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Qualitative damage of spider mites on selected medicinal plants and the corresponding biochemical changes

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Abstract
Adaxial feeding by spider mite, *Schizotetranychus baltazari* on M*urraya koenigii* resulted in grey speckling, discrete peeling of leaf cuticle, greyish-white appearance, withering and defoliation, decreasing the leaf biochemicals *viz*., chlorophyll (15.82%), carbohydrate (42.48%), proteins (55.1%), crude fibre (43.71%), flavonoids (26.83%), phenols (75.00%), potassium (7.37%) and sodium (23.18%). But alkaloid (92.44%) and tannin (12.50%) contents found increased. *Oligonychus mangiferus* and *Oligonychus thelytokus* feeding in *Ichnocarpus frutescens* plants caused yellowish-white spots, drying, withering and premature leaf-drop with significant reduction in chlorophyll (55.88%) and flavonoids (64.71%), but alkaloids (220%) found increased. Feeding by *Tetranychus hirsutus* on *Gymnema sylvestre* produced orange to yellow spots, profuse webbings with numerous mites congregating for dispersal. Chlorophyll (15.89%), carbohydrate (90.90%), proteins (69%) and flavonoids (17.60%) found reduced, but alkaloids (14.30%) increased upon mite feeding. This increase in secondary metabolites attributed to induced response of the plants to feeding by spider mites questioning the utility of such leaves in herbal preparations and therapeutic use.

Keywords: Spider mites, medicinal plants, symptoms of damage, biochemical profiling

Introduction

India is traditionally a treasure house of high value medicinal plants used in both folk health traditions and Indian System of Medicines. Like our food crops, Ayurvedic and commercial crops, medicinal plants are also prone to damage by pests particularly the spider mites causing substantial qualitative and quantitative changes. The members of spider mite family Tetranychidae are reported as major pests of medicinal plants in India (Gupta & Karmakar 2010) [9], pointing out *Tetranychus urticae* Koch as a major threat (Sharma and Agarwal 2010) [26]. The mite feeding caused yellowing of foliage, whitish streaks on stems with reduced foliage in *Pelarogonium* (Snetsinger *et al*., 1965) [28]; reduced growth was observed in ivy geranium plants (Opit *et al*., 2004) [20], also intense chlorosis and webbing damage in *Justicia adhatoda* (Sheela and Ramani 2012) [27]. Reports of mite-infested crop plants showing tolerance reaction to mite infestation or damage are available (Mutturaju, 2013; Rajagopal, 2015) [19, 21].

But no systematic study has been attempted to ascertain adverse biochemical changes subsequent to mite feeding in medicinal plant to quantify the damage. Recently, common medicinal plants like *Murraya koenigii* (also a spice crop), *Ichnocarpus frutescens* and *Gymnema sylvestre* were found damaged by spider mite species of *Schizotetranychus baltazari* Rimando, *Oligonychus mangiferus* (Rahman & Sapra) *& Oligonychus thelytokus* Guiterrez and *Tetranychus hirsutus* Zeity & Srinivasa, respectively. *Schizotetranychus baltazari* was originally described by Rimando (1962) [22] on *Citrus*, also found it damaging tropical, semi-tropical and medicinal plants like *Azadirachta indica*, *Holarrhena tubescens*, *Curcuma zedoaria* and *M. koenigii* (Ghosh and Gupta 2003; Gupta & Karmakar, 2011 and Roy *et al*. 2011) [8, 10, 23].

But systematic study of symptomology has been attempted on citrus only (Vacante, 2015) [29]. Similarly, *O. mangiferus* is a polyphagous pest with at least 50 host plants (Bolland *et al*., 1998) [4] and symptomological studies are available on its regular host plant mango. *O. thelytokus* that reproduces by thelytokus parthenogenetic mode of reproduction was originally described by Gutierrez in 1977 [11]. Recently, Zeity *et al*. (2016) [30] described a new species of mite *Tetranychus hirsutus* Zeity and Srinivasa from Bangalore, India and observed to cause sporadic damage on *Gymnema* plants in wild. In spite of significant damage caused by spider mites on *Murraya*, *Ichnocarpus* and *Gymnema*, data with regard to feeding damage by spider mites on these medicinal plants are scattered and scarce (Ahalya and Mikundan, 2009; Saini and Reddy, 2013; Karmakar *et al.*, 2014) [2, 24, 15]. Hence, an attempt was made to study characteristics of infestation by four different spider mite species on few common medicinal plants and to ascertain the impact of damage on leaf biochemicals.

