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Performance of *Schistocerca gregaria* haemocytes in response to entomopathogenic fungi *Metarhizium acridum* and *Beauveria bassiana* single and mixed infections

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Abstract:

Desert locust *Schistocerca gregaria* (Forskål) (Orthoptera: Acrididae) was infected with two entomopathogenic fungi *Metarhizium acridum* (Driver and Milner) (Ma), *Beauveria bassiana* (Bals.) Vuill (Bb). The haemocytes defense response was investigated using light microscope. Five types of blood haemocytes were recognized which were: prohaemocyte, plasmatocyte, spindelshiped haemocytes, spherulocyte and granulocyte. The first response was noted after 48 hrs. post infection as heamocytes aggregated in the haemolymph of infected nymphs, by the 3rd day the aggregation of haemocytes increased to form nodules specially in case of Bb infection. By the 4th day the aggregation and nodules were increased in the Bb and were less in Ma and Ma + Bb joint infection also the hyphal bodies were present for the first time in the haemolymph of Ma + Bb joint infection. The hyphal bodies increased during the 5th to 8th day post infection in the haemolymph of all infected nymphs accompanied with significant haemocytes destruction.

Introduction

Desert locust *Schistocerca gregaria* (Forskål) (Orthoptera: Acrididae) one of the most dangerous pests threaten planted crop in wide areas in Africa and Asia, its attacks cause huge destruction and required large amount of pesticides to control such attacks (Zhang *et al.*, 2019).

Entomopathogenic fungi, e.g. *Metarhizium acridum* (Driver and Milner) (Ma), *Beauveria bassiana* (Bals.) Vuill (Bb) confirmed as a good alternative to chemical insecticides in desert locust and grasshopper control in Egypt (El-Maghraby *et al.*, 2009).

Fungal infection starts via successful penetration of the insect's cuticle using extracellular cuticle-degrading enzymes (Pedrini *et al.*, 2007). Once the fungus enters the haemolymph, it stimulates the immune response of haemocytes (Strand, 2008 and Hillyer, 2016). Haemocytes defense response include Phagocytosis, encapsulation and coagulation (Strand, 2008).

The present work investigates the behavior of desert locust haemocytes in

response of *M. acridum* and *B. bassiana* infection.

Materials and methods

Desert locust *S. gregaria* 5th nymphal instars were obtained from the stock culture maintained for several generations at Locust and Grasshopper Research Department, Plant Protection Research Institute, Agricultural Research Center, Egypt. Spores of Ma and Bb were mass produced according to El-Maghraby *et al.* (2009). Three treatments were applied topically to 5th nymphal instar 1 day old after the fourth molting as a topical application according to Abdelatef *et al.* (2009), these treatments were: 1- (Ma) at a dose 1X 10^3 spores/nymph, 2- (Bb) at a dose 1X 10^3 spores/nymph and 3- combination of Ma and Bb at a dose 1X 10^3 spores/nymph of both fungi. Samples of haemolymph were taken daily after treatment till the 8th day, the nymphs were allowed to feed for 2 hrs., then were chilled on ice for 10 minutes, the arthrodial membrane of the hind leg of each nymph was pierced with sterile needle, the haemolymph was collected using 10 µl capillary pipette, small haemolymph droplet was placed on clear glass slide, then the drop was quickly smeared to a thin film on the slide by using an edge of another slide, the smear was then air dried, then fixed in 2-3 drops of methanol 95%. The smears were stained with diluted Gemsa stain for 15 minutes, then washed with distilled water. The haemocytes were examined and photographed using a light microscope under the oil immersion lens, the haemocytes were identified according to Gupta (1979).

Results and discussion

In the present work 5 types of heaymocyte were recognized, these types were: Prohaemocyte, plasmatocyte, spindelshiped haemocytes, spherulocyte and granulocyte as shown in Figure (1). The obtained results showed clearly that the treatment of 5th nymphal instar of *S. gregaria* with Ma and Bb caused several morphological and behavioral changes in the heaymocyte.

