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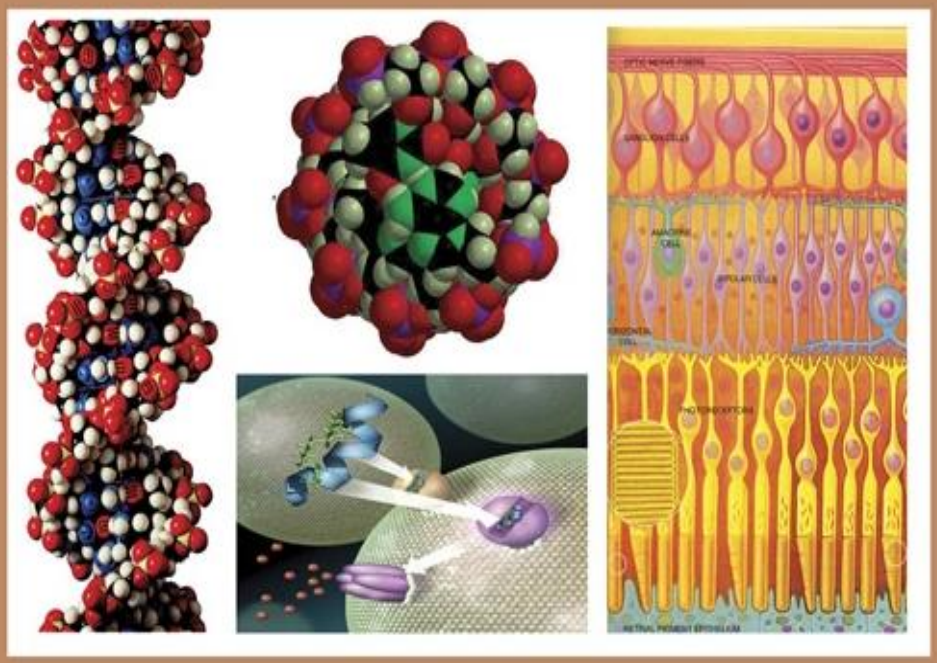

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The Monitoring and Molecular Identification of the Mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) on Okra Plants at Sharkia Governorate

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ABSTRACT

Mealybugs (Hemiptera: Pseudococcidae) are small piercing–sucking insects that infesting a wide range of crops and vegetables and their species are similar, which makes it difficult for non-specialists to distinguish between them. Samples were collected of infested okra plants from five districts at Sharkia Governorate, Egypt. This study aimed to study the distribution of the cotton mealybug, *Phenacoccus solenopsis* Tinsley and its associated parasitoid as well as the identification of this species by molecular characterization due to the high degree of morphological similarity between different closely related species of mealybugs. It was found that the Parasitism has a big influence on the occurrence of this insect infestation and this species of mealybug was confirmed by amplified and sequenced DNA loci known to be informative for species identification by provides information of 28S ribosomal RNA gene sequence. Consequently, the success of IPM programs against mealybugs depends on the correct definition of this pest through genetic characterization to choose the best control strategies. On the other hand, the difficulty of correctly identifying these species leads to its difficulty in choosing appropriate methods of control, especially biological control where each pest has a specific species of its natural enemy, which controls it biologically.

INTRODUCTION

Okra (*Abelmoschus esculentus* L.) is one of the major popular vegetables in Egypt. Incidence of mealybug pest, *Phenacoccus solenopsis* Tinsley on okra has been reported by many authors from different parts of the country while being responsible for considerable yield loss (Abd-Rabou *et al*., 2010; Ibrahim *et al*., 2015; Nabil *et al.*, 2015 and Rezk *et al*., 2019). Sample collections of mealybugs were surveyed from the okra plants at Sharkia Governorate. Dewer *et al*. (2018) recorded that the *Phenacoccus solani* Ferris is a new pest in Egypt. Mealybugs feed by piercing leaves and fruits and sucking plant sap from them, these cause direct and indirect damages while feeding and cause malformation for leaves, branches and fruits that lead to decreased the quality and marketing value of plants (Douglas and Kruger 2008; Meyer *et al.*,2008; Nakaune *et al.*,2008; Mahfoudhi *et al*., 2009; Garcia Morales *et al*., 2016 and Nabil and Hegab 2019).

