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Biological attributes and qualitative damage of *Oligonychus mangiferus* (Rahman & Sapra) (Acariformes: Tetranychidae) on the medicinal plant *Ichnocarpus frutescens* (L.) W.T. Aiton

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ABSTRACT: *Oligonychus mangiferus* (Rahman & Sapra) was found infesting the medicinal plant, *Ichnocarpus frutescens* L. Its biology and reproduction were studied at four different constant temperature conditions in the laboratory. *O. mangiferus* completed its development faster (7.10 to 8.77 days) at 30° - 32°C. Its egg-laying was highest at 20°C (31.03 eggs/female), but with similar progenial sex ratio (♂:♀) (1:2.68 to 1:2.84) across different temperatures. At 25°C, Mean Generation Time (T) and Doubling Time (DT) were lowest 15.26 days and 8.95 days, respectively, while, Intrinsic Rate of Natural Increase (r_m) was highest (0.085 female off-springs/female/day). Feeding damage by *O. mangiferus* resulted in apparent decline in chlorophyll and flavonoid contents, while alkaloid and terpenoid contents showed increase in mite infested leaves. Observed changes in the quantity of secondary metabolites like alkaloids, flavonoids and terpenoids, subsequent to mite feeding was significant, owing to the medicinal value of the herb. Further investigation on these biochemical changes may throw light on more advantageous medicinal use of *Ichnocarpus* for treating many human disorders.

KEY WORDS: *Oligonychus mangiferus,* medicinal plant, *Ichnocarpus frutescens*, life history, biochemical changes

INTRODUCTION

The spider mite family Tetranychidae comprises of significant number of species known to feed and damage almost all types of economically important cultivated crops (field crops, vegetable crops, fruit crops, ornamental and flower crops) including medicinal and aromatic plants (Gupta and Karmakar, 2010; Vacante, 2015). Quantitative losses due to mite feeding in the yield of food and commercial crops are well documented. Likewise, the mite damage on medicinal plants is expected to inflict variation in terms of its medicinal value. Both in India and outside except that of Ahalya and Mikundan (2009), Saini and Reddy (2013) and Karmakar *et al.* (2014), reports of mites occurring on medicinal plants are either scattered or meagre. Recently, a common medicinal plant *Ichnocarpus*

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frutescens L. (Apocynaceae) was found infested by *Oligonychus mangiferus* (Rahman & Sapra) (Acariformes: Tetranychidae) in the herbal garden of University of Agricultural Sciences campus at Bangalore, with significant damage on the green leaves. *I. frutescens* enjoys good prospects in the Indian medicine for the treatment of fevers, gout, rheumatism, arthritis, epilepsy, venereal diseases, herpes and skin diseases, blood purification, bleeding disorders, diarrhoea and protection of human fetal growth and development, and as cooling attribute (Sini and Malathyn, 2006; Pandurangan *et al.,* 2010; Joshi *et al.,* 2011). Many reports are available on the profile of secondary metabolites in mite-infested crop plants showing resistance or tolerance reaction to mite infestation or damage (Mutturaju, 2013; Rajagopal, 2015). But no systematic study has been attempted to ascertain biochemical changes subsequent to mite infestation or damage in any medicinal plant. Mamun *et al.* (2017) recorded lower amounts of polyphenols and catechins in green leaves of tea infested by *O. mangiferus*, when compared to mite-free fresh leaves. Black tea from severely mite infested leaves had low amounts of theaflavin (0.43%) and caffeine (54.68 ppm), accordingly the tea from mite-infested shoots was graded with inferior grade index of 31.65 to 32.90.

Oligonychus mangiferus is a polyphagous pest with at least 50 host plants (Bolland *et al*., 1998) and the incidence and damage of this mite on *I. frutescens* both in wild and planted situations is reported for the first time in our study. Information on growth, development and reproduction of *O. mangiferus* is available on its major host plants like mango and tea. While biological data of the mite were generated on an important medicinal plant *I. frutescens* for the first time from this study at four different constant temperature (20, 25, 30 and 32°C) and relative humidity (63-85%) conditions in the laboratory. Simultaneously, demography of the mite was also studied considering the significance of life table parameters in determining the population structure and performance of further generations that would ultimately damage the plants and appear as an emerging pest.