Such changes began after 48 hrs. post infection as aggregation of heaymocyte, as shown in Figure 2 (A, B, C), a multiplication in cells was noticed as in Figure 2 (A) a prohaemocyte was divided into 2 new cells. While at the 3rd day post infection the haemocytes began to form nodules, in treating insects, Figure 2 (D, E, F) shows more haemocytes aggregation at the 3rd day post infection with Ma and Bb mixture, Ma and Bb it could be noticed that more cell participate in aggregation specially in case of Bb infection.

There were no aggregations noticed in the non-infected nymph's haemolymph (Not shown). By the 4th day post infection, there were more aggregation forming nodules in case of Bb infection (Figure 3 A, B, C), while in case of Ma (Figure 3 D and E) and Ma and Bb mixture (Figure 3F) infection, there were less aggregation, also in Figure 3 (F) the first appear on the hyphal bodies in the haemolymph of infected insects with Ma and Bb mixture was noticed.

In Ma and Bb mixed infection, at the 5th, 6th and 7th day post treatment (Figure 4), there was a slight increase in the hyphal bodies (Figure 4 A) as well as distortion of the proheamocytes, granulocytes and spherulucytes at the 5th, while at the 6th day (Figure 4 B) more hyphal bodies occurred, some of the granulocytes lost part of its membrane, also pyconsis appeared obviously in the nuclei of spherulucyte, by the 7th day (Figure 4 C) the haeymolymph was full of hyphal bodies with lysed granulocytes and there was spherulucyte with obvious pyconsis in the nuclei. At the 8th day there was no haymocytes only hyphal bodies occurred in the haemolymph.

While in case of Ma infection at the 5th, 6th, and 7th day (Figure 5), at the 5th day post infection hyphal bodies were present for the first time (Figure 5 A) also there was

abnormalities in the granulocytes and spherulucytes, moreover, there were a lot of vacuoles in the cytoplasm of the granulocytes and pycnosis in the nuclei of the spherulucyte, on the 6th day more hyphal bodies occurred surround the haemocytes, also some lysed spherulucytes were present (Figure 5 B), by the 7th day post infection (Figure 5 c) few haemocyte were found surrounded with more hyphal bodies, there were also lysed granulocytes and plasmatocytes, the cytoplasm of the plasmatocytes was full of vacuoles. While at the 8th day post infection there were no sign of haemocytes and the haemolymph was full of hyphal bodies. Figure (6) shows the effect of Bb infection on the 5th and 6th day post infection. At the 5th day (Figure 6 A) post infection there were many plasmatocytes with large vacuoles, distorted granulocytes showing vacuolization in the cytoplasm, and abnormal spherulucytes, also aggregation of few cells, although on the 6th day (Figure 6 B) post infection there were abnormal spherulucytes, granulocytes and prohaemocytes with lysed cytoblasm, the hyphal bodies were first occurred. Figure (7) illustrates the effect of Bb infection on the 7th and 8th day, there were abnormal granulocytes and spherulucytes with obvious pycnosis in the nuclei, plasmatocytes with large vacuoles and abnormal prohaemocytes, also more hyphal bodies occurred on the 7th day (Figure 7 A). On the 8th day (Figure 7 B) there were distorted in the granulocytes with obvious pycnosis in the nuclei, abnormal plasmatocytes, spherulucytes and proheamocytes, more hyphal bodies occurred.

Lackie *et al.* (1985) observed a greater number of plasmatocytes in control desert locust, which in harmony with the present work. During the infection, there was an increase in the proportion of spherulucytes and granulocytes, this may be due to their role in production of humoral defense reaction. The insect immune system is subdivided into humoral and cellular defense responses. Humoral defenses include the production of antimicrobial peptides and the complex enzymatic cascades that regulate coagulation or melanization of hemolymph. In contrast, cellular defense refers to hemocyte-mediated immune responses like phagocytosis, nodulation and encapsulation (Lavine and Strand 2001). The failure of the immune system of mycosed desert locust *S. gregaria* nymphs may be due to the secretion of mycotoxins by the entomopathogenic fungi, Vey *et al.* (2002) suggested that the destruxin may intervene during the disease by true immune-inhibitory effect occurring at doses which do not cause paralysis or any general sign of toxicity. It's clear that combined infection of both used fungi caused a greater reduction to the 5th nymphal instar of *S. gregaria* immune system followed by *M. acridum* then *B. bassiana* infection, such effect may explain the superior effect of the combined infection and *M. acridum* treatments as previously reported in work of (Abdelatef *et al.,* 2009 and El-Maghraby *et al.,* 2009).