The morphological identification of scale insects can be difficult particularly for non- specialists. On the other hand, the taxonomic identification methods are currently based on keys dealing with different external body structures of adult female species but the use of this method is impossible to distinguish nymphs and males of this pest. The difficulty of correctly identifying these species affected selecting the suitable control strategies, particularly biocontrol. This problem has led to the development of molecular tools used to obtain accurate identification of species of Pseudococcidae using DNA barcoding (Park *et al*., 2011; Abd-Rabou *et al.*, 2012; Correa *et al*., 2012 and Pacheco Da Silva *et al*., 2014). In this study, the authors have focused on the use of DNA markers to distinguish between closely related species of mealybugs for agronomic applications (Cavalieri *et al*., 2008; Rung *et al*., 2008 and Saccaggi *et al.*, 2008). This study helps us to further track the spread of this species in the region and provides 28s ribosomal RNA gene sequence of its. Therefore, this investigation has contributed to the development of new tools for mealybug characterization, but it remains difficult to evaluate the efficacy of different markers for distinguishing between closely related species of Pseudococcidae.

MATERIALS AND METHODS

Plant Material:

Sample Collection:

Twenty-five samples were surveyed from different five districts; Zagazig, Hihya, Abo-Hammad, Mina–Elkamh and Abo-Kabier, at Sharkia Governorate, Egypt during 2020 season. Each sample consisted of twenty-five leaves transferred to the laboratory and examined using a stereomicroscope on the same day. The different stages of *P. solenopsis* on both surfaces of the leaves were counted and recorded.

To study the percentage of parasitism of *P. solenopsis*, the insects on each sample were separated into healthy alive insects and mummies of its insect. The percentages of parasitism were recorded. Specimens were conserved in ethanol (95%) and stored at 20°C for identification and molecular analyses.

DNA Extraction, Amplification And Sequencing:

1. DNA Preparation and PCR Amplification:

Genomic DNA was extracted from insect samples using a DNeasy Blood & Tissue extraction kit (Qiagen, CA, USA) according to the manufacturer's instructions. In order to identify the samples by molecular methods, PCR amplification and sequencing of 28S rRNA were carried out.

The primers were used for amplification of the 28S rRNA gene using 28S-D2 primers forward and reverse F (5′-AGAGAGAGTTCAAGAGTACGTG -3′) and R (5′-TTGGTCCGTGTTTCAAGACGGG -3′) according to Malausa *et al*., 2011.

DNA amplification was performed in a 50µL volume containing 30ng of DNA template, 250mM each dNTP, 25pmol each primer, 2.5mM MgCl2, 10µL of 5x PCR buffer, 2.5 U *Taq* polymerase (Promega) and dist·H_2O to a final volume of 50µL. The reactions were performed in an automatic thermal cycler (GeneAmp1 PCR System 9700, Perkin-Elmer) under the following conditions: initial denaturation at 94 °C for 3 min; 35 cycles of 94 °C for 40 s, 58 °C for 50 s, and 72 °C for 60 s; and a final extension at 72 °C for 7 min. An aliquot of 10µL PCR products was analyzed on a 1% agarose gel.

2. Sequencing and Data Analysis:

Sequencing analysis was performed on a 300 bp PCR product. The purification of the PCR product was carried out using a high pure PCR purification kit (Qiagen). DNA sequences were determined by the automated DNA sequencing method. The automated DNA sequencing reactions were performed using an ABI PRISM Big Dye

Terminator Cycle Sequencing Ready Reaction kit (PE Applied Biosystems, USA) in conjunction with ABI PRISM (310 Genetic Analyzer). Alignment of The sequencing data was compared with other rDNA sequences in GenBank using the NCBI Basic Local alignment search tools (BLAST) program (http://www.ncbi.nlm.nih.gov/BLAST).

The top five homologous hits were selected for neighbor-joining and multiple alignments with Clustal W methods. The alignment results were used to construct a Phylogenetic tree using DNAStar, version 7.1, software.

