



RESEARCH PAPER

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Morphometric study of *Tetranychus urticae* (Acariformes: Tetranychidae) in Ziarat district, Baluchistan, Pakistan

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Abstract

Tetranychus urticae is one of the most important economic pest feeds on a wide range of plants. Our research concerns with identification and morphometric studies of collected Tetranychid female mites. Collection was made at the interval of two distinct peak occurrences, one in June and the other in the mid-autumn from apple orchards (*Malus domestica*). Mites were isolated by adopting Sieve and Berlese funnel methods from infested leaves. Morphometric measurements were examined under camera fitted Leica microscope (LM) and electron micrographs were taken by Scanning electron microscope (SEM). Black spots on dorsal side of the body are the main particular and distinguishable identification feature for comparison with other mite species of family Tetranychidae. Wavy cuticle presence in the genital region was the prominent feature that conformed that all collected specimens were females. It was concluded that measurements taken by SEM found more understandable and accurate to study morphology of female mites than LM.

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Introduction

Mites are among the most diverse and successful of all the invertebrate groups. Some mites are considered as vector source for transmission of viruses such as Eriophyid mites carried wheat streak mosaic virus (Hong *et al.*, 1999) and Tetranychid mites carried potato virus (Jeppson *et al.*, 1975) respectively. The phytophagous mites belong to the family Tetranychidae which feed upon on crops, vegetables, fruits, stored grains and food products. Paired needle like structure called stylets is used to rupture the leaf cells having depth reached approximately is 70-120µm (Avery and Briggs, 1968; Naher and Haque, 2007).

Two spotted spider mites commonly known as *T. urticae* is the most prominent member of family Tetranychidae, subfamily Tetranychidae, order Acarina and genus Tetranychus. It is extensively acknowledged as a serious pest of economic importance in agriculture and distributed worldwide (Pritchard and Baker 1962; Zhang, 2003). Forghani and Honarparvar (2012) explained that *T. urticae* is not only harmful to fruits trees and ornamental flowers but their severe damage can be seen on cotton crops at great extent up to 40% infestation of crops was recorded in Iran. It was observed by Sances *et al.*, (1982) that *T. urticae* population density can reduce; the significant photosynthesis, productivity, transpiration and vegetative growth. Its feeding habit can cause injury to the mesophyll cells that contained chlorophyll and cause the continuous stomata closure (Farouk *et al.*, 2014).

Sarwar, (2014) observed that *T. cinnabarinus* and *T. urticae* has the same common morphological features except the black spots present on their dorsal side of the body. Mixed population of some species like *T. urticae* and *T. turkestanii* are found on watermelon, overlapping of habitats helps in the interaction especially during reproduction, such habit greatly affect the fitness (Benadavid, 2013). *Tetranychus urticae* can be distinguished from other family members in body colour appearance as it appears in red and green colours (Ehara, 1999; Zhang, 2003).

So far limited research has conducted to explore mite fauna from Ziarat district of Baluchistan and most of the mite species in this region are still remain uncertain for acarologist.

This study is therefore aimed to identifying and providing detailed morphometric study on *T. urticae*. The criteria examined for measurements include morphological features like the colour of the mite, number of legs, metapodal and sternal plates, hypostome, chelicerae, gnathosoma, idiosoma and genitalia.

Materials and methods

Collection of mite's specimens

Presence of mites was confirmed on undersides of infested leaves using double convex lens. Sieve and Berlese funnel methods were adopted for isolation of mite's specimens from infested leaves of apple orchards (Dietick, 1959; Whitehead and Hemming, 1965). Henderson and Mc Burnie (1943) method was also adopted, where infested leaves were passed by a mite-brushing machine having circular glass plate; coated with thin layer of glycerol to catch mites. Plates were placed on same sized circles of paper divided into 16 sectors with alternating black and white arcs to facilitate counting sub samples under a binocular microscope.