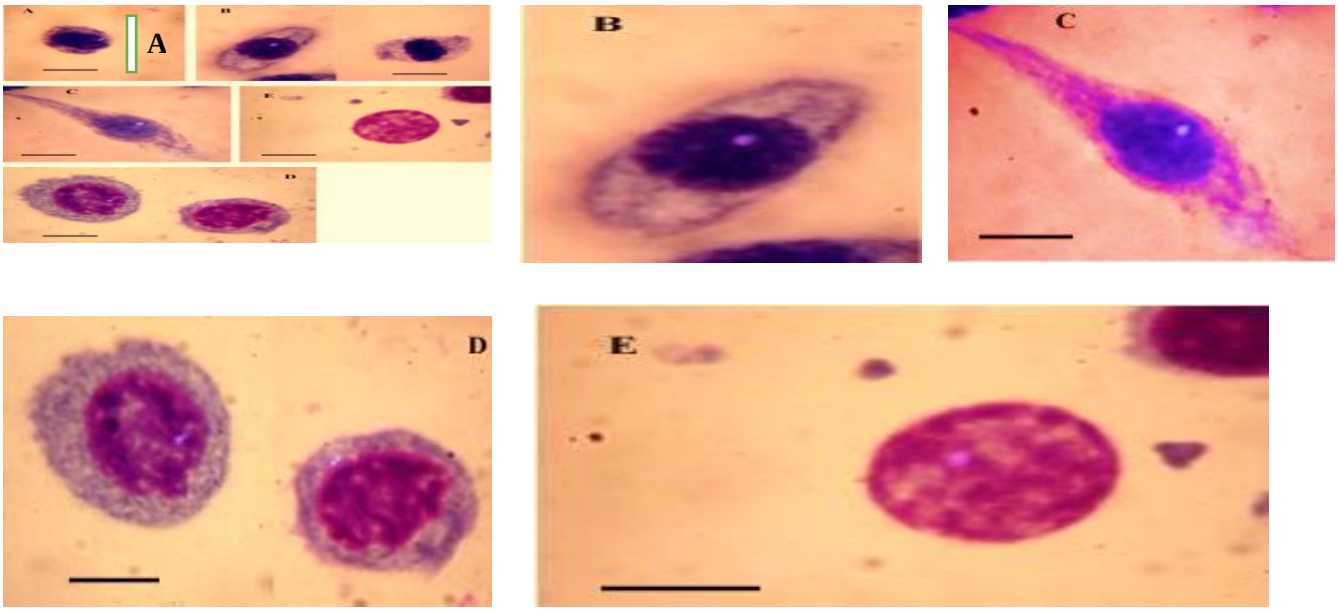

Figure (1): Haemocytes types of *Schistocerca gregaria*. (A) Prohaemocyte (B) Plasmatocyte (C) Spindleshaped cell (D) Spheriloucytes (E) Granulocyte, Bar= 8µ.