Material and methods

Symptomology

The medicinal plants selected for the study *viz., M. koenigii, I. frutescens and G. sylvestre* were maintained in earthen pots in the polycarbonate house. To enable mite infestation, the healthy potted plants of *M. koenigii* were placed in close proximity of mite infested potted plants and were undisturbed until the *S. baltazari* mites got transferred from infested plants to healthy plants. The newly infested plants were observed daily for the progressive development of symptoms subsequent to feeding damage by mites.

Potted plants of *I. frutescens* were artificially infested by fastening *O. mangiferus* and *O. thelytokus* mite infested leaves and likewise, wild vines of infested *G. sylvestre* were collected and were loosely tied to healthy potted plants to facilitate the transfer of mites. Freshly infested plants were observed every 24 hours for the development of damage symptoms up to 10-15 days.

Estimation of phytochemicals

Total chlorophyll content in healthy and mite-infested leaves of *M. koenigii, I. frutescens* and *G. sylvestre* was estimated using the standard procedure of Arnon (1949) [3] by incubating one gram of fresh leaf sample overnight in Dimethyl sulfoxide and 80% Acetone mixture in 1:1 ratio, diluting the supernatant extract and recording the absorbance at 645 and 663 nm wavelengths in a Spectrophotometer (Hitachi U-2900). Healthy and mite infested leaves of *M. koenigii, I. frutescens* and *G. sylvestre* were shade dried and powdered separately using a Waring blender and subjected extraction using Soxhlet apparatus with organic solvent methanol for *M. koenigii* & *G. sylvestre* and with solvent ethyl acetate for *I. frutescens.* The extract was evaporated using a Rotary vacuum flash evaporator and concentrated extract was subjected to biochemical analysis following standard analytical procedures. Whenever necessary the concentrated extracts were stored in airtight glass bottles at -20ºC and analysed further for primary metabolites, secondary metabolites and nutrient elements contributing to the medicinal property of test plants. Total carbohydrate content was estimated following Anthrone method (Hedge and Hofretter, 1962) [12] by recording the absorbance at 630 nm and Total protein content by Lawry's method (660nm), Total crude fibre content was estimated by boiling leaf powder samples with sulphuric acid, sodium hydroxide washing and finally igniting the dried samples at 130º±2ºC and 600º±15ºC (Maynard, 1970) [18]. For total alkaloid content procedure of Agarwal and Murali (2010) [1] by Gravimetric method was followed. Estimation of total flavonoid content by the method of Samata *et al.* (2012) [25] and absorbance reading at 510 nm in the Spectrophotometer (Hitachi U-2900).

Total phenol content was estimated measuring the absorbance at 765nm (Jalal *et al.*, 2015) [13], saponin content by absorbance reading at 544 nm (Khan and Choudhary, 2010) [16] and tannin content using Folin's reagent-Tannic acid standards (absorbance at 725 nm). Estimation of total terpenoid content (expressed as Ursolic acid) was by High Performance Liquid Chromatograph (Agilent) using methanol - water (95:5) as mobile phase at a flow rate of 1ml/minute and absorbance measured at 205 nm and using standard ursolic acid calibration curve obtained at the retention time of 8 minutes. Potassium and sodium contents in the leaf samples were determined using Flame photometer. The biochemical data from healthy and mite-infested leaves were compared for interpretation.

