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Research Article

SEM study on morphological changes in *Metarhizium anisopliae* infected *Aphis craccivora* Koch.

SARODEE BORUAH, PRANAB DUTTA*, JOYARANI PEGU, HIMADRI KAUSHIK, N. GOGOI[1], K. C. PUZARI and G. N. HAZARIKA

Department of Plant Pathology, Assam Agricultural University, Jorhat-785013, India.
[1]Department of Environmental Science, Tezpur University, Assam, India.
**Corresponding author Email:* pranabdutta74@gmail.com

ABSTRACT: Morphological changes in *Metarhizuim anisopliae* (Metschnikoff) Sorokin infected cow pea aphid, *Aphis craccivora* Koch were studied by scanning electron microscope (SEM). Aphids were caged on paired lantern chimney over cowpea twigs dipped in conical flasks with water infected and inoculated with fungal propagules of *M. anisopliae* at the concentration of $1X10^6$ spores /ml of water. Infected aphids were observed under SEM and it showed severe cuticular damage, abnormalities in sensory systems as well as deformation of all the body parts. The hydrophobic conidia of *M. anisopliae* were found to attach to all body regions. It was evident that mycelial growth and conidiophores with conidia of *M. anisopliae* covered the body surface and penetrates inside the body of infested aphid causing damage to the pest by disturbing its major physiological activities leading to its death.

KEY WORDS: *Aphis craccivora, Metarhizuim anisopliae*, scanning electron microscope

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INTRODUCTION

Cow pea aphid, *Aphis craccivora* (Koch) is a major pest of legumes in India. Both nymphs and adults suck plant sap and cause serious damage right from the seedling to pod bearing stage. Due to heavy infestation, young seedlings die, whereas the older plants show symptoms such as stunting, crinkling and curling of leaves, delayed flowering, shrivelling of pods and finally reduction in yield. Damage by cow pea aphid is as high as 80-100 per cent if not controlled effectively. Cowpea aphids are also known to transmit a number of plant viruses. Considering the adverse effect of insecticides, pest management through biological control is now-a-days encouraged using predators, parasites and pathogens. Entomopathogens are emerging as potential biocontrol agents among which *Metarhizium anisopliae* (Metschnikoff) Sorokin is one of the highly promising fungal bio-agent. *Metarhizium* species, also known as green muscardine fungi, have long been recognized for their biological control potential against arthropods. They infect more than 200 species of insects in over 50 families including termites (Moore *et al*., 1996) and are currently being used as a biological insecticide to control a number of pests such as termites, thrips, aphids etc. Foster (1975) has recorded naturally infecting aphid populations of lettuce root aphid, *Pemphigus bursarius* (trehernei) (L.) by *Metarhizium* sp. in Norfolk, UK. Reports on post infection morphological changes of insects with entomopathogenic fungi are very few.

Scanning electron microscope (SEM) is used to observe the mode of action of entomopathogenic fungi and to see how they are able to colonize and infect the host (Neves and Alves, 2004; Pedrini *et al*., 2007; St. Leger *et al*., 1991). Moino *et al*. (2002) studied the external development of *Beauvaria bassiana* and *M. anisopliae* on the subterranean termite *Heterotermes tenuis* (Hagen) using SEM, determining the duration of the different phases of fungal infection. With this background the present study was conducted to see the morphological changes of *A. craccivora* due to infection and infection process of *M. anisopliae*.

MATERIALS AND METHODS

The experiment was carried out at Mycology Research Section, Assam Agricultural University (AAU), Jorhat, Assam and in collaboration with Department of Physics, Tezpur University, Assam, India.

Fungal isolate

The isolate *M. anisopliae* (ITCC No. 8882.12) was isolated from naturally infected *A. craccivora* from Majuli island of Assam (Pegu *et al*., 2012). Mass production to obtain dry spore powder was carried out at Mycology Research Section, Assam Agricultural University (AAU), Jorhat in liquid medium. The dry spore powder was stored at 4-8°C.

