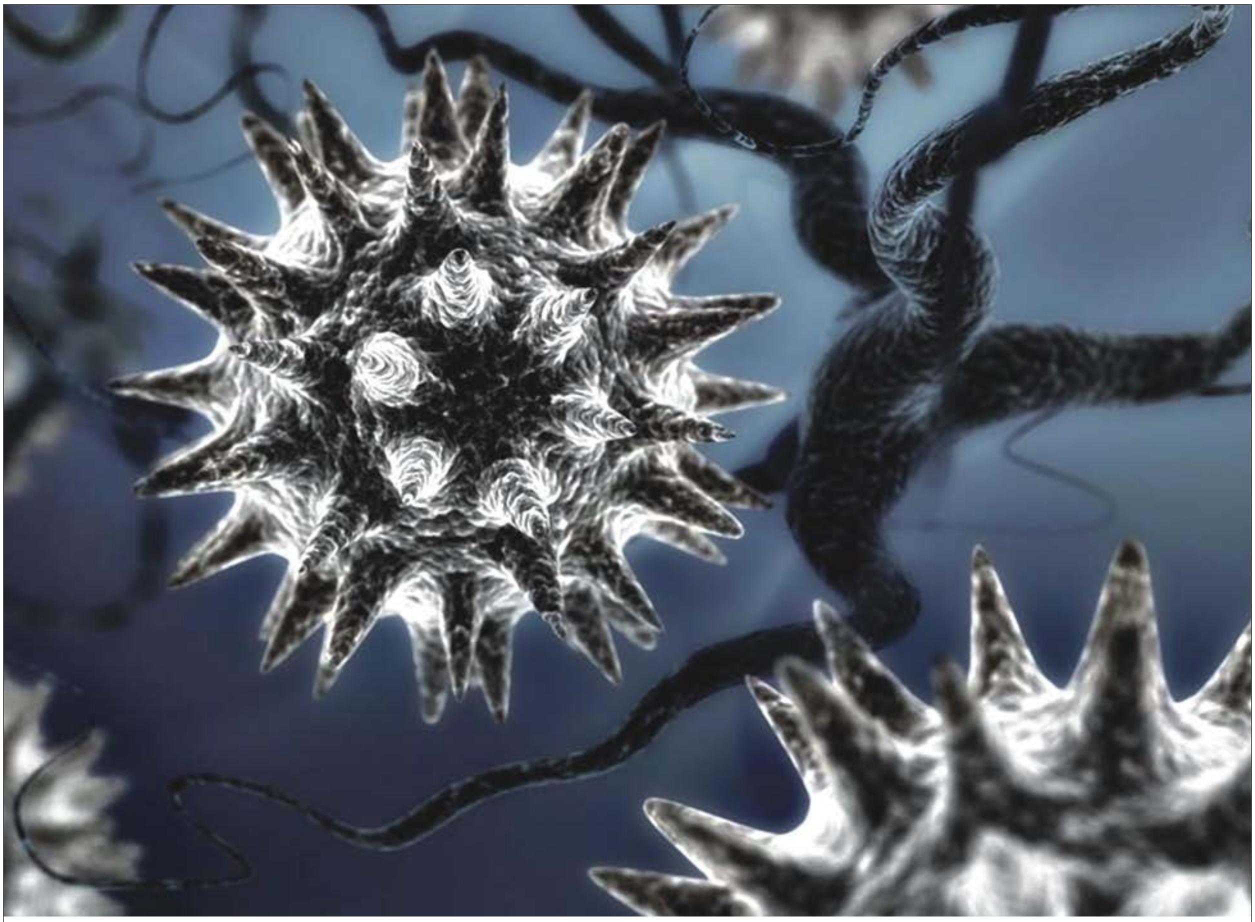

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Malformations Characterization of the Potato Tuber Moth (PTM) Pupae Infested by *Phthorimaea operculella* Granulovirus (POGV) using Image Processing Based Analysis

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ABSTRACT

Malformative features of the Potato Tuber Moth (PTM) pupae infested by *Phthorimaea operculella* Granulovirus (POGV) were shown to little extend by the macro morphological criteria depending on the naked eye based vision to follow changes in size, shape and color. As defined in this work, image processing and analysis proved to be useful for evaluating representative parameters of morphological characters such as size (area, area fraction, feret measurements, perimeter, major and minor axis and Aspect Ratio (AR) and shape parameters (circularity and round parameters). Thus, the demonstration of morphological changes in terms of numerical analysis (mathematical morphology) could be adopted to express variations at the structural level which was enriched by other operations as Pixel Plot Profile and histogram. Thus, Using image processing and analysis in morphometry or mathematical morphology was intense and promising.