Results

Symptoms of damage

Murraya koenigii* infested by *Schizotetranychus baltazari: The mite feeds from the upper surface of leaf and damage was evident on the lower surface of the leaf as the mite increased in number and started congregating. Grey irregular spots appear on the leaves with thin or less dense webbings, which developed into more number of spots. Leaf cuticle and upper epidermal layers were affected most by mite feeding. Discrete leaf peelings were evident and entire leaf appeared pale due to depletion of chlorophyll. Complete withering and defoliation were prominent in young seedlings due to severe mite feeding damage (Fig. 1a).

Ichnocarpus frutescens infested by Oligonychus mangiferus and Oligonychus thelytokus: O. mangiferus was recorded on leaves of top and middle canopy, while O. thelytokus was on the lower canopy. Both the mite species were found feeding on the upper surface of the leaves, removing a notable amount of cell contents. As a result, orange – yellow spots appeared on the leaves (Fig. 1b), became prominent as the number of mites and feeding damage increased. Severe infestation resulted in drying and premature dropping of the leaves.

Tetranychus hirsutus infesting Gymnema sylvestre: This tetranychid mite species feeds from the upper surface of leaves, moving on to the lower surface with increase in the mite population. Feeding damage was evident usually a month after infestation, which was characterised by the development of yellowish spots on the leaves, proceeding towards yellowing and drying of leaves at the later stages of infestation. At higher mite population, the symptoms were so evident even from a distance due to profuse webbings, where the mites congregate in large numbers in order to get disperse to other nearby plants. Severe infestation lead to complete drying and ultimately leading to plant death (Fig. 1c).

Biochemical profile spider mite infested of plants

Murraya koenigii versus infestation of Schizotetranychus baltazari: As result of mite feeding decrease in almost all the leaf biochemicals was observed. Chlorophyll content decreased from 1.77 mg/g (healthy leaves) to 1.49 mg/g (infested leaves) showing 15.82 per cent reduction, carbohydrates from 1.53 mg/g to 0.88 mg/g (42.48% reduction), proteins from 0.02 µg/mg to 0.01 µg/mg (55.1% reduction), crude fibre from 8.42 per cent to 4.74%per cent (43.71% reduction), flavonoids from 0.41µg/mg to 0.30 µg/mg (26.83% reduction), phenols from 0.012 µg/mg to 0.003µg/mg (75% reduction), potassium from 12.49 ppm to 11.57 ppm (7.37%) and sodium from 6.60 ppm to 5.07 ppm (23.18% reduction). Whereas, alkaloids and tannin contents increased in mite infested leaves 92.44 per cent and 12.50 per cent respectively (Fig. 2). Increase in the concentrations of secondary metabolites can be attributed to their definite role as plant defensive chemicals against mite infestation.

Ichnocarpus frutescens versus infestation of Oligonychus mangiferus and Oligonychus thelytokus: The mite feeding damage showed significant reduction in chlorophyll (55.88%) and flavonoid content (64.71%) from 0.17µg/mg in the healthy leaves to 0.06µg/mg in the infested leaves. Alkaloid content increased enormously (220%), while terpenoid content increased marginally (0.29%) in mite infested leaves (Fig. 3).

***Gymnema sylvestre* versus infestation of *Tetranychus hirsutus*:** *Gymnema sylvestre* leaves infested by *T. hirsutus* showed reduction in major leaf biochemicals.

A reduction of 15.89 per cent in chlorophyll, 90.90 per cent in carbohydrates, 69 per cent in proteins, 17.60 per cent in flavonoids and 2.90 per cent in saponin contents was recorded except with alkaloids, which showed 14.30 per cent increase upon mite feeding (Fig. 4).

This can be related to induced plant defense due to feeding damage by mites.

