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Morphological characterization of *Varroa* mite of honey bee *Apis mellifera* using light and scanning electron microscopy collected from Sambhal (U.P.)

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Abstract
Pollinators play a crucial role in maintaining existence of life on the earth. These insects pollinate a wide variety of plants and crops. Recent reports suggest that if honey bees get disappeared from earth, there would be no life within 4 years. Products like honey, bees wax, propolis and pollens serve as a natural food supplement for humans. Bee keeping industries have provided employment to millions of people across the globe. But now-a-days, there is a continuous deflation in honey bee population. This could be possible due to various factors like-human practice, deforestation, land change, lack of flowers and parasitic invasion. One of the important factors is *Varroa destructor* (an ectoparasite of honey bees). *Varroa* (mites) feed on the haemolymph of honey bees and weaken the bees with in no time. The broods are majorly infected with the disease Varroosis and needs to be explored for treatment at an alarming rate. The present work is conducted to characterise *Varroa* mites morphologically using light and Scanning electron microscopy to examine the morphological characters involved in causing Varroosis.

Keywords: pollinators, *varroa destructor,* haemolymph, scanning electron microscopy, light microscopy, varroosis, employment

Introduction
Honey bee is an important insect for pollination and ecological functions, as well as for the production of colony products such as wax, royal jelly, bee venom, honey, pollen, and propolis (Klein *et al*., 2007) [10]. Honey bees are the most significant plant pollinators, and they pollinate about a large number of agricultural food sources which constitutes about 1/3 of the globe's diet relying on pollination by bees, which is worth billions of dollars (T.O nail *et al.,* 2018). The maintenance of their health most important because they contribute in food production and in ecosystem a lot (Ullah *et al*., 2020). *Varroa* mites (*Varroa destructor* and *Varroa jacobsoni*) are little red-brown honey bee parasites. *Varroa* mites can feed and live on adult honey bees, but they prefer to feed and multiply on larvae and pupae in the growing brood, causing honey bee malformation and weakness as well as transmitting a variety of diseases (Ratnieks *et al*., 2010). From long history of apiculture there are no other parasite which ever impact on honey bee and beekeeping industry (Webster *et al.,* 2001) [20]. The honey bee *A. mellifera* has a novel parasite, *V. destructor* as a result, there isn't a healthy host–parasite interaction, and beekeepers haven't dealt with this issue for very long and thus *Varroa destructor* has expanded practically worldwide in a short period of time (De la Rua *et al*., 2009) [7]. Most honey bee colonies in cooler climates would die out in 2–3 years if they weren't treated on a regular basis (De la Rua *et al.,* 2009) [7]. The *Varroa* mite is thought to be a big contributor to decline beekeeping industries and have negative impact on global reduction of natural pollinators, the *Varroa* mite can arise future pollination issues (Rosenkranz *et al.,* 2010). The western honey bee (*Apis mellifera*) is the most common single species of pollinators for crops globally, provides pollination services to crops and flowers. History of cultivation and its relation with honey bees have been exploited; *A. mellifera* now has a worldwide range that covers all continents except Antarctica and many oceanic islands. Given the current degree of information about this species and its role in agriculture, it's remarkable that its usefulness as a pollinator in natural habitats is known and exploited for greater good (Hung.*et al.,* 2018) [9]. Bees are the most efficient and the only dependable pollinators, because they visit flowers methodically to collect nectar and pollen and do not destroy the flower or the plant in the process (Devillers *et al.,* 2002). The relationship between different species in which one organism lives on or in the other organism and benefits from it by causing some harm is called parasitism (Pauline 2007). The parasite is derived from of the Greek word, meaning “one who eats at the table of another’’. The parasite that lives outside the body or on the host body is called ectoparasite, the main cosmopolitan ectoparasite of honey bee is *Varroa destructor* (*Varroa* mite) which is an external parasitic mite that attacks and feeds on the honey bees *Apis cerana* and *Apis mellifera* and causes

varroosis (Allen *et al.,* 1986) [1]. Recent observation depicts that ecto-parasite of honey bees are playing role in the destruction of bee hives, and it is also observed the mite create a change in their strain diversity (Martin *et al.,* 2012). If the wild population of honey bee is not treated the hive may be get unprotected (webster *et al.,* 2001) [20]. This ectoparasite of honey bee mainly feed on the haemolymph of bee (Bailey 1991). *Varroa* mite act as a vector for many diseases (Chen *et al.,* 2007) [5].