RESULTS

Occurrence of *Phenacoccus solenopsis* Tinsley and its Parasitoid:

Data in Table (1) give some remarks on the distribution of mealybug species inhabiting okra crops in different localities (Mina-Elkam, Zagazig, hihya, Abo-Hammad and Abo-Kabier districts) at Sharkia governorate, Egypt . The Parasitism has a high effect on the occurrence of the infestation with mealybug, whereas, the highest incidence of infestation arranged in descending in the Zagazig, followed by Mina al-Qamh then Abo- Hammad then Abo-Kabier and Hihya districts, respectively. The highest numbers of adult females were recorded in Mina-Elkamh distract and represented by 471 individuals, while the highest numbers of nymphs occurred in Zagazig distract with values of 3488 individuals. On the other hand, the lowest numbers of adult females and nymphs occurred in hihya distract and were represented by 21 and 399 individuals, respectively.

Table 1: Occurrence of *Phenacoccus solenopsis* Tinsley infesting okra plant at Sharkia Governorate during the 2020 season**.**

Distracts	Number of insects			Percentage of parasitism (%)
	Females	Nymphs	Total alive stages	
Mina-Elkamh	471	2128	2599	3.809
Zagazig	36	3488	3524	1.022
Abo-Hammad	93	1044	1137	9.234
Hihya	21	399	420	33.571
Abo-Kabeer	336	2028	2364	3.087

It was found that the increase in population of tested insect was related to the decreased percentage of parasitism.Whereas, the percentage of parasitism reached to maximum in Hihya district together with the lowest numbers of alive stages. While, the lowest percentage of parasitism was recorded in Zagazig district with the minimum numbers of insect stages. Nabil and Hegab (2019) reported that the population density of tested insect was indirectly correlated with the parasitism .

28S Ribosomal RNA Gene Analysis:

PCR products of approximately 300 bp amplified with the 28S-D2 F and R primers and corresponding to the 28S ribosomal RNA gene were obtained from all strains (five selected strains) (Fig.1). After purification of PCR products and sequencing, the results of the BLAST-n alignment showed that all five sequences were associated with high levels of sequence similarity (98-100%) with the 28S ribosomal RNA gene sequences for the species, *P. solenopsis*. So, the samples collected were diagnosed as similar to this species.

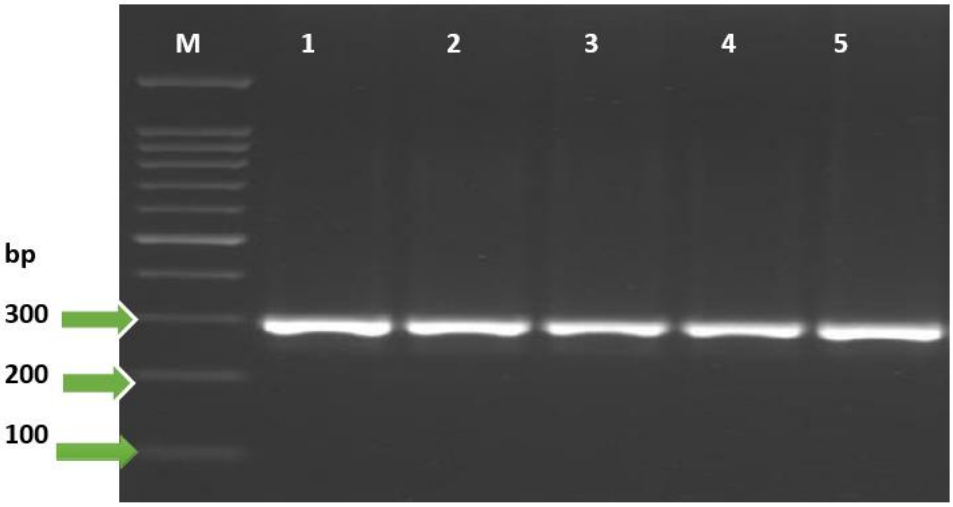

Fig.1: Amplification of 28S r RNA gene from of five strains Sh1, Sh2, Sh3, Sh4, and Sh5. M: 100 bp DNA ladd.