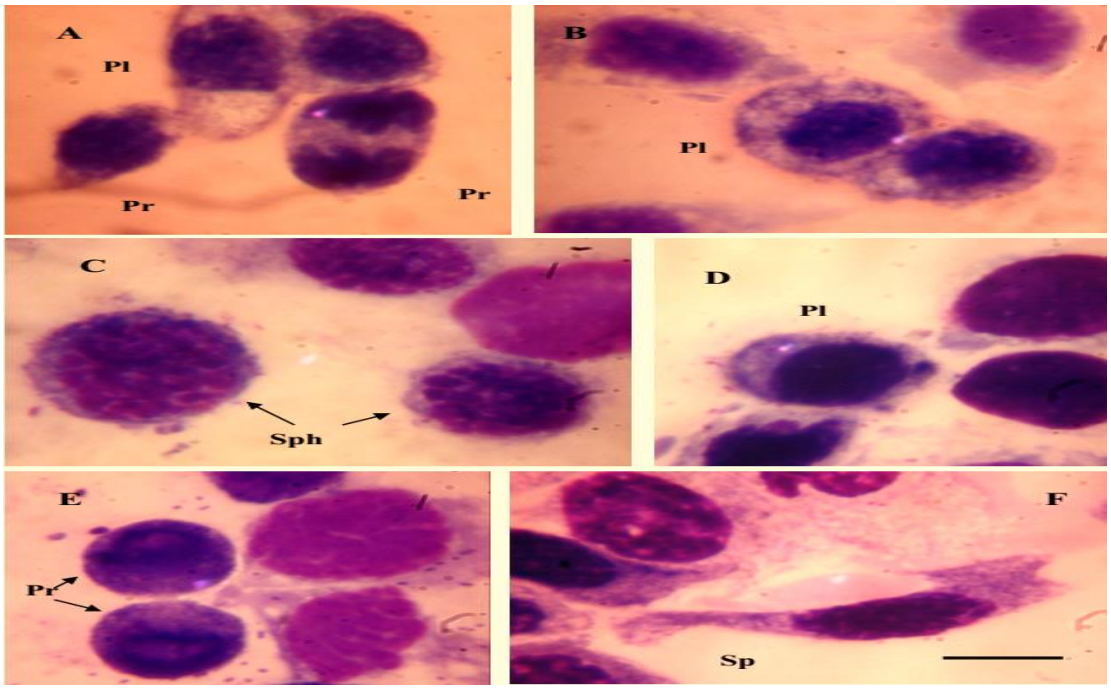

Figure (2): Haemocytes of *Schistocerca gregaria* at 48 hr. (A,B,C) and 3 days (D, E, F) post infection. *M. acridum* (B, E), *B. bassiana* (C, F) *M. acridum* and *B. bassiana* mixture (A, D) infection. (Pr=proheamocyte Pl= plasmatocyte Sp= spindle shaped cell Gr= granulocyte Sph= spherulucyte) Bar= 8µ.