Table 1: Showing classification of *Varroa destructor*

Classification of *Varroa destructor*-	
Kingdom	Animalia
Phylum	Arthropoda
Subphylum	Chelicerata
Class	Arachnida
Order	Mesostigmata
Family	Varroidae
Genus	*Varroa*
Species	*V.destructor*

Due to the constant decline of honey bee colonies, there is a current need to investigate about the threats, diseases and consequences caused due to the same. Keeping the above facts in mind the current study is designed to investigate more about the external morphology of ectoparasite *Varroa* of honey bees using light and Scanning electron microscopy.

Materials and Methodology

Study area

To perform the morphological characterization of honey bee, bees were captured from many sites in Asmoli, District- Sambhal apiary in the month of February, March and April under proper care and condition. Sample collected was preserved in 70% ethanol. Mites were collected and data was recorded in a tabular format accordingly.

Table 2: Showing *Varroa* mite collection in month of February 2022

Mite collection from different apiary in month of February-					
DATE	**SITE -1**	**SITE- 2**	**SITE -3**	**SITE-4**	**Total**
14/2/22	5	0	0	0	5
15/2/22	1	2	2	0	5
16/2/22	4	0	0	4	8
17/2/22	0	2	5	0	7
18/2/22	0	1	0	0	1
19/2/22	2	0	0	5	7
20/2/22	6	0	3	0	9
21/2/22	0	3	0	0	3
22/2/22	4	2	0	0	6
23/2/22	2	0	0	0	2
Grand total- 53					

Table 3: Showing *Varroa* mite collection in month of March 2022

Mite collection from different apiary in month of March-					
Date	**Site-1**	**Site-2**	**Site-3**	**Site-4**	**Total**
15/3/22	3	1	1	0	5
16/3/22	4	0	0	0	4
18/3/22	0	0	0	5	5
19/3/22	5	3	0	0	10
20/3/22	2	0	0	0	1
Grand total - 25					

Table 4: showing *Varroa* mite collection in month of April 2022

Mite collection from different apiary in month of April-					
Date	**Site1**	**Site-2**	**Site-3**	**Site-4**	**Total**
6/4/22	0	2	0	1	3
7/4/22	0	0	0	5	5
8/4/22	0	4	1	0	5
9/4/22	5	1	3	0	9
10/4/22	7	3	1	0	11
11/4/22	6	2	0	1	9
Grand total -42					

Light microscopy

For light microscopy, samples were cleaned with distilled water then boiled in 15% KOH for 15 minutes, after washing the KOH treated sample were dehydrated with different type of grades of alcohol (30%, 50%, 70%, 90%, 95% and 100%) and left for one minute in xylene. Later sample were mounted in DPX.

Scanning electron microscopy

For scanning electron microscopy, the sample were first cleaned with PBS (1%) solution. Primary fixation was done by using 2.5% glutaraldehyde for overnight. After primary fixation the sample were washed three time in 1% PBS for 15 minutes each. For post fixation sample were put in 1% osmium tetroxide for overnight. Washing process were done for 7 time in 1% PBS for 15 minutes after washing sample were dehydrated with different type of grades of acetone (30%,50%,70%,90%,95% and 100%). After dehydration sample were mounted on stub and coated with platinum and image were obtained.

Results

Following are the images captured during the study.

Light microscopy revealed about the external morphology of the mite. Proper KOH boiling enabled the sample to become transparent that helped to visualize the clear morphology of the mite.