Slide mounting

Slides were prepared for isolated specimens of the mites from leaves applying different concentration of methyl alcohol. Specimens were dehydrated by adding different concentration of alcohol; 30%, 50%, 75% and 100% with an interval of 20 to 25 minutes. Cleaning of the specimens was performed by placing samples in the clove oil for about 24 hours. Permanent slides were prepared by transferring the specimen on clear slide and slides were fixed with Canada balsam. Mounted slides were placed in drying oven at 40°C for about two days for final fixation, while un-mounted specimens were stored in 70 % methyl alcohol for further study.

Morph metric studies

Mounted specimens of *T. urticae* were identified with the help of taxonomic keys developed by Rowell *et al.*, (1978); Lindquist (1985) and Daly *et al.*, (1998). Identification key of Pritchard and Baker (1955) was also used to identify dorsal striation and setal patterns of adult female. Collected specimens were also examined under Compound microscope (Olympus-CH10 Japan) and photographs were taken at 40X Magnification through camera fitted Leica microscope (DM, 4000-B) and 100µm-300µm in Scanning Electron Microscope (Hitachi S3400-N, type-II) available at CASVAB, University of Baluchistan, Quetta. Specimens samples on dehydration were mounted on SEM stubs provided with double sided carbon tapes. Micrographs of entire body were taken between the magnifications of 100µm to 300µm. In addition, oil immersion was used to visualize the morphological characters like limbs, mouth parts, tenant hairs on claws, striation pattern, shape of female genitalia, setae and other minute areas. The length of idiosoma was calculated between the anterior most border line of propodosoma and furthest back edge of ophiosoma.

Results and discussion

Temperature variations

In this research, observation was made on optimum temperature for their development was recorded around 30-32°C and this temperature variation was found almost quite suitable temperature for their rapid growth in the Ziarat district. High frequency distribution of red coloured *T. urticae* was conformed during mid-autumn than during spring season (Fig. 1). Jeppson *et al.*, (1975) and Zhang (2003) made their observation that low moisture and dry weather facilitate their movement and rapid growth.

Seasonal colour variation

Most of the specimens of *T. urticae* during autumn season appeared as strong red colour with two dark spots, although they show greenish brown coloured appearance during summer season. These spots can be seen clearly on the dorsal side of the body. It possesses several colours such as yellow,

green and brownish, but our collected female mites were observed only in red colour having black form of spots indicates vigorous feeding. This coloration is developed due to internal pigments and feeding habit because cuticle is not coloured (Geest, 1985).

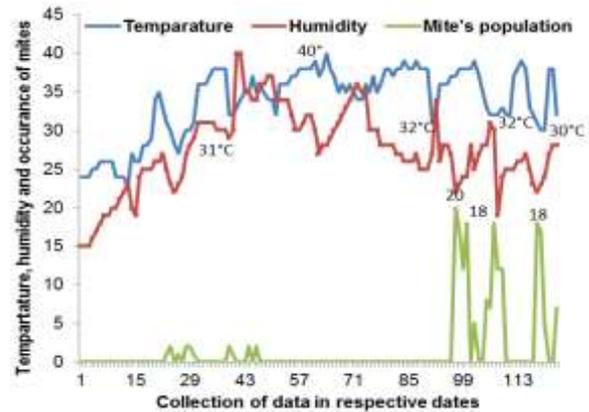


Fig. 1. Data indicates the peak occurrence of mites on average temperature and humidity with an interval of 2 weeks taken for consecutive five months (June till September, 2016).

It was also noticed by that collected female were red in colour with broad body and having four pair of legs on propodosomal region. *Tetranychus urticae* of red colour is formerly known as the carmine spider mite or *T. cinnabarinus* (Auger *et al.*, 2013). It was also noticed that red coloured *T. urticae* tends to live in groups and form complicated silky webs (Fig. 2) on undersurface leaves of the apple (Yoshioka and Yano, 2014).