MATERIAL AND METHODS

Culturing of mite: The excised leaf disk technique was used for rearing and maintaining mite culture in the laboratory. Initially, *I. frutescens* leaves infested by *O. mangiferus* were brought to the laboratory in polyethylene bags, from which females along with one to two attending males were transferred separately on to 2.5cm X 2.5cm individual leaf disks (by changing at 10-12 days interval) placed on wet cotton wad in 15 inches Petri plates and allowed to colonize for at least 10-15 days and were further used in our studies.

Life history and Life table: The life history of the mite was studied separately at four different constant temperatures of 20±1°C; 75-85% RH, 25.3°C; 67-77% RH, 30±1°C; 64-73% RH & 32±1°C; 62-70% RH and 14h: 10h L: D conditions in a BOD incubator. Initially a cohort of eggs laid on leaf disks was transferred individually using a fine camel hair brush on to 30 separate 1.5 cm × 1.5 cm fresh leaf disks kept on wet sponge placed in 20cm X 15cm polyethylene trays. Development from egg hatching to adult emergence was observed periodically (every 3 to 6 hours) using a stereo-binocular microscope and after transferring hatched larvae on to individual leaf bits separately, duration of each stage of development was recorded. Duration of different developmental stages such as larva, protonymph and deutonymph was computed for male and female separately. Other observations such as longevity and oviposition pattern were also recorded.

To study the reproduction parameters and population characteristics, 30 teleiochrysalis females along with two males (to ensure mating) released separately on individual leaf disc were made used. After the emergence of the female mite reared on individual leaf bit, oviposition pattern (pre-oviposition, oviposition and post-oviposition periods) and daily egg laying were recorded at 24 hours interval, till the female mite stopped laying eggs and died naturally. Since the life span of male was shorter, as and when they were found dead or not seen on the leaf discs, fresh ones were released in the initial period of 10 days. Loss of any female

mite was made-up by replacing with a fresh virgin female/deutonymph or by increasing the number of replications. After recording egg-laying every day, the female mite was carefully transferred on to fresh leaf bit. The eggs laid were reared till adult emergence and sex of the emerging adult mite was recorded. Similarly, reproduction attributes of unmated females were recorded by maintaining female deutonymph separately on individual leaf bits without the release of males to such bits.

Reproduction attributes such as oviposition, fecundity and proportion of male and female off-springs (♂:♀) were recorded and compared across different rearing temperatures. Age specific life table data were computed and demographic characteristics such as, Mean Generation Time (*T)* (average age of parenthood in days), Net Reproduction Rate *(R_o)* (no. of female off-springs/ female/generation as the average number of new born females produced by a female during her entire life time), Finite Rate of Increase (λ) as no. of female off-springs/female/day, Intrinsic Rate of Natural Increase (r_m) (no. of female off-springs/ female/day as the maximal rate of increase by the combination of food, temperature, quality of food, *etc.*) and Doubling Time in days (*DT)* were calculated following the procedure suggested by Birch (1948) and Atwal and Bains (1974) as below;

Net Reproductive Rate, $R_o = \sum l_x m_x$

Mean Generation Time, $T = \frac{\Sigma xlxmx}{Ro}$

Finite Rate of Increase in number, λ =

anti ln $\left[\frac{\log eRo}{T}\right]$

Intrinsic Rate of natural Increase, $r_m = \ln(\lambda)$

Doubling time, $DT = \frac{\ln 2}{r_m}$

Where,

l_x = proportion of females alive at age interval x

m_x = number of female off-springs produced by the surviving female at the age interval x

$l_x m_x$ = product of the proportion of females live at age interval x and the number of female off-springs per original female produced at the age interval x

Duration of different developmental stages and reproduction attributes were expressed as mean ± SE, while data of total development (female and male) were analysed following One-way ANOVA. The standard error of different demographic parameters was estimated by bootstrapping technique and for comparison across different rearing temperature conditions, data were analysed using Tukey's HSD test in the statistical software SPSS 23.