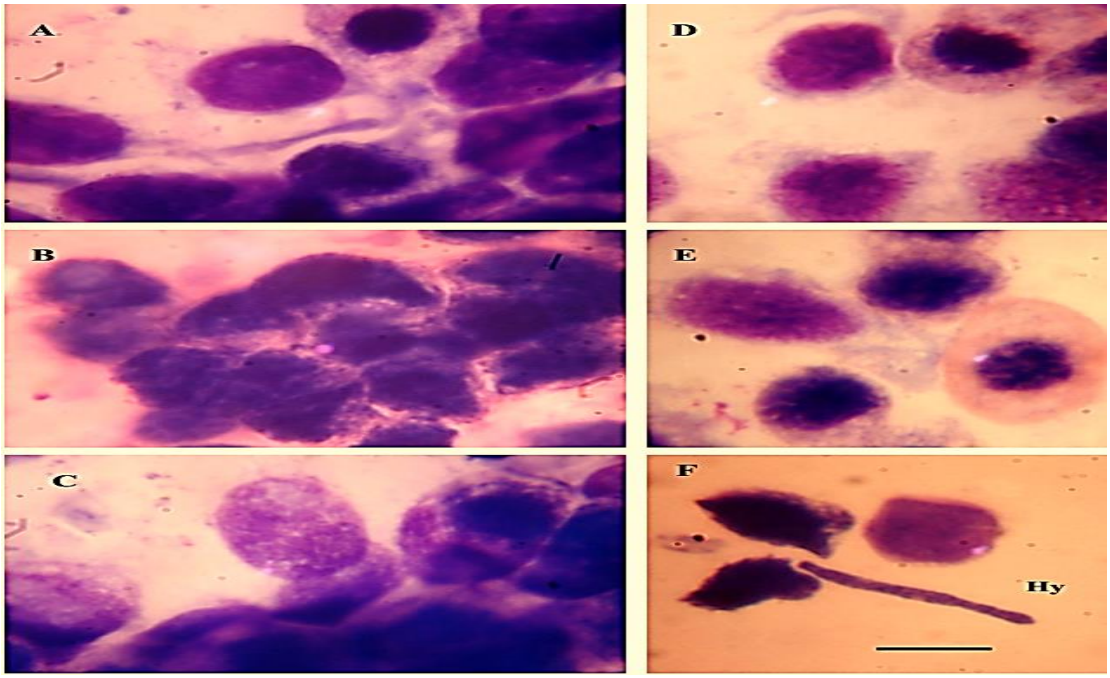

Figure (3): Haemocytes of *Schistocerca gregaria* at 4th day post infection with *B. bassiana*, (A,B,C) *M. acridum* (D, E),. and *M. acridum* and *B. bassiana* mixture (F) (Hy= hypha) Bar= 8µ

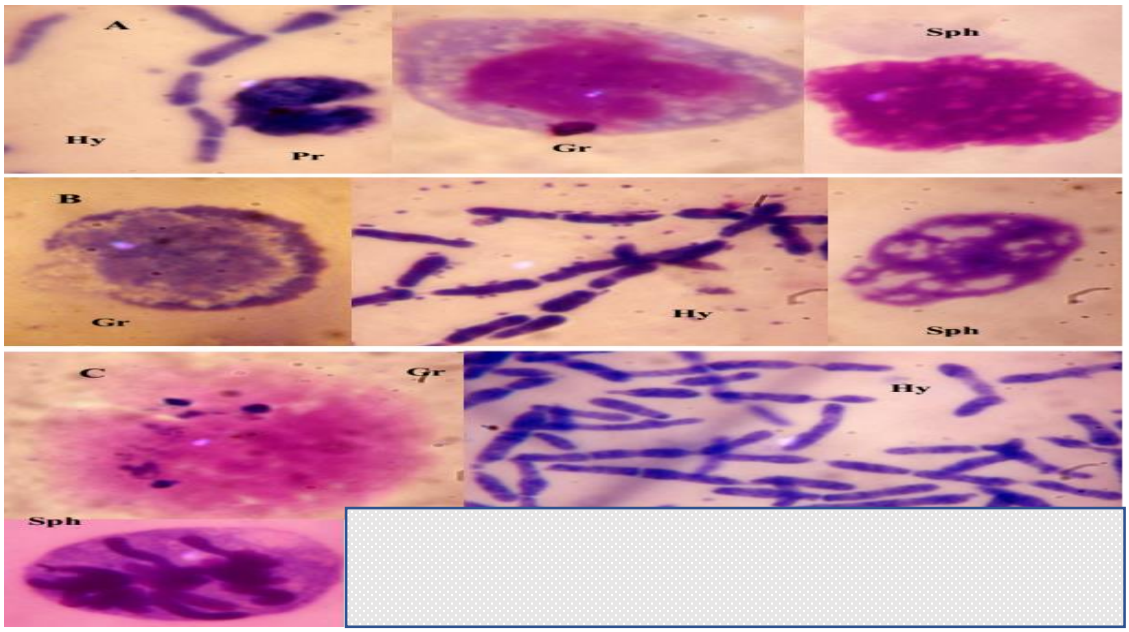

Figure (4): Haemocytes of *Schistocerca gregaria* at 5th (A) , 6th (B) and 7th (C) day post infection with *M. acridum* and *B. bassiana* mixture. (Pr=proheamocyte Pl= plasmatocyte Gr= granulocyte Sph= spherulucyte Hy= hypha) Bar= 8µ.