Light microscopy

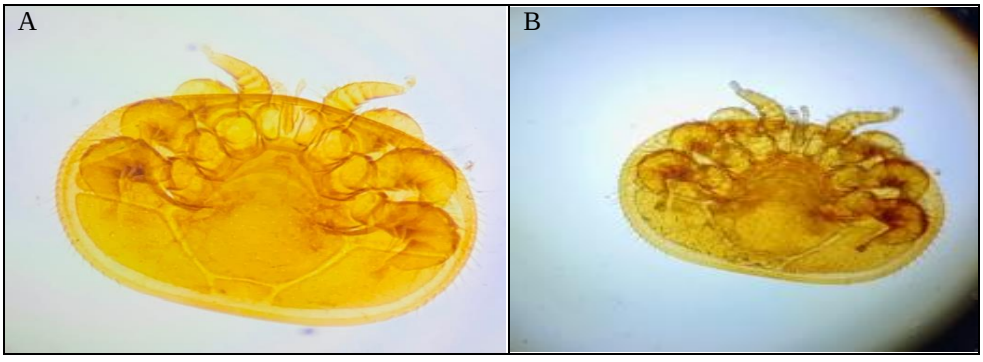

Fig 1: (A) Showing light microscopic image of the dorsal side of *Varroa* mite, (B) Showing a ventral side of *Varroa* mite.

