b*).

Estimation of Total Phenols: Phenols are wide spread in plant kingdom, the role of which in the metabolism of the plant has not been adequately explained, though are believed to offer resistance to pests and diseases. Because certain phenolics have the ability to precipitate plant proteins and render them indigestible, they have been considered as defense compounds. Analysis of feeding response of *O. biharensis* in terms of phenol content of cassava leaves provided better picture on the impact of their feeding. Significant increase of 11.67 + 0.17 mg phenol/ gm plant material of M^+ plants was observed following infestation by *O. biharensis* (Table 2). Ananthakrishnan *et al.* (1992) recorded a similar increase in the production of phenolics in cassava, castor and eucalyptus during pest attack and concluded that increase in total phenols induced resistance in hosts against herbivory. Such an increase in phenolic content due to spider mite feeding in 'Conica' leaves was reported earlier (Puchalska, 2006), estimating up to 50% reduction in photosynthesis rate after 3 weeks of heavy infestation by *O. ununguis.* Accumulation of phenolic compounds in plant tissues is reported to be one of the causes of photosynthetic suppression (Puchalska, 2006). These results reflected on the innate response of the cassava plants against *O. biharensis* attack.

Table 1: Quantitative difference in chlorophyll content (mg/gm tissue) of *M. esculenta* leaves due to infestation by *O. biharensis.*

Chlorophyll	*Milligram Chlorophyll /gram tissue*		*Loss in chlorophyll*	*% chlorophyll loss*
	M^+	M^-		
Chlorophyll *a*	1.49±0.20	9.05±0.24	7.56±0.04	86.40±1.6
Chlorophyll *b*	1.35±0.13	8.04±0.23	6.70±0.10	81.03±1.2

n = 25

Table 2: Quantitative difference in total phenol content (mg/100 gm tissue) of *M. esculenta* leaves due to infestation by *O. biharensis.*

Milligram Phenol/gram tissue		*Increase in total phenol*
M+	M^-	
17.72 ± 0.41	6.05 ± 0.24	11.67 ± 0.17

n = 25

Biochemical responses from mite feeding

Estimation of Protein profile: Preliminary studies on the protein profile of *M. esculenta* leaves revealed 5 prominent bands each, both in M^+ as well as M^- samples (Fig. 1). However, the positions of these bands were identical in both the leaf samples. Of the 5 bands, the third

band from the top (62 kDa) was quantitatively the major one as indicated by the band width and staining intensity. The other bands recorded substantially very low concentration in comparison to the major band. Further, the M^- leaf samples invariably had a higher protein concentration than the M^+ samples as the former had more intensity of staining than the latter. Endogenous degradation of existing proteins might have occurred following mite infestation in M^+ leaves. Apparently neither de novo synthesis of proteins occurred nor was there a selective depletion of any protein. Following infestation and damage by *O. biharensis*, a slight decrease in the protein concentration in the M^+ leaves was observed though no significant change in the protein profile could be recorded in the present investigation. However, studies undertaken on this aspect were preliminary and first of its kind and in-depth studies are underway to substantiate the results. Yet the results gained by SDS PAGE provide a simple picture which makes it possible to understand the biochemical changes that followed infestation by *O. biharensis.*

The feeding activity of these mites also induced mechanical injury to epidermal and mesophyll tissue and hence heavy water loss from the leaf tissues. These results clearly reflect on the capacity of *O. biharensis* in damaging the host plant, *M. esculenta* by inducing mechanical damage aggravated by biochemical alterations.

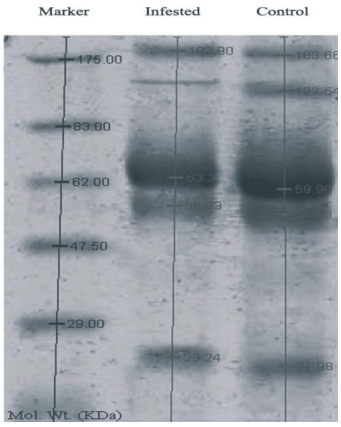

Fig. 1. Protein profile of *M. esculenta* leaves infested by *O. biharensis.*

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