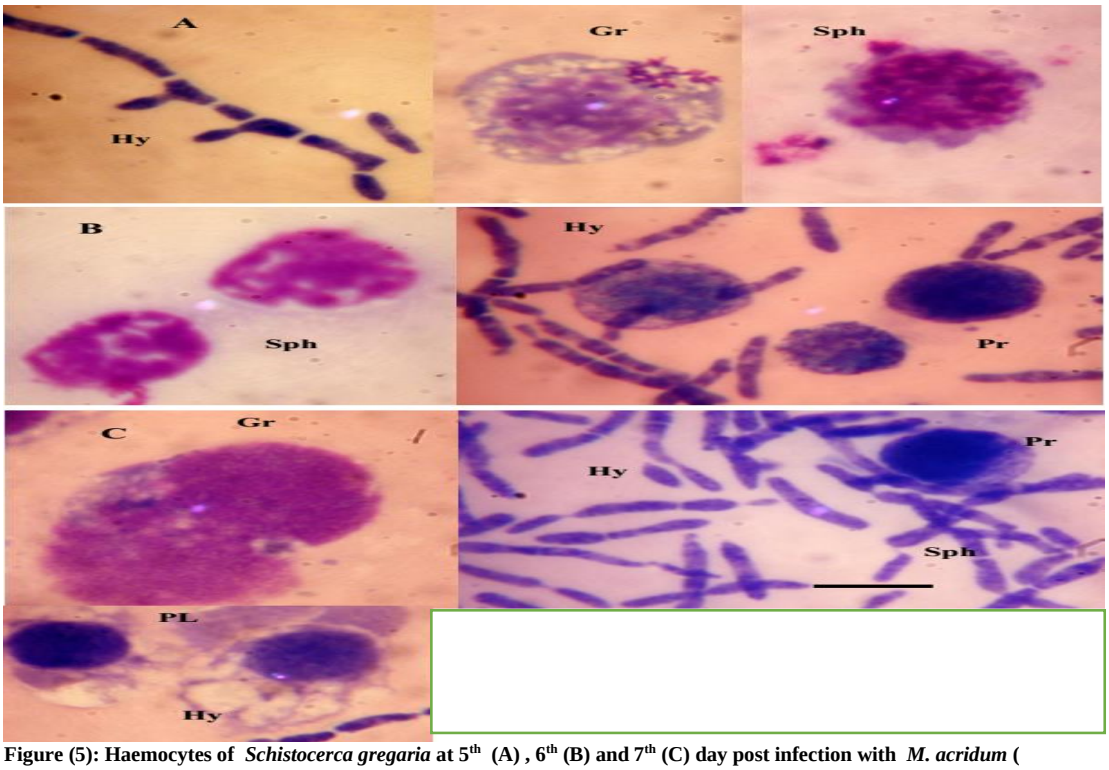

Figure (5): Haemocytes of *Schistocerca gregaria* at 5th (A) , 6th (B) and 7th (C) day post infection with *M. acridum* (Pr=proheamocyte Pl= plasmatocyte cell Gr= granulocyte Sph= spherulucyte Hy= hypha) Bar= 8µ