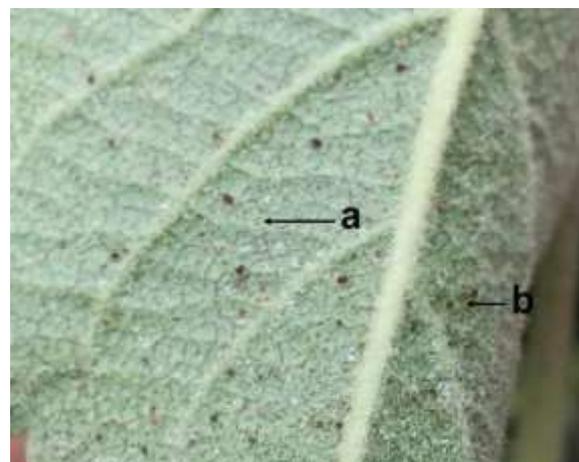


Fig. 2. Indicate a. *Tetranychus urticae* colonies b. web formation (arrows) on undersurface leaves of the apple.

Morphological description with LM

LM and SEM were used to observe important morphological characterization such as legs, metapodal and sterna plate, hypostome, chelicerae, gnathosoma and idiosoma (Fig.3). The measurement taken from SEM was cross checked with the handmade diagram from LM (Fig. 4). Our results revealed that palp was noticed strong; having well developed thumb claw complex and sejugal furrow was not observed under LM.



Fig. 3. *Tetranychus urticae* with dorsal view; a1. First leg, b2. Second leg, a3. Third legs, a4. fourth leg, ap. anal plate, mp. metapodal plate, sp. sterna plate, h1. hypostome, h2. chelicerae, g. gnathosoma (capitulum), i. idiosoma.

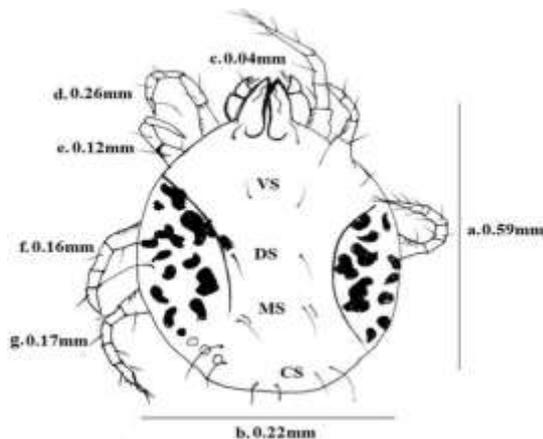


Fig. 4. Drawing of female *Tetranychus urticae*; VS= Vertical setae, DS= Dorsal setae, MD= Median setae, CS= Clunal setae, a. body length, b. body width, c. Chelicera, d. first leg, e. second leg, f. third leg, g. fourth leg.

Body of the male mite was examined ovoid or sack like, having empodium with 3 proximoventral hairs (Collier, 1998). The average length calculated for female was recorded 0.59mm in length and 0.22mm in width respectively. Patel *et al.*, (2016) finding was similar to those of our measurements such as the average length and width of female were measured 0.44-0.50mm and 0.18-0.23mm, respectively. The gnathosoma was measured 0.1mm in length; while chelicera was measured 0.4 mm long appeared as needle like structure.

The idiosoma was located at the end of gnathosoma having striations with not more than 16 pairs of dorsal setae and total 10 pairs of dorsal setae were seen during this course of research (Kuang and Cheng, 1990). The idiosoma consists of further vertical, dorsal, median, and clunal prominently observed setae.

The tarsus contained proximal hairs at the anterior margin and clunal setae were located at posterior anal region. It was noticed during this morphological study, legs of the mites were quite long, covered with straight setae. Mounted specimens examination showed four pairs of legs in adult female having the length of first leg 0.26mm, second 0.12mm, third 0.16mm, and four the 0.17mm in measurements respectively.

LM image revealed that the presence of large dark coloured spots were clearly visible on the dorsal side of the body. These black coloured spots are aggregation of wastes of the body such kind of spots cannot be seen in newly molted mites.