Scanning Electron Microscopy

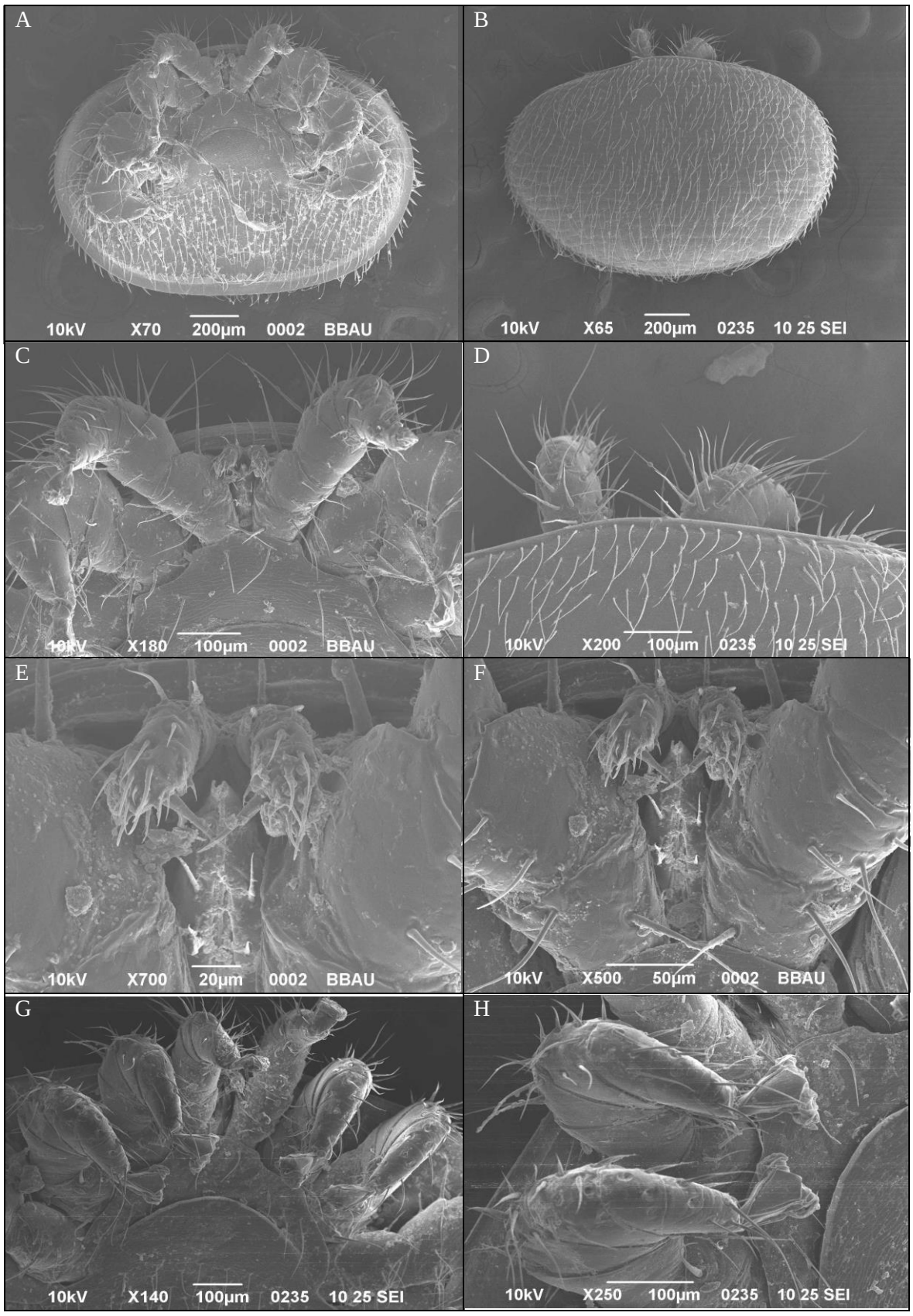

Fig 2: (A) Magnified image of the ventral side of the *Varroa* mite (B) magnified image of dorsal side of *Varroa* mite -dome shaped body having sparsely covered by setae (C) magnified image of mouth with two pair of leg in ventral side of *Varroa* mite, (D) magnified image of first pair of leg with different setae in the dorsal side, (E) highly magnified image of only mouth part hypostome *Varroa* mite. (F) magnified image of Cheliceri and apotele claws in mouthpart, (G) magnification of the whole set of legs of *Varroa* mite with their setae, (H) magnified image of empodium at anterior part of leg under Scanning electron microscopy.

The scanning electron microscopy revealed about the external properties of the mite. Mature *Varroa* mites are oval in structure with flattened dorsoventrally. The dorsal side is convex dome-shaped appearance and its whole body is covered by varying lengths of setae.

Sample collection was done from the apiary of Asmoli (Sambhal) locality. Four different apiaries were located in the middle of a vast farmland. Sample collection was done in month of February, March and April 2022 during which temperatures was ranging from moderately cold to warm. Infected and healthy honey bees were present in same apiary box and there was no clear-cut separation for infected and healthy honey bees. The farm land was specifically of mustard plant and honey bees were seen pollinating the flower.

There were a few mango trees under which some apiary boxes were placed and honey bees present there showed aggressive, virulent behaviour. It could be possible that aggression existed was because of the high prevalence of ectoparasitic mites present on honey bees. They could be altering the behaviour of honey bee most of the mites were seen in the mesothoracic area of honey bees. The other three apiaries were exposed to sun light; also showed lesser prevalence of mites and the mites collected showed lesser virulence.

Honey bees which were infected with the ectoparasite showed some specific behaviour like slow flying, aggressive behaviour and lesser coordination among each other. During the brood examination, it was seen that the brood had minute pores in it. SEM study showed series of plates forming ventral surface. Respiratory structures are seen laterally as coxa III. The parts of the mouth of ectoparasite *Varroa* were seen modified that can easily attach to bee. Males are seen to be reddish brown colours in variety of shades. Body is very small i.e., smaller than 2mm and width is also about in the same dimensions. *Varroa* mites do not have eyes and they possess 8 legs (Fig-2.A).

It was observed that *Varroa* mite possess setae. These setae are of different type will specific function (Fig- 2 B). In the detailed view, it was seen that pedipalp were segmented and the segmented number was 5; also, along with this pedipalp tarsi showed to have long claws as well as apotele claws which was found to be shorter.