Key words: Potato tuber moth, *Phthorimaea operculella* granulovirus (POGV), image processing, numerical analysis

INTRODUCTION

The Potato Tuber Moth (PTM), *Phthorimaea operculella operculella* (Zeller), is a pest of potatoes in the field and storage, in North Africa. The availability of potatoes when coupled with favorable conditions for the development of PTM even in winter, allow for tremendous build up of populations of PTM. Overlapping of potato growing seasons (winter, autumn, spring and summer) creates ideal conditions for this pest to reproduce and migrate between crops. Migrating adults, coming from surrounding fields are the most source of PTM invasion into newly planted crops. However, weeds and other crops (tomato or eggplants) are also a potential source of migrating adults. PTM damage to potatoes both in the field and in store is a major constraint for potato production and storage in North Africa and the Middle East. PTM is active through the year, but is most active from April to late August. In winter and early spring, PTM populations remain low and don't cause significant damage to potato crops (Hanafi, 2005). Baculoviruses are pathogens that attack insects and other arthropods. The majority of baculovirses are used as biological control agents in the genus *Nucleopolyhedrovirus*, so "*Baculovirus*" or "virus" will refer to *Nucleopolyhedroviruses*. These viruses are excellent candidates for species specific, narrow spectrum insecticidal applications (Reardon *et al*., 1996). It was mentioned that granulovirus (POGV) is a promising candidate to substitute for chemical insecticides in Integrated Management (IPM) of the potato tuber moth *P. operculella* (Sporleder *et al.*, 2005). In our present work, we intended to reveal the malformative effects of viral infected pupae of *P. operculella* using image processing and analysis. Computerized image analysis is a powerful tool for exactly determining elaborate morphological traits in microscopic pictures (Newton and Kendrick, 1990).

MATERIALS AND METHODS

Data presented in (Shimaa, 2011) showed morphogenetic effect of PoGV isolate on pupal stage of PTM after treatment of newly hatched larvae of *P. operculella* using tubers dipping technique in different viral concentrations, from which (10^9) viral concentration showed extreme detrimental influence on the infested pupae which were selected here to image processing and analysis. Image analysis systems facilitate the recording of various parameters describing the form and size of objects. Parameters evaluated for differentiating infected and healthy *P. operculella* in this study are listed and some complex morphological parameters are shown. Most of them, even widely used parameters such as length, width and length width ratio, were dropped in further evaluation. Differences were best described by the shape factors. The selected images were analyzed using Image J software after numerous preprocessing operations such as noise reduction, contrast enhancement and grey level transformation.

Area: The area of subset S is the total number of pixel in the abject.

Coordinates (X, Y): x-coordinates, y-coordinates, often implemented with the origin of the pixel.

Centre of mass measures (Xm and Ym): The centre of mass of object from X and Y axis.

Perimeter: The contour length of a target objects this is calculated by contour trace alogrithms of image.

Major axis: The longest dimension of the target object.

Minor axis: The breadth of the target object.

Fitting angle: The angle at which the object orientates.

Circularity: A shape factor describing the deviation of an object in an image from a true circle.

Feret diameter: A diameter of object measured using calipers.

Area fraction: The actual area occupied by the object.

Feret x: The average distance across the object from x-axis.

Feret y: The average distance across the object from y-axis.

Min feret: The shortest feret distance.

Aspect Ratio (AR): The ratio of width to length.

Round: A degree of irregularity of object from sphericity.

Solidity: It is a measure of voidage or compactness of object.

The histogram of a digital image with grey level in the range [0, L-1] is a discrete function h (rk) = nk, where rk is the kth grey level and nk is the number of pixels in the image having grey level rk. The horizontal axis of each histogram plot corresponds to grey level values, rk. The vertical axis corresponds to values of h (rk) = nk, simply plots of h (rk) = nk versus rk. Each pixel is surrounded by eight neighbouring pixels and identified as Structuring Elements (SE). The histograms determined for the two PTM pupae represented their elementary map via histogram (Gonzalez and Woods, 1992).