***Phenacoccus_solenopsis*_Sh1**
TTTCTCTTTGGGTACGGAAGAGCC
CGTGATCCGGGCGGCAGAATTCAG
AGACGATGGCGGCAACGTTGTCGG
TTCGATATTTCTGTCGCCGGCACG
GACGTCGCGACCCGTTTGGTGTCG
GTCTCAGGAGACGCGTTTCACGTT
CGTCGACGCGTCGTCTGCCTCGGT
AGGCGCGCGTTGCGAGTACGCGTT
CGTGTTCCGGCCGACTCGCCAGAC
GGTAGGTTAATGGTGGCCGCGGCG
GTCGTTCGCTTCGCGGCGATCAGC
GCCGTCCGCGGTGCCGGTTTGCGA
CGAATCTTCGGGCCTCTTTCCGAC
CCGTCTTGAAACACCGGACCAA

***Phenacoccus_solenopsis*_Sh2**
TTTTCGCGCCGTGATCCGGGCGGC
AGATTAGAGACGATGGCGGCACGT
TGTCGGTCGATATTTCTGTCGCCG
GCACGGACGTCGCGATCGTTTGGT
GTCGGTCTCAGGAGACGCGTTTCA
CGTTCGTCGACGCGTCGTCTGCCT
CGGTAGGCGCGCGTTGCGAGTACG
CGTTCGTGTTCCGGCCGACTCGCC
AGACGGTAGGTTAATGGTGGCCGC
GGCGGTCGTTCGCTTCGCGGCGAT
CAGCGCCGTCCGCGGTGCCGGTTT
GCGACGAATCTTCGGGCCTCTTTC
CGACCCGTCTTG
ACAACGGGACCAA

***Phenacoccus_solenopsis*_Sh3**
CCCGGATTCAGGTACGGACAGAGC
CCGTGAATCCGGGCGGCAGAATTC
AGAGACGATGGCGGCAACGTTGTC
GGTTCGATATTTCTGTCGCCGGCA
CGGACGTCGCGACCCGTTTGGTGT
CGGTCTCAGGAGACGCGTTTCACG
TTCGTCGACGCGTCGTCTGCCTCG
GTAGGCGCGCGTTGCGAGTACGCG
TTCGTGTTCCGGCCGACTCGCCAG
ACGGTAGGTTAATGGTGGCCGCGG
CGGTCGTTCGCTTCGCGGCGATCA
GCGCCGTCCGCGGTGCCGGTTTGC
GACGAATCTTCGGGCCTCTTTCCG
ACCCGTCTTGAAACACGGACCAA

***Phenacoccus_solenopsis*_Sh4**
CCGGATTCGGGGTACGGACAGAGC
CCGTGATCCGGGCGGCAGAATTCA
GAGACGATGGCGGCAACGTTGTCG
GTTCGATATTTCTGTCGCCGGCAC
GGACGTCGCGACCCGTTTGGTGTC
GGTCTCAGGAGACGCGTTTCACGT
TCGTCGACGCGTCGTCTGCCTCGG
TAGGCGCGCGTTGCGAGTACGCGT
TCGTGTTCCGGCCGACTCGCCAGA
CGGTAGGTTAATGGTGGCCGCGGC
GGTCGTTCGCTTCGCGGCGATCAG
CGCCGTCCGCGGTGCCGGTTTGCG
ACGAATCTTCGGGCCTCTTTCCGA
CCCGTCTTGAAACACGGACCAA

***Phenacoccus_solenopsis*_Sh5**
CCCGGATCAGGTACGGAAGAGCCC

GTGATCCGGGCGGCAGAATTCAGA
GACGATGGCGGCAACGTTGTCGGT
TCGATATTTCTGTCGCCGGCACGG
ACGTCGCGACCCGTTTGGTGTCGG
TCTCAGGAGACGCGTTTCACGTTC
GTCGACGCGTCGTCTGCCTCGGTA
GGCGCGCGTTGCGAGTACGCGTTC
GTGTTCCGGCCGACTCGCCAGACG
GTAGGTTAATGGTGGCCGCGGCGG
TCGTTCGCTTCGCGGCGATCAGCG
CCGTCCGCGGTGCCGGTTTGCGAC
GAATCTTCGGGCCTCTTTCCGACC
CGTCTTGAAACACCGGACCAA