Treatment of insects

Adult insects of *A. craccivora* were used which were reared at 18°C, 70 % relative humidity. Aphids were caged on paired lantern chimney over cowpea twigs dipped in conical flasks with water and inoculated with fungal propagules of *M. anisopliae* at the concentration of $1X10^6$ spores /ml of water (Puzari *et al*., 1994). Twenty ml of the suspension was sprayed over 20 insects. Spraying was done through an atomizer No 600 (Holmspray T.J. Holmes. Co. Inc. Charly. Mass.). Control twigs were sprayed with equal amount of distilled water. Aphids used were zero to two week old nymph and adult. Sprayed aphids were kept for 4-7 days to get the infection of *M. anisopliae* and development of fungal growth.

Sample cleaning, drying

Before characterization in SEM, samples were thoroughly degreased and dried to eliminate any outgasing from organic contamination and water. There after the infected aphids were cleaned ultrasonically by using alcohol then subjected to "critical drying". For critical drying, the infected insects were held in plastic vials (2 ml) containing absolute ethanol for at least a month. Samples were checked periodically to make sure the ethanol does not evaporate from the container. Infected aphids were then removed, washed gently with absolute ethanol, and allowed to dry in a covered cavity slide (to avoid dust) for at least a few days. Few samples of infected aphids were dissected into three parts (head, thorax and abdomen) with the help of a sharp blade. Dissection was done under the sterioscopic microscope (Carl Zeiss).

Coating of the sample

Whole and sections of infected aphid bodies were coated with Gold or gold-palladium to make them conductive. Coating was applied at a thickness of about 20 nanometers, which was too thin to interfere with dimensions of surface features.

Mounting and loading of sample

Sample holders of 76 mm and thin plate were used according to the sample size. Samples were attached to the thin plate using double- side carbon or copper tapes. Sample plates were seated in the corresponding sample holder securely, and fastened with setscrews, then transferred the sample holder into or out of the sample chamber by using the sample exchange tool. After loading of the sample, pressed the release button of the sample exchange tool, and then removed the holder from the hook.

Image under scanning electron microscope (SEM)

Samples were observed under SEM (JEOL, JSM-6390LV) and photographs were taken to get detailed morphological changes of the insect body at Department of Physics, Tezpur University, Assam. For each insect an assessment of the penetration of mycelia and development of conidia on three different areas of the body was made.

RESULT AND DISCUSSION

To study the morphological changes of aphid and to determine possible cause of its mortality due to infection of *M. anisopliae*, infected *A. craccivora* were observed under scanning electron microscope and photographs were taken. Moino *et al*. (2002) determined the duration of different phases of fungal infection by studying the external development of *Beuvaria bassiana* and *M. anisopliae* on the subterranean termite *Heterotermes tenuis* using scanning electron microscopy.

We found that the host's whole body was invaded by hyphae between five to six days after inoculation. Three different body areas, head, thorax and abdomen were examined after treatment with the conidial suspension of *M. anisopliae*. The SEM photographs showed that the hydrophobic conidia of *M. anisopliae* were able to attach to all body regions as was reported by Boucias *et al*. (1988). The mode of action of *M. anisopliae* begins with spore attachment, germination and penetration through insect cuticle, followed by a rapid proliferation of fungal cells within the body of the host which ultimately results in the death of the host. Similarly, Gabarty *et al*., (2014) did scanning electron microscopy (SEM) study of *B. bassiana* and *M. anisopliae* infected larvae of greasy cut worm, *Agrotis ipsilon* (Hufnagel) which showed adhesion and penetration structures of these fungi.

Growth of the fungus on the infected larvae and signs of hyphal penetration of insect cuticle as well as proliferation of the cuticle were also appearing. On the other hand, the fungus, *M. anisopliae* as declared by SEM showed a dense network together and cause the green spores on the insect cuticle. Also, SEM allowed observing the spores and hyphae of the fungus in the body cavity of infected larvae. The insect body parts with stronger cuticle, such as the head, were less susceptible to the fungus as Agullo *et al*. (2010) also reported.