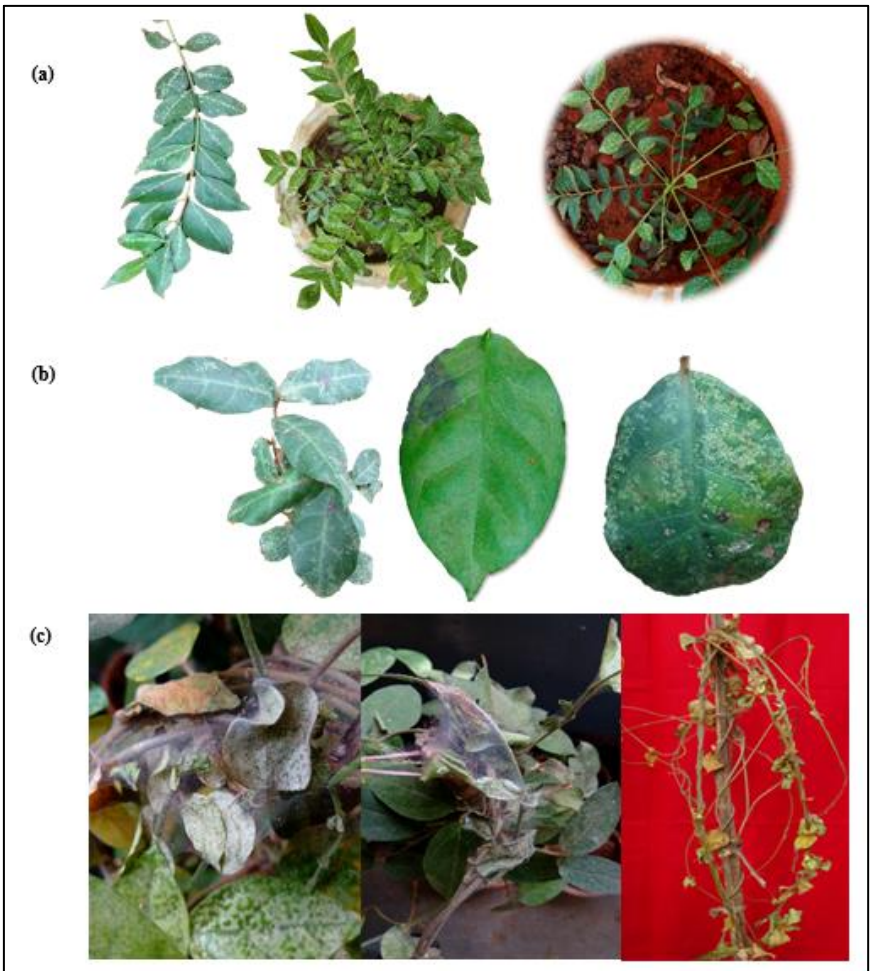

Fig 1: Progress of damage by spider mites: (a) On *M. koenigii* by *S. baltazari* (b) On *I. frutescence* by *O. mangiferus & O. thelytokus* and (c) On *G. sylvestre* by *T. hirsutus*

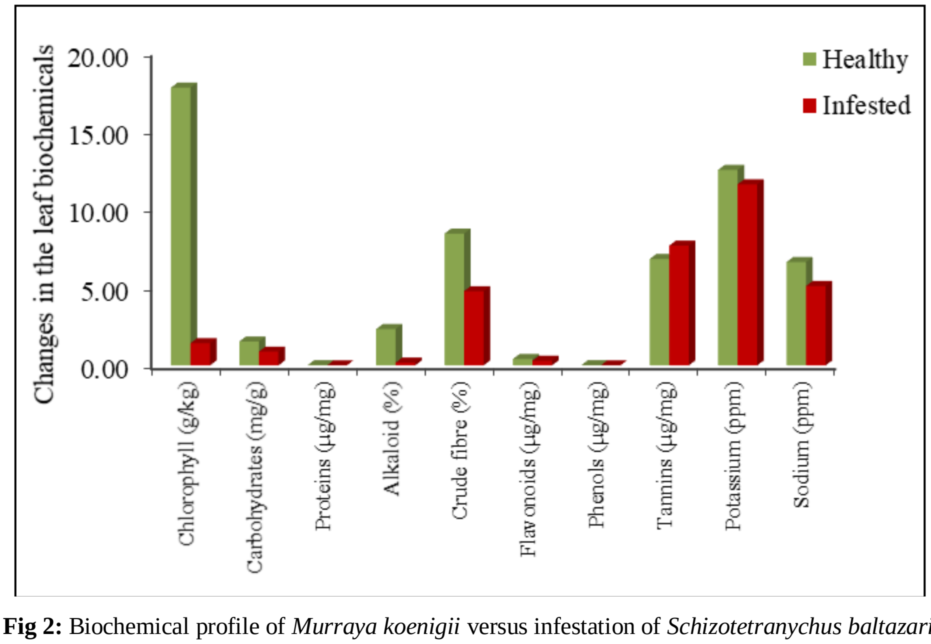

Fig 2: Biochemical profile of *Murraya koenigii* versus infestation of *Schizotetranychus baltazari*