The sequences have been submitted to GenBank database and accession numbers were obtained (MN887775, MN887776, MN887777, MN887778 and MN887779). Mega-alignment was applied among the five nucleotide sequences that were found to be *P. solenopsis* in our study and the top five homologous hits of 28S rRNA from the NCBI databank. Additional five sequences of 28S rRNA of *P. solani* were used to extend the genetic variation. The data in the phylogenetic tree showed that the sequences can be divided into two major branches. One major branch has comprised all the *P. solenopsis* including those of the current study. While the other major branch has comprised the sequences of *P. solani*. The results proved that sequences of the current study are truly more related to *P. solenopsis* than *P. solani.* Also, the sequences were highly related with 98.9 % of similarity to each other and a maximum deviation 1.1% (Fig.2).

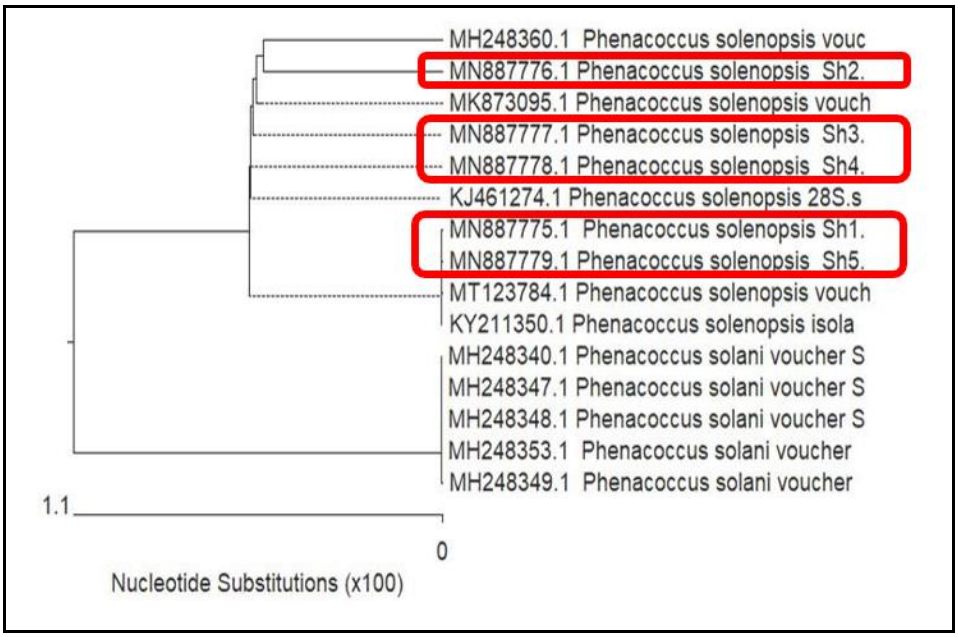

Fig. 2. Phylogenic tree based on the 28 rDNA gene sequences and relationship among the five accession numbers of identified strains (red squared) and the top homologous on the NCBI data base.

In conclusion, the aim of the study is to identify the sequencing of 28S rRNA of the cotton mealybug, *P. solenopsis*, due to the presence of other morphologically similar genera with a high percentage of this species that was discovered in Rashid Governorate where, the solanum mealybug, *P. solani*, is similar in appearance to the cotton mealybug *P. solenopsis*, and there is some evidence to suggest they might represent environmentally induced forms of the same species according to Hodgson *et al.* (2008) pointed out in their detailed study of

morphological variation that these three species might be environmentally induced variants of a single species. In addition to the correct identifying of the mealybugs is important for succeeding in the controlling, as biological control depends mainly on biological enemies or pheromone traps that are specific to certain species. (Dewer *et al*., 2012 and Mani and Shivaraju 2016). Therefore, the aim of this research reported that the populations of insect were indirectly related with the percentage of parasitism and confirmed that the dominant species at Sharkia Governorate was *P. solenopsis.* In addition, the PCR was useful for the rapid and cost-efficient identification of this species.