Electron micrograph study

The body of female mite examined under SEM, showed that body was ovoid or sack in shaped; measured 219µm in length and 152µm in width with maximum and bristle length was recorded up to 73µm. The SEM micrograph provides similar observation with LM determined for gnathosoma was 59.0µm long and 41.2µm in width.

Legs were observed long and wrapped with setae. The lengths of legs were measured as first leg 206.2 μ m, second 115.9 μ m, third 143.4 μ m, and fourth 225.7 μ m respectively. The maximum length of bristle was calculated 73 μ m. It was observed that idiosoma consist of approximate 16 pairs of dorsal setae (Fig.5). This valuation of measurements for *T. urticae* was found In accordance with Patil *et al.*, (2014). In the mite species studied, wavy cuticle was clearly visible around the genital region with its two setae (Fig. 6) conformed that all collected specimens were female.

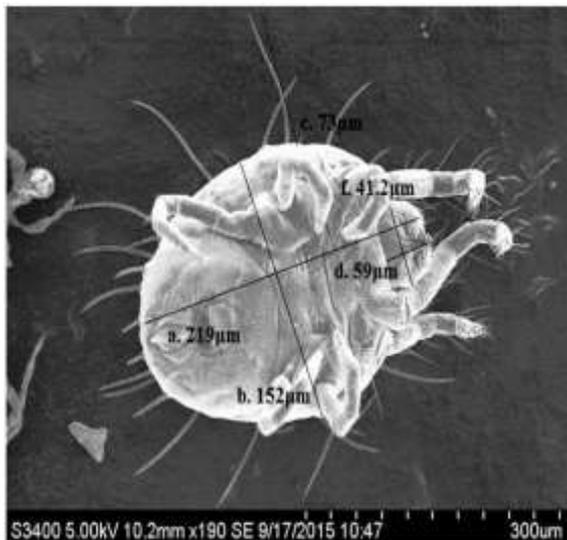


Fig. 5. Electron micrograph taken from SEM; a. showing body length, b. width, c. bristle d. gnathosoma length, and e. gnathosoma width of adult female of *T. urticae*.

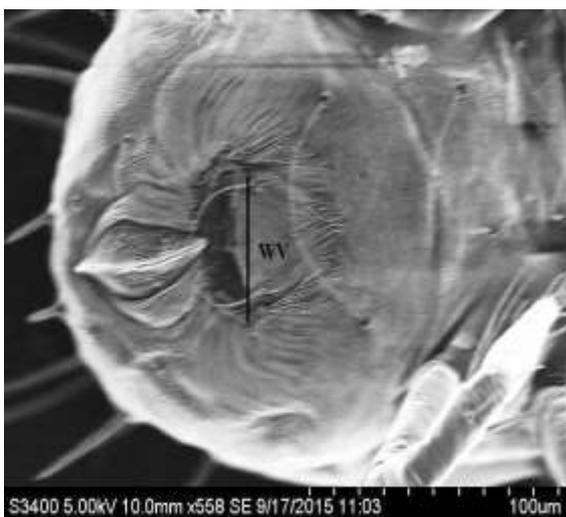


Fig.6. Female genital opening clearly marked with prominent wavy cuticle (wv).

Overall, comparatively SEM measurement was found more accurate and can provide detail morphological study than LM. Salient features for identification of this spider mite were based on shape, length and position of the; setae, gnathosoma, chelicera, hypostome and idiosoma. Presence of black coloured spots on the dorsal side of the body is the main diagnostic feature for comparison with other family member of Tetranychidae.

Conclusion

Tetranychus urticae is an economically important pest having cosmopolitan position and feeds on a wide range of plants. The overall finding of this study indicates that SEM provides better and accurate measurement than LM for this species.

It was unfortunate only female mites were collected as arrhenotoky; a parthenogenesis process is more common in this specie. It is expected that examination of more characters and other specific differences will be helpful for identification and comparison with newly identified species.

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