Biochemical analysis: In order to ascertain the qualitative damage to *I. frutescens* plant, biochemical analysis was carried out for only those biochemicals, which are significantly contributing to the medicinal property in the plant. Total chlorophyll content of healthy and mite-infested leaves was estimated following the standard procedure of Arnon (1949) by using one gram of fresh leaf sample incubated 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 *I. frutescens* were shade dried and powdered separately using a Waring blender. Dry leaf powder was used for ethyl acetate extraction in Soxhlet apparatus and evaporated in Rotary flash evaporator. The concentrated extract stored in airtight glass bottles at -20ºC was used for the estimation of total alkaloid content following the procedure of Agarwal and Murali (2010) by Gravimetric method, estimation of total flavonoid content following the method of Samata *et al.* (2012) by absorbance reading at 510 nm in a Spectrophotometer (Hitachi U-2900) and estimation of total terpenoid content (expressed as Ursolic acid) by a High Performance Liquid Chromatograph (Agilent) using methanol - water (95:5) as mobile phase at a flow rate of 1ml/minute and absorbance was measured at 205 nm using the standard ursolic acid calibration curve obtained at the retention time of 8 minutes. Further, the biochemical data from healthy and mite-infested leaves were compared.

RESULTS AND DISCUSSION

Life history

Developmental time of *O. mangiferus* from egg to adult for female was 15.50 days, 12.20 days, 7.10 days and 8.56 days, while it was 14.67 days, 11.99 days, 7.32 days and 8.77 days for male on *I. frutescens* at four temperatures of 20°C, 25°C, 30°C and 32°C, respectively. Developmental time for male was shorter than that of the female at all the rearing temperatures. 30°C was found more suitable for faster mite development and the next best rearing temperature was 32°C (Fig.1).

Reproduction and demography

Age specific fecundity pattern revealed that egg laying by *O. mangigerus* reached peak on 25th, 5th, 18th and 11th day of its emergence over its survival period of 55, 29, 30 and 26 days at 20°C, 25ºC, 30°C and 32º C, respectively (Fig. 2). Each mated female laid as many as 31.03, 21.04, 24.16 and 26.52 eggs over a period of 25.13, 11.52, 10.28 and 10.96 days, respectively, while males lived for a shorter period of 12.75, 8.40, 12.00 and 11.67 days correspondingly. The sex ratio (♂:♀) in the succeeding progeny was 1:2.71, 1:2.84, 1:2.71 and 1:2.68. Virgin females (emerged from female teliochrysalis) maintained separately on individual leaf bits without males laid significantly less number of eggs at 20°C (17.82 eggs) and 32°C (17.55 eggs), but it was highest *i.e*. 36.20 eggs at 30°C. Mated females laid significantly a greater number of eggs at 20°C and 32°C compared to those at 25°C and 30°C (Table 1).

Daily female production by a mated female (rm) was 0.049, 0.085, 0.068 and 0.083 at the rearing temperatures of 20°, 25, 30 and 32°C, respectively. The corresponding Net Reproduction Rate (Ro) was 26.57, 16.29, 17.74 and 18.84 female offsprings/female/generation and the Mean Generation Time (T) were 29.50, 15.26, 19.20 and 16.76 days. Mite's doubling time was relatively shorter at 25°C and 32ºC *i.e.,* 8.95 days and 9.38 days, respectively (Table 2).

Qualitative damage due to mite feeding

O. mangiferus successfully completed life cycle on the medicinal plant, *I. frutescens* at all the test temperatures. The feeding damage of *O. mangiferus* on *I. frutescens* with respect to biochemical constituents was studied. Primary metabolite (chlorophyll) and secondary metabolites (alkaloid, flavonoids, terpenoids) were estimated from both healthy and mite infested /damaged leaves for comparison.

Feeding damage caused due to *O. mangiferus* resulted in 55.88 per cent decline in total chlorophyll content affecting the photosynthetic efficiency of infested leaves and it is obvious that, spider mites will suck away or remove chlorophyll along with the plant sap. Flavonoid content, an important secondary metabolite recorded a decrease of 64.71 per cent in mite infested leaves. But alkaloid and terpenoid contents were found increased in mite infested leaves (Fig. 3).

The herb *I. frutescens* was found infested by *O. mangiferus* as a predominant species and is the first report on this medicinal plant and hence, no earlier report of either biology or demography of the mite on *Ichnocarpus* is available. It is opined that the life table parameters are important indicators of population growth efficiency of a pest species on its host plant (Southwood and Henderson 2000; Islam *et al.,* 2017) and also provide information about survival and multiplication. This is more often useful in the prediction of population size on a host plant, which helps in ascertaining the damage intensity of the mite and growth as well as quality parameters of the medicinal plant.