From the Fig 1 (at lower magnification) it was evident that ventral cuticle and abdomen of infected aphid were totally distorted with deformation of all the body parts. SEM study revealed adhesion, growth of the fungus as well as proliferation inside the body of infected aphid (Fig 2 A-C). Schneider *et al*. (2013) also found similar results in case of pupae of *Diatraea saccharalis* F. (Lepidoptera: Crambidae) due to infection of *M. anisopliae.*

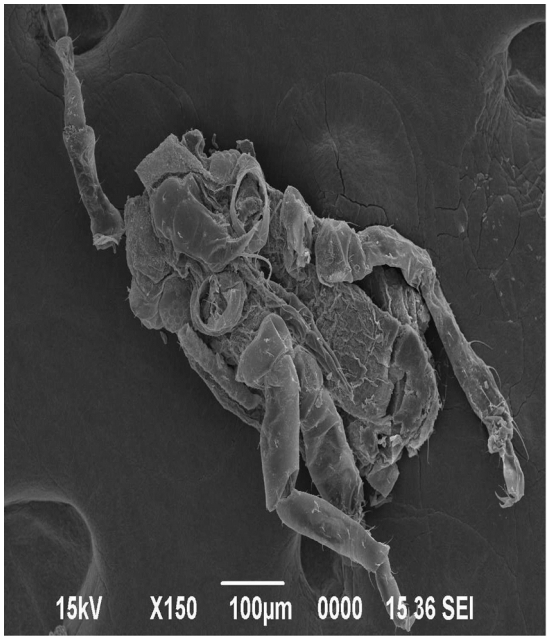
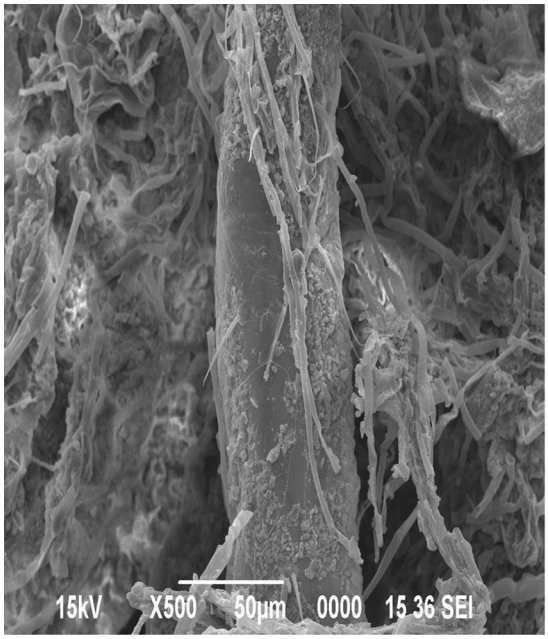
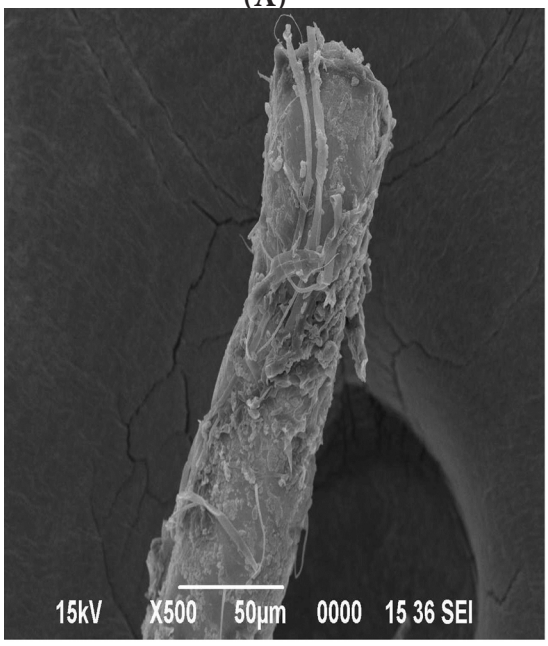


Fig. 1. ***Metarhizium anisopliae*** **infected deformed** ***Aphis craccivora*** **under SEM.**

Severe cuticular damage and abnormalities in sensory systems were observed due to the infection. The cuticle of aphid body falls under soft cuticle category. Hence, it is easily susceptible to fungal growth resulting in chemical as well as morphological abnormalities as reflected in the scanning electron micrographs. At 550X magnification, conidiophores with conidia of *M. anisopliae* were observed inside the body of infected Aphid (Plate 2 D). Highest concentration of hyphae was detected in the terminal regions of the abdomen. In the colonization events observed in this study, the formation and multiplication of hyphal bodies of *M. anisopliae* inside the host body was noted. This suggests that fungal growth can cause serious damage to the pest disturbing its major physiological activities resulting in its death.