REFERENCES

Abd-Rabou, S., J.F. Germain and T. Malausa (2010). *Phenacoccus parvus* Morrison et *P. solenopsis* Tinsley, deux *Cochenilles nouvelles* pour l'Egypte (Hemiptera: Pseudococcidae). *Bulletin de la Société Entomologique de France, 115(4): 509- 510.*

Burland, T.G. DNASTAR's Lasergene sequence analysis software. *Methods in Molecular Biological*, 2000;132:71–91

Cavalieri, V., G. Mazzeo, G.T. Garzia, E. Buonocore, A. Russo (2008). Identification of *Planococcus ficus* and *Planococcus citri* (Hemiptera: Pseudococcidae) by PCR-RFLP of coigene. *Zootaxa*, 1816, 65–68.

Correa, M.C.G., J-F. Germain, T. Malausa and T. Zaviezo (2012). Molecular and morphological characterization of mealybugs (Hemiptera: Pseudococcidae) from Chilean vineyards. *Bulletin of Entomological Research* , 102: 524–530.

Dewer, Y., S. Abdel-Razak and A. Barakat (2012). Comparative efficacy of some insecticides against purple scale insect, *Lepidosaphes beckii* (Hemiptera: Coccoidea) and its parasitoid in citrus orchard in Egypt. *Egyptian Academic Journal of Biological Sciences,A.Entomology*, Vol. 5, 121–127.

Dewer,Y., R. S. Abdel-Fattah and S. A. Schneider (2018). Molecular and morphological identification of the mealybug, *Phenacoccus solani* Ferris (Hemiptera: Pseudococcidae): first report in Egypt. *Bulletin OEPP/EPPO*, 48(1), 155–159.

Douglas, N. and K. Kruger (2008). Transmission efficiency of grapevine leafroll-associated virus 3 (glrav-3) by the mealybugs *Planococcus ficus* and *Pseudococcus longispinus* (Hemiptera : Pseudococcidae). *European Journal of Pathology*, 122, 207–212.

Garcıa Morales, M., B.D. Denno, D.R. Miller, G.L. Miller, Y. Ben-Dov and N.B. Hardy (2016). ScaleNet: A literature-based model of scale insect biology and systematics. *Database*, 2016, bav118.

Hodgson, C.J., G. Abbas, M.J. Arif, S. Saeed and H. Karar (2008). *Phenacoccus solenopsis* Tinsley (Sternorrhyncha: Coccoidea: Pseudococcidae), a new invasive species attacking cotton in Pakistan and India, with a discussion on seasonal morphological variation. *Zootaxa*, 1913: 1-35.

Ibrahim, S.S., F.A. Moharum and N.M. Abd El-Ghany (2015). The cotton mealybug *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) as a new insect pest on tomato plants in Egypt. *Journal of Plant Protection Research*, 55 (1): 48-51.

Mahfoudhi, N., M. Digiaro and M. Dhouibi (2009). Transmission of grapevine leafroll viruses by *Planococcus ficus* (Hemiptera: Pseudococcidae) and *Ceroplastes rusci* (Hemiptera: Coccidae) .*Plant*

Disease, 93, 999–1002.

Malausa, T., A. Fenis, S. Warot, J.F. Germain, N. Ris, E. Prado, M. Botton, F. Vanlerberghe-Masutti, R. Sforza, C. Cruaud, A. Couloux and P. Kreiter (2011). DNA markers to disentangle complexes of cryptic taxa in mealybugs (Hemiptera: Pseudococcidae). *Journal of Applied Entomology*, 135, 142–155.

Mani, M. and C. Shivaraju (2016). Mealybugs and their management inagricultural and horticultural crops. Part I: Biology (Eds. Mani M &Shivaraju C), pp. 87–106. Springer, India.

Meyer, J.B., G.G.F. Kasdorf, L.H. Nel and G. Pietersen (2008). Transmission of activated-episomal banana streak ol(badna)virus (bsolv) to cv. Williams banana(musa sp.) by three mealybug species. *Plant Disease* , 92,1158–1163.

Nabil, H. A. and M.A.M. Hegab (2019). Impact of Some Weather Factors on the Population Density of *Phenacoccus solenopsis* Tinsley and its Natural Enemies. *Egyptian Academic Journal of Biological Sciences ,A.Entomology,* 12(2): 99–108.