Total developmental period from egg to adult of *O. mangiferus* on the leaves of *I. frutescens* was 12.20 days at 25ºC, while a study by Abu-shosha *et al.* (2017) recorded a lower total developmental period of 7.51 days on mango leaves. This variation in development is attributed to difference of host plants. Mani *et al.* (2014), who studied the life cycle of the mite on grapevine at 31ºC temperature, recorded a longer total life cycle of 27.3 days, while it has been recorded as a low of 7.10 days at 30ºC rearing temperature in the present study. From this it is evident that *I. frutescens* might be a more preferred host of *O. mangiferus* than grapevine on which the mite took more time to develop and complete its life cycle. Gotoh and Gomi (2003) and

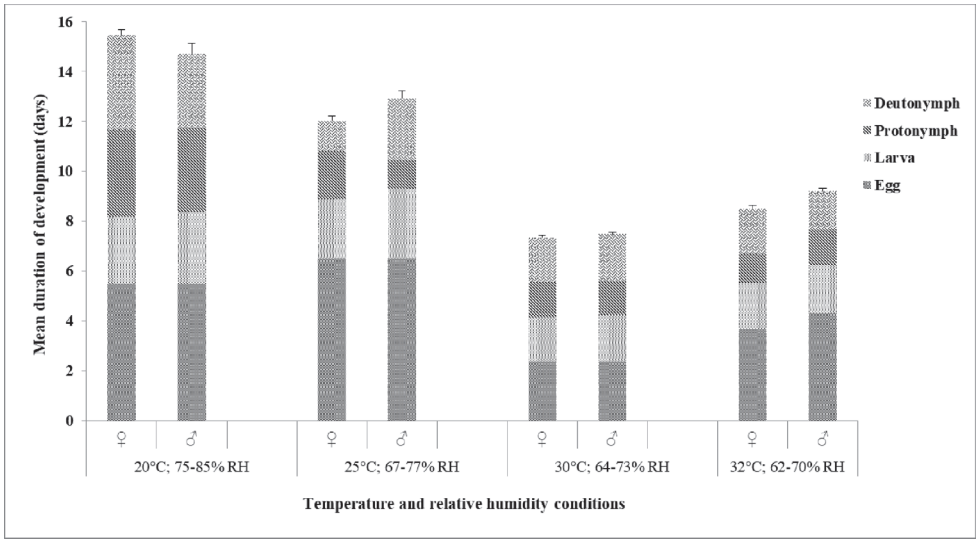

Fig. 1. Development of *Oligonychus mangiferus* on *Ichnocarpus frutescens* at different constant temperature and humidity conditions