Legs setae was less rough less complex and spiny form, also the legs have shown different length (Fig-2 G). The position of hypostome was found to be in between the first pair of legs which was accurately confirmed by SEM (Fig-2 E). Ambulacral sucker is found on tarsi leg 1-5, which is a structure that helps in walking and also attachment as per the need. Basal tritosternal showed teeth. At the distal end of pedipalps apotele claws are seen (Fig-2 F).

Through research techniques and using molecular characterization, new researchers can try to find out about the unusual behaviour of bees. *Varroa* has the capacity to twist its legs segments and that too about 180º during locomotion. Adult tarsal claws and setae which are present on the legs play role in attachment during the dispersal. Body and leg setae of adults appear to be used as a tactile tool to sense amount of space within tracheal tubes.

Discussion

Mites cause a severe threat to survival and majorly affect honey bees' populations. The haemolymph sucking mechanism exhibited by *Varroa* mites makes worker bees weak due to which they fail to gather pollen and nectar. And at last, the colony starts to suffer. All the bees show a slow movement and incapability in flight. The mechanism could be a serious concern for bees. In India, bee keeping is providing employment to millions of people but if bees are not healthy then the nation would face economic losses and as well as unemployment. Many of the researchers have found about the specificity related to *Varroa* through the studies done with the help of SEM, like Ammar *et al*., (2015) found that adult female body is flattened with dome shaped having 5 rows of small chemoreceptor papillae, unique respiratory structure and setae distributed on the body. Ceciro *at al.,* (2010) talked about the role of *Varroa* as vector which may transmit diseases with a notion that disease transmission may involve the salivary glands. Virus along with some of the endosymbionts were seen in the glands. Liu and Tang, (2012) performed SEM and phase contrast microscopy to examine the structure of sensory hairs found on palptarsus of female *Varroa*. Ruttner and Hanel, (1992) worked on the defence system present in Carniolan strain of honey bee against *Varroa*. They observed about 700 hives to study the decline in population of *Varroa* mortality. They found that destruction to *Varroa* mite followed by the death was being done by the mandibles of workers honey bees which indicated that mandibles act like scissors to protect them. Thapa *et al.,* (2015) performed SEM to observe Korean haplotype of *Varroa* which showed that K-type *Varroa* is more oval and possess three kinds of setae that is spiny, serrated and smooth in which margin was flexible. The peritreme is smaller than other *Varroa* species. The K-type *Varroa desrtuctor* showed the absence of sternal pores and presence of more metapodal setae. Bruce *et al.,* (2009) talked about the morphology of peritremes of *Varroa* and *Euvarroa*. In *Varroa* movable peritreme lies flat. sclerotized peritremal groove has ascending and descending arm which forms a hook at tip of the peritremes. Open stigmata are present but they were found not to be continuous with peritremetic groove as in *Euvarroa.*

Therefore, all the researches performed earlier suggested that by performing SEM, it is possible to get detailed structure and information related to *Varroa destructor*. Though further investigations may be conducted for future developments and deciphering of the structure and anatomy whenever required.

Conclusion

Present study was conducted to examine the morphological characters of honey bee ectoparasite *Varroa* mite. While performing the work few steps of protocols were modified, like fixation in glutaraldehyde and osmium

tetroxide was allowed to done overnight rather than few hours; washing was done 7 times (prescribed three times). Earlier it was quite difficult to obtain clear image because dehydration lining was present in the image. To remove this problem, acetone treatment was done for 40 minute (not for 30 minutes), by which dehydration lining disappeared while taking images. Also, primary fixation in glutaraldehyde helped in proper fixation of proteins of the mites which enabled to have very clear mouth parts (Figure 2). Osmium works as a stain of heavy metals and is also used as a fixative in SEM. Osmium tetroxide is good as fixative and stains lipids which are constituents of plasma membranes or other membrane bound structures. With this it can be said that, with modification at different steps clearer image can be obtained and observed properly. This paves the way in future for protocols to be revised from time to time according to the sample and the environment. Also, research concludes that dehydration step was found to be at most importance.

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