Nabil, H.A., A.SH. Hassan, and SH.A.A. Ismail (2015). Registration of the cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Sternorrhyncha: Coccoidea: Pseudococcidae) for the first time on four economical crops in Egypt. *Zagazig Journal of Agriculture Research*, 42(6): 1555-1560.

Nakaune, R., S. Toda, M. Mochizuki, A. Nakano Rung, S.J. Scheffer, G. Evans and D. Miller (2008). Molecular identification of two closely related species of mealy-bugs of the genus *Planococcus* (Homoptera: Pseudococcidae). *Annals Entomological Society America* , 101, 525–532.

Pacheco da Silva, V.C., A. Bertin, A. Blin, J-F. Germain, D. Bernardi, G. Rignol, M. Botton and T. Malausa (2014). Molecular and Morphological Identification of Mealybug Species (Hemiptera: Pseudococcidae) in Brazilian Vineyards. *Plos One,* 9(7): e103267.

Park, D.S., S.J. Suh, P.D. Hebert, O.h. HW and K.J. Hong (2011). DNA barcodes for two scale insect families, mealybugs (Hemiptera: Pseudococcidae) and armored scales (Hemiptera: Diaspididae). *Bulletin of Entomological Research*, 101, 429–434.

Rezk, M., A.T. Hassan, M.F. El-Deeb, N. Shaarawy and Y. Dewer (2019). The impact of insecticides on the cotton mealybug, *Phenacoccus solenopsis* (Tinsley): Efficacy on potato, a new record of host plant in Egypt. *Journal of Agriculture Research*, 59(1): 50–59.

Rung, A., S.J. Scheffer, G. Evans and D. Miller (2008). Molecular identification of two closely related species of mealy-bugs of the genus *Planococcus* (Homoptera: Pseudococcidae). *Annals Entomological Society America*, 101, 525–532.

Saccaggi, D.L., K. Kruger and G. Pietersen (2008). A multiplex PCR assay for the simultaneous identification of three mealybug species (Hemiptera: Pseudococcidae). *Bulletin of Entomological Research* , 98, 27–33.

ARABIC SUMMARY

الرصد والتعريف الجزيئى للبق الدقيقى *Phenacoccus solenopsis* Tinsley على نباتات الباميه فى محافظه الشرقيه (Hemiptera: Pseudococcidae)

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4- قسم وقاية النبات – كلية الزراعة – جامعة الزقازيق – مصر

البق الدقيقي (Hemiptera: Pseudococcidae) عبارة عن حشرات ثاقبة ماصه وصغيرة الحجم وتصيب مدى واسع من المحاصيل والخضروات وتتشابه أنواعها، مما يجعل من الصعب على غير المتخصصين التمييز بينها. تم جمع العينات من نباتات الباميه المصابة من خمس مراكز بمحافظة الشرقية ، مصر .هدفت هذه الدراسة إلى دراسة توزيع حشرة البق القطن الدقيقى *Phenacoccus solenopsis* Tinsley وطفيلاتها المصاحبه لها. وكذلك التعرف على هذا النوع عن طريق التوصيف الجزيئي بسبب الدرجة العاليه من التشابه المورفولوجى مابين الإنواع المختلفة ذات الصله الوثيقة من بق الدقيقى. فوجد ان التطفل له تأثير كبير على حدوث الإصابة بتلك الحشرة وتم تأكيد هذا النوع من البق الدقيقي من خلال تضخيم وتسلسل مواقع الحمض النووي المعروف بأنها مفيدة لتحديد الأنواع من خلال توفير معلومات عن تسلسل الجين S28 الريبوسومي. وعلية فإن نجاح برامج المكافحة المتكاملة للآفات (IPM) ضد البق الدقيقي يعتمد على التعريف الصحيح لهذه الآفة عن طريق التوصيف الجيني لإختيار إفضل استيراتيجيات المكافحة ، من ناحية أخرى، فإن صعوبة تحديد هذه الأنواع بشكل صحيح يؤدى الى صعوبه اختيار الطرق المناسبة للمكافحه وخاصه المكافحة الحيوية حيث لكل آفة نوع معين من عدوها الطبيعى الذى يكافحها بيولوجيًا.