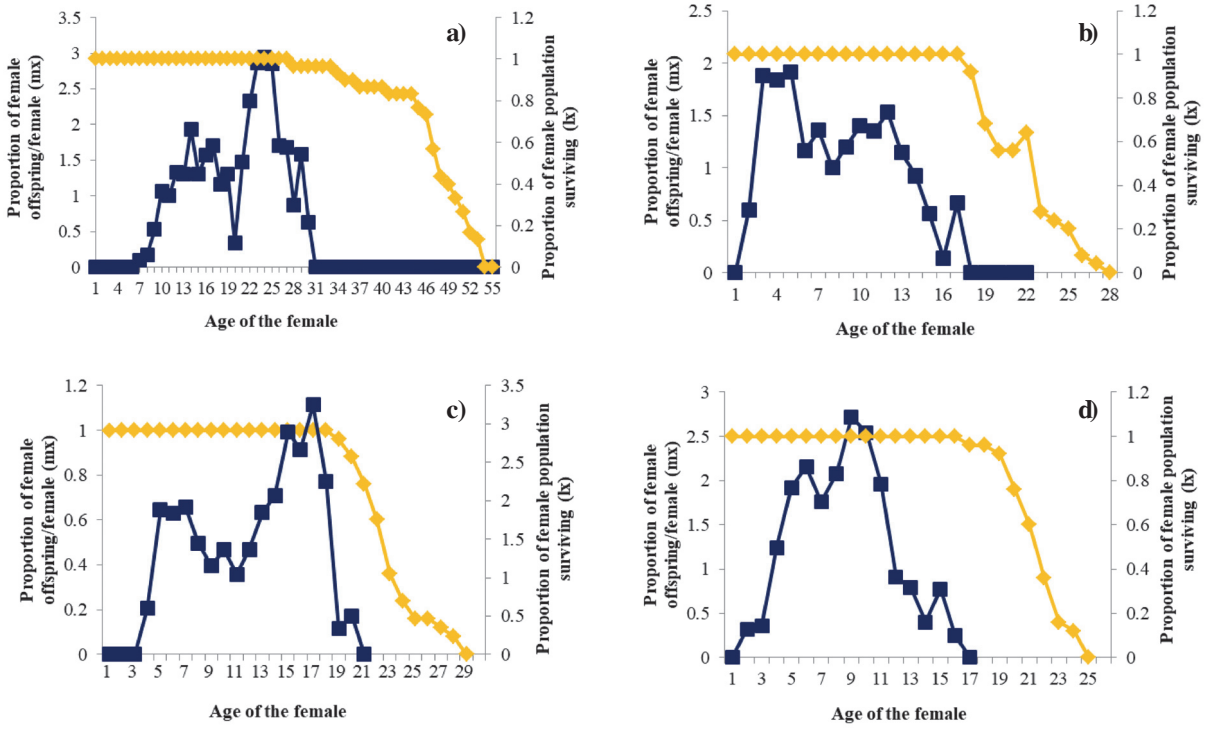

Fig. 2. Age specific survival and fecundity of *Oligonychus mangiferus* on *Ichnocarpus frutescens* at different constant temperature and humidity conditions:

a) 20° C; 75-85% RH, b) 25° C; 67-77% RH, c) 30° C; 64-73% RH and d) 32° C; 62-70% RH

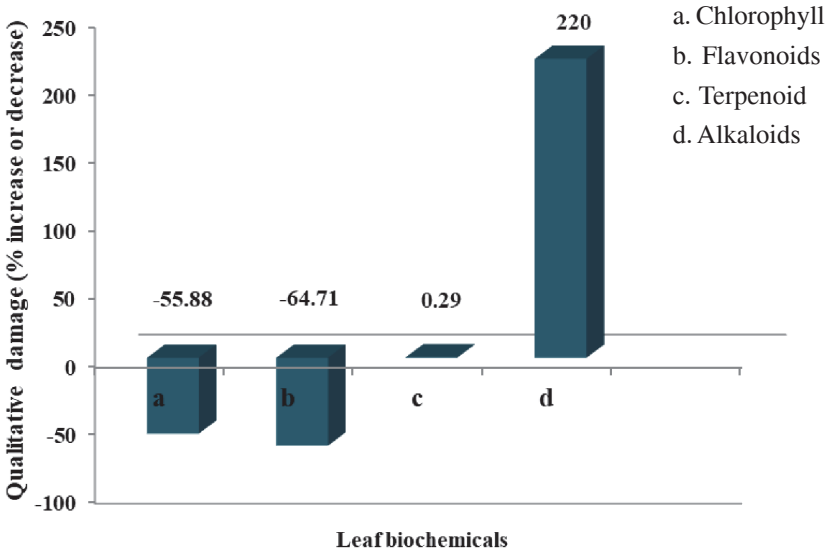

Fig. 3. Biochemical profile of *Ichnocarpus frutescens versus* infestation of *Oligonychus mangiferus*

Table 1. Reproduction of *Oligonychus mangiferus* on *Ichnocarpus frutescens* at different temperature and humidity conditions

Temp. & RH	Pre-oviposition (days)	Oviposition (days)	Post-oviposition (days)	Female longevity (days)	Fecundity (no. of eggs/ female)	Progenial sex ratio (♂:♀)
1 (n=25)	2.90±0.13	25.13±1.50^{b}	4.67±0.33	32.70±1.41^{b}	31.03±1.83^{c}	1:2.71^{a}
2 (n=23)	2.76±0.12	11.52±0.58^{a}	2.92±0.13	17.20±0.54^{a}	21.04±1.15^{a}	1:2.84^{a}
3 (n=25)	2.00±0.13	10.28±0.34^{a}	2.28±0.09	14.56±0.30^{a}	24.16±0.84^{b}	1:2.71^{a}
4 (n=30)	1.61±0.10	10.96±0.40^{a}	1.87±0.14	14.43±0.41^{a}	26.52±0.97^{c}	1:2.68^{a}

n: number of mites observed
1. 20°C; 75-85% 2. 25°C; 67-77% 3. 30°C; 64-73% 4. 32°C; 62-70%
Mean values with same alphabetical superscript within the column are not significantly different as per Tukey's HSD test (p<0.05)