RESULTS

The two raw images analyzed are listed in Fig. 1. A considerable damage appeared by comparing healthy and infested pupae in color, shape and size. Such properties were estimated numerically via morphometric analysis aided by image analysis and computer assistance. The size parameters revealed sharp decrease in the total area of the infested PTM pupa (32280 pixels) as compared to healthy (58630 pixels). The total length contour and diameter (denoted by feret diameter, min feret, feret y, major axis and minor axis) also exhibited similar pattern to the total area. The conclusive Length to Width ratio determined by Aspect Ratio (AR) addressed the significant lowered size of infested pupa due to viral infection. The shape parameters regarded as Circularity factor and round parameter showed the irregularity and extensive malformation of the infested pupa when compared to control, all size and shape parameters estimations are summarized in Table 1. The structuring elementary profile of healthy and infected pupae scanned individual

Table 1: Morphometric parameters for healthy and viral infested pupae

Parameters	Healthy	Infected
Area	58630.000	32280.000
X	84.500	76.000
Y	264.000	187.500
Xm	76.947	74.087
Ym	259.735	178.678
Perimeter	1106.000	778.000
Major axis	462.635	303.534
Minor axis	161.358	135.406
Angle	90.000	90.000
Circularity	0.602	0.670
Feret diameter	434.222	294.552
Area fraction (100%)	100.000	100.000
Feret X	13.000	16.000
Feret Y	56.000	53.000
Feret angle	109.228	114.041
Min feret	143.000	120.000
AR	2.867	2.242
Round	0.349	0.446
Solidity	1.000	1.000

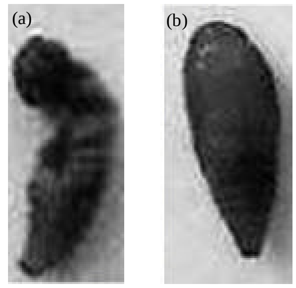

Fig. 1(a-b): (a) Grey level images of healthy and (b) PoGV infested pupae

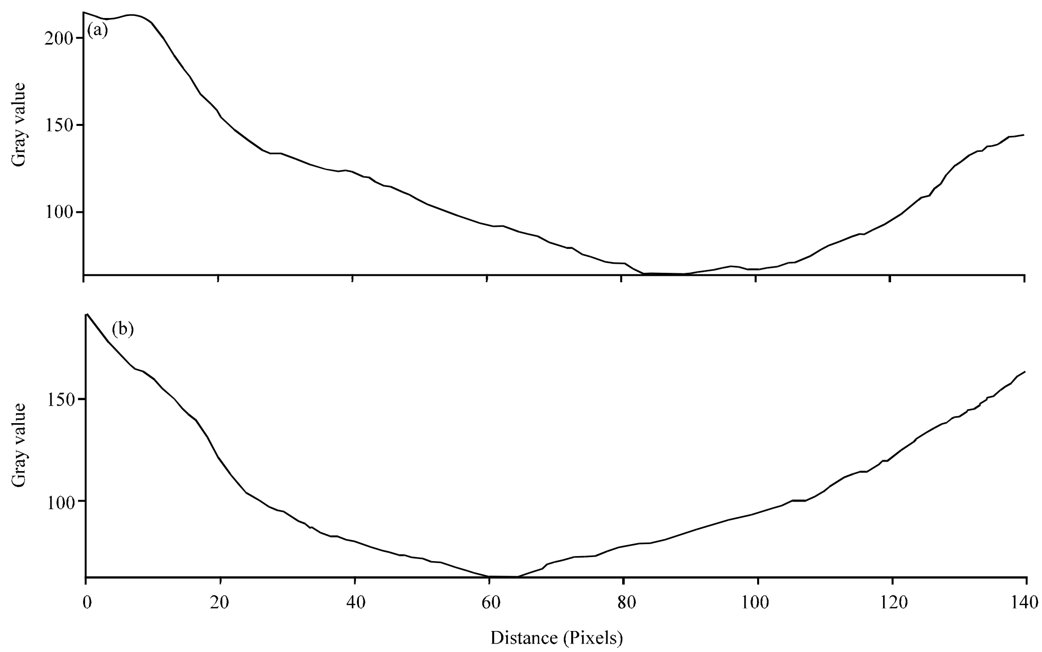

Fig. 2(a-b): Pixel plot profiling of pupae healthy (a) Infected and (b) PTM

structures considering both distance (by pixels) from unified axis from the image plane and the corresponding gray values. The significance was to be exploited from comparing the scanned elements at each single point to reveal consistence of structure or vice versa; the matter to be noticeable in our case here; is that to be greatly inconsistent. The grey level components for both pupae are different in value which clarifies the changeable color due to PoGV infection. All the variable color components are displayed as profile map as in Fig. 2. As shown from that figure, almost of the grey pixels lies between 0 to 85 at the distance axis with gradual decrease in the grey value for the healthy pupa while for the PoGV infested PTM, there was rapid decrease in grey intensity followed by sharp increase of the grey values at 60 pixels distance. All these different organizations of gray components at different unit distances accounted for the uncompatibility of the two structural systems and configurations.

The corresponding histogram for each pupa showed in Fig. 3 exhibited differential pattern concerning with their elementary map from pure black grey level (0) to pure white grey level (255).