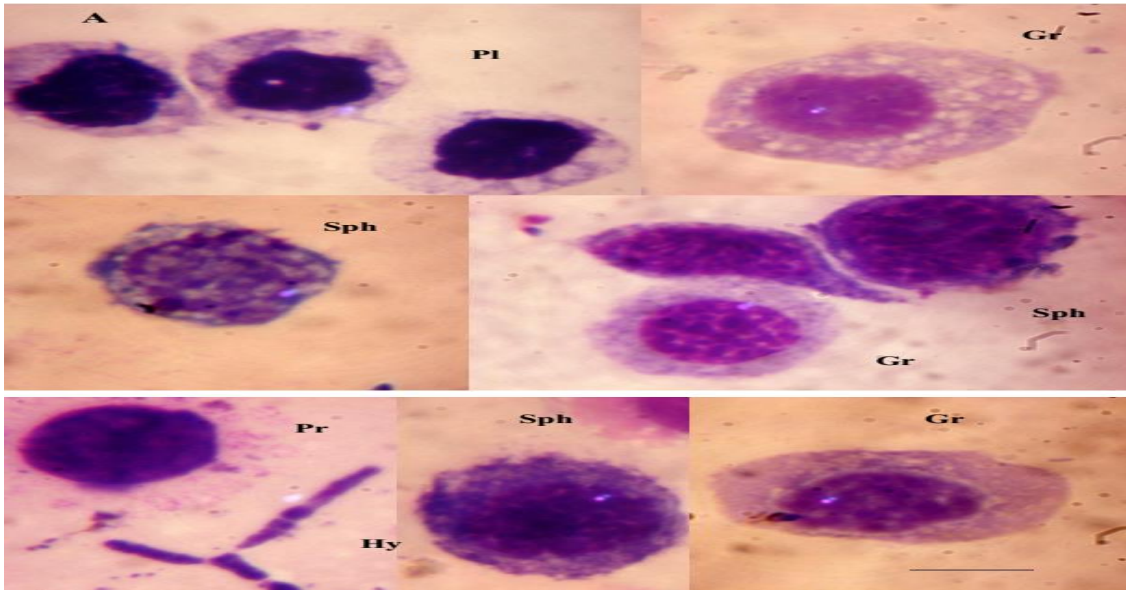

Figure (6): Haemocytes of *Schistocerca gregaria* at 5th (A) and 6th (B) day post infection with *B. bassiana*. (Pr=proheamocyte Pl= plasmatocyte Sp= spindle shaped cell Gr= granulocyte Sph= spherulucyte) Bar= 8µ

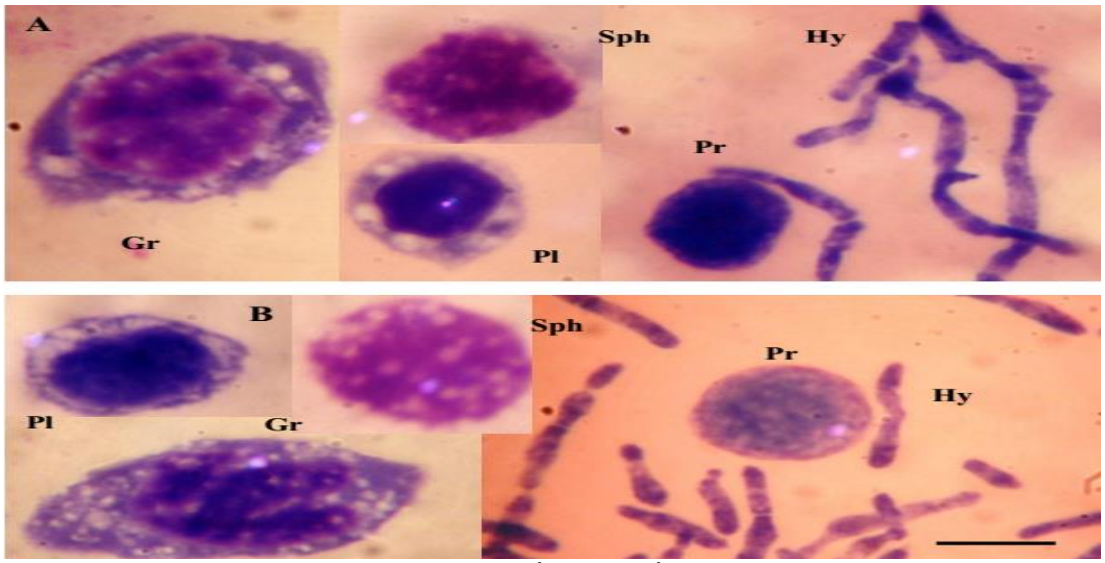

Figure (7) Haemocytes of *Schistocerca gregaria* at 7th (A) and 8th (B) day post infection with *B. bassiana*. (Pr=proheamocyte, Pl= plasmatocyte, Gr= granulocyte, Sph= spherulucyte Hy= hypha) Bar= 8µ

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