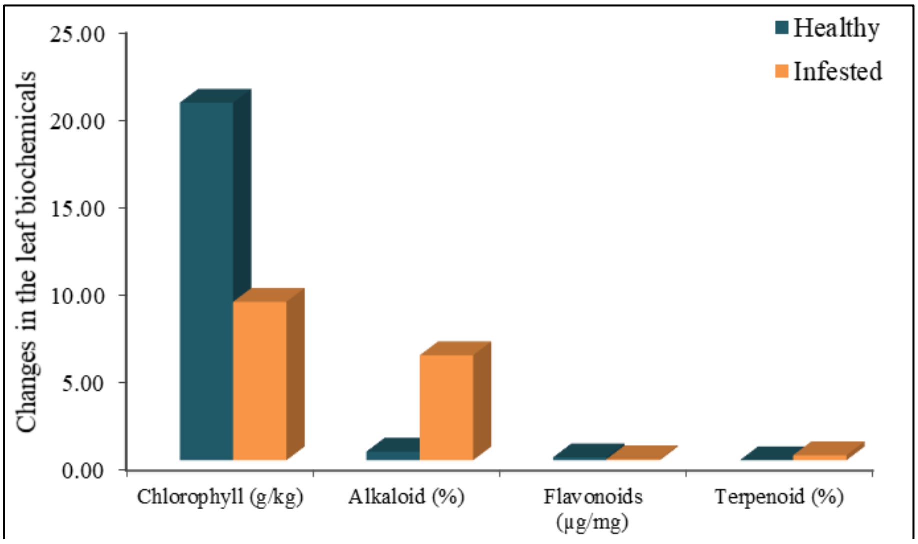

Fig 3: Biochemical profile of *Ichnocarpus frutescens* versus infestation of *Oligonychus mangiferus* and *Oligonychus thelytokus*

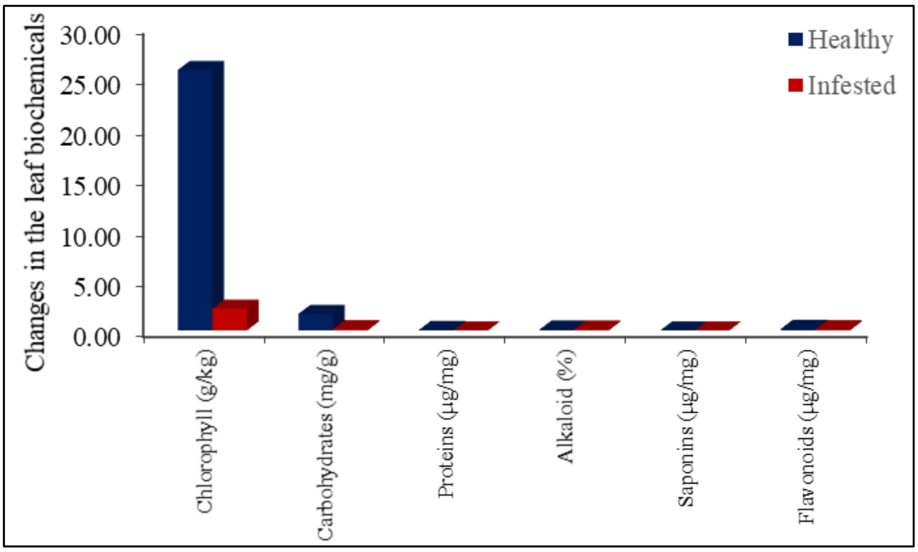

Fig 4: Biochemical profile of *Gymnema sylvestre* versus infestation of *Tetranychus hirsutus*