Murungi *et al.* (2010) attributed great variation in development and reproduction of spider mites to the quality of their host plants. Thus, it is inferred that *I. frutescens* would be more preferred by *O. mangiferus* particularly at higher temperatures of 30º to 32ºC, as the mite could complete its development much faster *i.e.,* 7.10 to 8.77 days. Bayu *et al.* (2017) opined that higher rearing temperatures accelerated the biochemical processes of growth and development of two–spotted spider mite, *Tetranychus urticae* Koch.

Our demographic study of *O. mangiferus* is compared with that of Badawi *et al.* (2011) on mango leaves, who recorded the Intrinsic Rate of Natural Increase of the mite as 0.125/female/day at 32ºC, whereas it was 0.083/female/day on a different host *Ichnocarpus* in the present study. The fecundity of 26.52 eggs/female recorded at 32ºC is much lower than that, 46.43 eggs/female recorded by Badawi *et al.* (2011) at the same temperature and this was evidenced from the better reproduction performance of *O. mangiferus* on its original host plant mango. At 25°C rearing temperature, the mite had both Mean Generation Time and Doubling Time as shortest, while its Intrinsic Rate of Natural Increase being highest at this temperature (Table 2).

Table 2. Demography of *Oligonychus mangiferus* on *Ichnocarpus frutescens* at different constant temperature and humidity conditions

Temp. &RH	Mean Generation Time (days)	Doubling Time (days)	Net Repro. Rate (No. of female offsprings/female/ generation)	Gross Repro. Rate (No. of offsprings/ female /generation)	Finite Rate of Increase (No.of female off-springs /female/day)	Intrinsic Rate of Natural Increase (No. of female off-springs/ female/day)
1 (*n=30*)	29.50±0.16[d]	14.49±0.09[d]	26.57±0.11[d]	28.00±0.09[c]	1.051[a]	0.049[a]
2 (*n=25*)	15.26±0.12[a]	8.95±0.09[a]	16.29±0.09[a]	18.61±0.09[a]	1.089[d]	0.085[d]
3 (*n=25*)	19.20±0.12[c]	10.82±0.08[c]	17.74±0.08[b]	28.73±0.11[d]	1.070[b]	0.068[b]
4 (*n=23*)	16.76±0.15[b]	9.38±0.11[b]	18.84±0.11[c]	20.17±0.01[b]	1.0875[c]	0.083[c]

n: number of mites observed

1. 20°C; 75-85% 2. 25°C; 67-77% 3. 30°C; 64-73% 4. 32°C; 62-70%

Mean values with same alphabetical superscript within the column are not significantly different as per Tukey's HSD test (p<0.05)

Apart from the associated host plant, adaptability of the mite to prevalent local climatic conditions would greatly influence the growth, development and multiplication performance of the mite. Even on the same host, rearing of mites at different temperature has varying influence on development and reproduction potential of the mite, which again confirmed by the present study.

Feeding damage by *O. mangiferus* resulted in apparent decline in total chlorophyll and total flavonoid contents. Primary metabolites like chlorophyll have direct influence on plant's photosynthetic efficiency, which is responsible for overall growth and ultimately the medicinal qualities of the plant. In spite of being a secondary metabolite, quantity of flavonoids showed a decline in mite infested plants which should have rather increased. This may be answered by further investigation on the action and role of such biochemicals in the plant system. But alkaloid and terpenoid contents showed increase in mite infested leaves owing to their defensive role. However, observed changes in the quantity of secondary metabolites like alkaloids, flavonoids and terpenoids, subsequent to mite feeding damage may be significant, in view of their definite role in the medicinal value of the herb, therapeutic use and application. The study focused on the importance of feeding damage of mite as a sucking pest on the medicinal quality characteristics of the plant. How these biochemical changes be exploited to the best advantage of medicinal use of *Ichnocarpus,* needs further investigation.

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