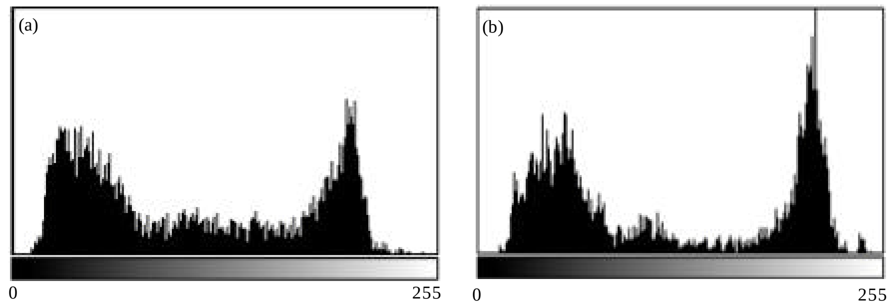

Fig. 3(a-b): Histogram display of the two grey level PoGV viral infected (a) Healthy and (b) PTM pupae

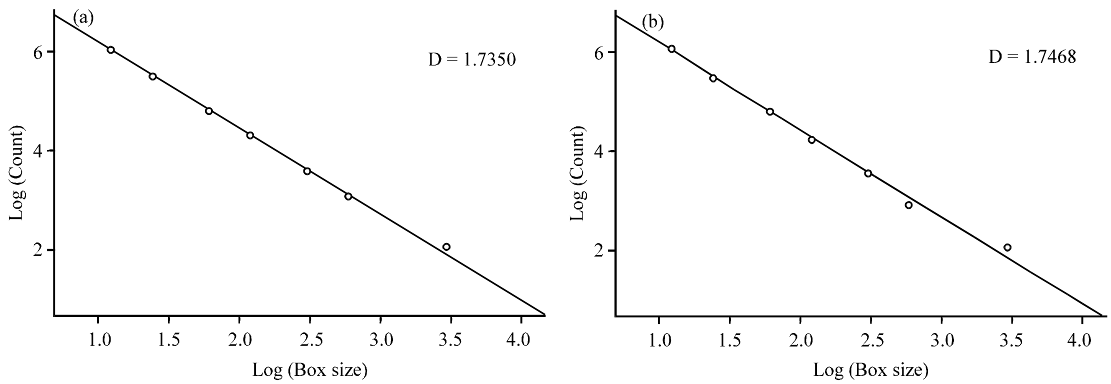

Fig. 4(a-b): Fractal Dimension (FD) parameter for PoGV infested (a) Healthy and (b) PTM pupae

The histogram corresponding to the healthy showed high dynamic range, nearly towards white scale, in contrast, the infected histogram exhibited slightly low dynamic range and general dissimilar peak pattern.

Other morphological indicator for malformations held by viral infested pupa is contributed by Fractal Dimension (FD) parameter. FD quantifies morphology of irregular and complex objects and non linear systems. As shown in Fig. 4 the D value corresponding to healthy PTM pupa (1.7468) is higher than that of the infested one (1.7350) that showed to what extend the former is complicated than the last one.

DISCUSSION

Malformative effects of the PTM pupae infested by PoGV were shown to little extend by the macro morphological criteria depending on the naked eye based vision to follow changes in size, shape and color. As defined in this work, image processing and analysis proved to be useful for evaluating representative parameters of morphological characters such as size (area, area fraction, feret measurements, perimeter, major and minor axis and AR) and shape parameters (circularity and round parameters). The pixels plot profile enriched our information about the differential color components exhibited by the two analyzed grey level images which provided a great impact on the differential structural properties as colors always are correlated with structural irregularities. The decrease in elementary map dynamic range exhibited by infected PTM pupa accounted for disappearance of some structuring elements and co-occurrence of new structures indicated by novel positions on the histogram horizontal axis when compared to healthy one which could be viewed in terms of structural disorganization exploited from viral infection. To, valuably, quanitify morphology numerically by image analysis, FD parameter was measured and showed complexity of healthy PTM system as compared by PoGV infested pupa. The detailed distortion of edge features and extensive irregularity as compared to normal features exhibited by healthy ensured the morphodeformative effect of the invading virus. Thus, the demonstration of morphological changes in terms of numerical analysis (mathematical morphology) could be adopted to express variations at the structural level. Using image processing and analysis in morphometry or mathematical morphology was intense and promising. The basic objectives of object measurement are application dependent. It can be used simply to provide a measure of the object morphology or structure by defining its properties in terms of area, perimeter, intensity, color, shape, etc. It can also be used to discriminate between objects by measuring and comparing their properties (Gonzalez and Woods, 1992).

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