Discussion

Feeding damage by *S. baltazari* produced greyish spots, leading to complete leaf withering and defoliation when young seedlings of *M. koenigii* were infested by the mite. Jeppson *et al.* (1975) [14] observed similar feeding damage with discoloured greyish spots on both leaves and fruits of citrus; Gerson (1985) [7] reported this mite feeding caused greyish colouration on mite infested citrus leaves and Karmakar *et al.* (2014) [15] noticed *S. baltazari* to cause yellowish white striplings on citrus leaves, with severe infestation leading to defoliation. Our studies concluded that *S. baltazari* produces characteristic greyish spots, withering and ultimate defoliation in all the known host plants.

FanggPing and RuiGuan (2006) [6] reported that the mango mite (*O. mangiferus*) turns infested mango leaves to grey-white; Ghosh and Gupta (2003) reported then its infestation on *Mangifera indica* and *Syzygium cumini* caused yellowing on the upper surface of the leaves and Jeppson *et al.* (1975) [14] reported it to produce drying effect and leaf drop on citrus. Channabasavanna and Banu (1972) [5] recorded typical feeding damage characterized by pale yellow colouration and yellow speckling on *Syzygium jambulana* and *Jambosa vulgaris*. In the present study more similar symptoms were observed due to feeding by *O. thelytokus* on the upper surface causing discolouration, yellowish–white spots, while *O. mangiferus* with orange – yellow spots, leading to drying, withering and premature leaf-drop of *I. frutescens* leaves, suggesting that polyphagous mite, *O. mangiferus* produces yellow to whitish yellow spots with little modification depending on the host damaged.

Feeding damage of *T. hirsutus* on *G. sylvestre* was characterized by yellowish spots on the adaxial leaf surface, yellowing and drying of leaves with profuse webbing as the mite population increased enormously. No data is available for comparison in this regard and since feeding damage of this species was reported for the first time from this study.

Feeding by *S. baltazari* on *M. koenigii* significantly altered both primary & secondary metabolites of the leaves. Chlorophyll, carbohydrate, proteins, crude fibre, phenols and sodium found decreased, while alkaloids and tannin content increased in mite infested leaves. Mutturaju (2013) [19] studied the effect of *T. urticae* mite feeding on the leaf biochemical profile of brinjal, which revealed significant reduction in protein contents (48.33%), but increase in total phenols (51.30%) and the activity of phenylalanine ammonia lyase enzyme (51.86%) subsequent to mite feeding was attributed to plants induced defense. Significant increase in alkaloid content and significant decline in total phenol upon mite feeding or damage in the present study could be possibly due to the accumulation of these secondary metabolites due to mite damage.

Feeding by *O. mangiferus & O. thelytokus* showed apparent decline in chlorophyll (55.88%) and flavonoid content (64.71%), but other leaf biochemicals like alkaloid and terpenoids were found increased in the infested leaves. Mamun *et al.* (2017) [17] investigated the effect of *O. mangiferus* on the physico-biochemical parameters of tea and found that chlorophyll a (2.0 mg/g) and b (1.05 mg/g), carotenoids (0.55 mg/g), polyphenols (45.56 ppm), catechins (10.40 ppm) and reducing sugar (34.57 ppm) on severely infested leaves lower than in the fresh uninfested tea leaves. *Gymnema sylvestre* leaves infested by *T. hirsutus* showed more apparent reduction of 15.89% in chlorophyll, 90.90% in carbohydrates, 69% in proteins, 17.60% in flavonoids, while alkaloid content showed 14.30% increase upon mite feeding.

The qualitative damage in the present study is comparable to many earlier studies, by justifying the difference in the role or content of these biochemicals in the respective plant system. Increase in the concentration of secondary metabolites might be attributed to their role in induced plant defence as a result of mite feeding damage. These alterations in the biochemistry of plants, like chlorophyll and other primary metabolites, which contributed to their normal growth and development and also the up regulated defensive pathways like alkaloids and tannins, definitely questions the usage of such mite infested plants in herbal preparations need to be answered in future studies.

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