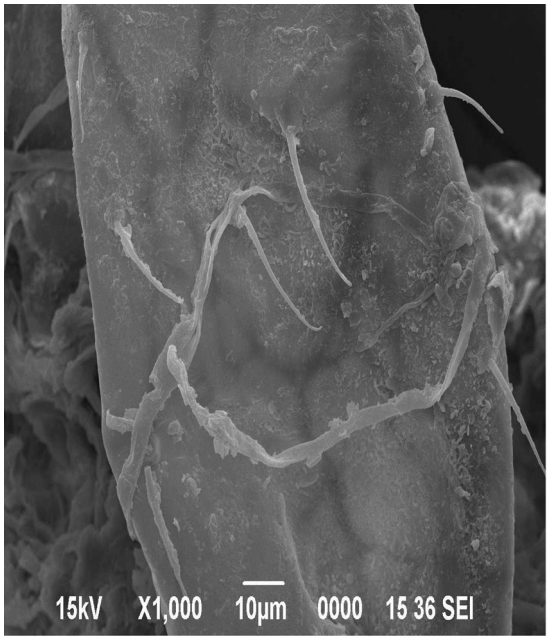
(A)



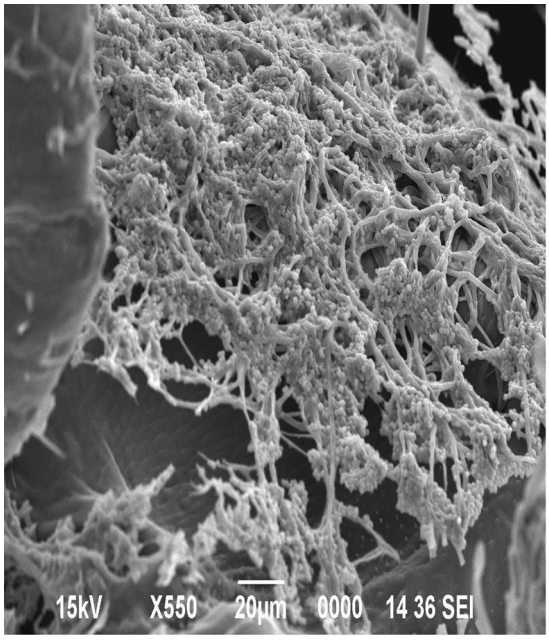
(B)

Penetration through the cuticle was the most frequent method of penetration in case of *M. anisopliae.* Usually, penetration occurs through intersegmental regions, cornicles, joints of legs, antennae etc in *A. craccivora*. Zhang *et al*. (2010), Vestergaard *et al*. (1999), Toledo *et al*. (2010) investigated penetration of the cuticle of *Locusta migrato-*

ria (L.), Frankliniella oxidentalis (Pergande) and in adult planthopper*, Peregrinus maidis* (Ashmead) *M. anisopliae* and *B. bassiana* using scanning and transmission electron microscopy.



(C)



(D)

Fig. 2. (A-D). Mycelial growth and conidiophores with conidia of *Metarhizium anisopliae* inside the body of infected *Aphis craccivora*. (A) & (B): Mycelial growth of *M. anisopliae* on infected body parts (Tibia, anternae), (C): Conidiophores with conidia of *M. anisopliae* inside the infected cells of *Aphis craccivora*.

For clear observation on penetration of the fungus through insect cuticle further investigations are required. It may be possible through transmission electron microscopy (TEM) study.

Scanning electron microscopy study documented good adherence of entomopathogenic fungi to the insect cuticle. This established it as a positive host for the fungi. As the adhesive processes involve both physical and chemical interactions therefore, further biochemical studies were required for confirmation of exact nature of the toxins released by *M. anisopliae.*

Nevertheless, from this study we can say that *M. anisopliae* is a potential biocontrol agent which could be an important component of 'Organic Agriculture'.

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