

/ Taxonomical Studies On Some Aphid Species By Using Modern Techniques

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الحجم / 104 p ; أبعاد الوعاء / 28 cm.	حقل الوصف المادى
College Of Agriculture. Department Of .Thesis (D. S.) - University Of Alexandria. Damanhour Branch / تبصرة أطروحة / .Economic Entomology	تبصرة نوع درجة الرسالة العلمية - أطروحة
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morphological features of alate adults. Identification of different aphid species mostly depends on the / الخ / species. have proven inadequate in differentiation between closely related Morphological characters alone and molecular genetic techniques in insect Throughout the last few years, many authors used biochemical ten aphid and molecular genetic fingerprint was started with differentiation among differentiation. Biochemical study has continued to complete aphid biochemical and species belonging to genus Aphis in Egypt, so the current .fingerprints with other aphid species molecular genetic -:The current studies aimed to study the following points – .Survey of some Macrosiphini and Rhapalosiphina aphid species in Egypt (1 Macrosiphini and Sub-tribe: Rhapalosiphina by using :Identification of eighteen aphid species belonging to Tribe (2 (markers Molecular genetic markers (Isozyme, SDS-Page total protein and RAPD-PCR Biochemical and Evaluation the efficiency of the used biochemical and molecular genetic techniques in aphid species (3 .discrimination construct a molecular key to identify some of Survey of biochemical and RAPD–PCR species – specific bands to (4 .those tested species .Studying the phylogenetic relationships among the eighteen tested aphid species (5 -:Survey of Aphid Species Under Consideration and New Record Species in Egypt .1	التبصرة الخاصة بالتعليق أو التلخيص

Sub-tribe: Rhopalosiphina) were) aphid species belonging to Tribe: Macrosiphini and Tribe: Aphidini Common years plants at some localities of Egypt, through out about two successive surveyed and collected from main host that fourteen aphid species belonging to extended from December, 2005 to June, 2007. Survey studies showed *Brachycaudus schwartzi*, *Acyrtosiphon pisum*, *Brevicoryne brassicae*, *Brachcaudus helichrysi*. tribe Macrosiphini i.e *Doctynotus sonchi*, *Myzus persicae*, *Nasonovia (Hyperomyzus)* ,, *Capitophorus elaeagina*, *Chomaphis inculata rosae*, *Macrosiphum (Sitobion) Pentalonia nigronervosa*, *Pleotrichophorus chrysanthemi*, *Macrosiphum ,lactucae* :four aphid species belonging to Tribe: Aphidini (Sub-tribe avenae and *Metopolophium dirhodum* , and *Rhopalosiphum maidis* and *Rhopalosiphum padi* were ,Rhopalosiphina) i.e *Hyalopterus pruni*, *Schizaphis graminum* .surveyed

during the present study. This *Appelia) shwartzi (Borner)* was recorded for the first time in Egypt) *Brachcaudus Prunus armeniaca* and peaches *Prunus persica* with high density during ,species was attacking leaves of apricot was confirmed at British Museum by Prof. May, 2006 at El-Tahrir, El- Behera Governorate. Identification procedure species was drafting descriptions for alate viviparous female of this new recorded R. Blackman. Verbal and .identify different species of Genus *Brachycaudus* in Egypt presented. Moreover a bracket key was constructed to

:Biochemical Genetic Characterizations .2

.Isozyme Electrophoresis .2.1

electrophoresis (Native-PAGE) to identify eighteen aphid species were subjected to Native – polyacrylamide gel The α -esterase, β - Four enzymatic systems were chosen for this purpose, which were .isozyme variations among them

Isozyme electrophoresis analysis showed 96.88 % of .esterase, Acid phosphatase, and Alkaline phosphatase observed in the the eighteen tested aphid species that twenty seven different bands were polymorphism among esterase was detected among the eighteen tested aphid -four enzymatic systems. Only one common band in β highest percent of the lowest polymorphism (87.5 %.) in all enzymatic systems, while the species to achieve .polymorphism was 100% in the other enzymatic systems

.SDS- Protein Electrophoresis .2.2

aphid species. Thirty two electrophoresis pattern analysis was also subjected among the eighteen Total protein SDS-Polyacrlamide gel. Molecular weights of recorded bands ranged from protein bands were recorded along addition one band was species – to 245.578 KDa. Two of them were recorded as monomorphic band; in 14.366 the eighteen electrophoresis analysis showed the lowest level of polymorphism among specific band. Total protein .(tested species (93.75%

:Molecular Genetic Characterizations .3

.RAPD-PCR analysis for the tested Aphid species .3.1

study, to analyze molecular marker and eighteen tested aphid species were subjected to RAPD-PCR fingerprint The with So, ten arbitrary primers were used for this purpose. The RAPD analysis .estimate polymorphism among them range of molecular sizes. Four monomorphic the ten primers gave 101 different DNA fragment bands with wide recorded; most of them occurred with primer B10. So, the lowest value of distinct fragment bands were polymorphic bands were produced by polymorphism (80%) was generated by it. On the other hand, the highest and Z1 to achieve polymorphism levels reach 100%. The highest number primers C15, D2, I17, L12, L20, UBC75 lowest number was five bands, DNA fragment bands (fifteen bands) was observed in primer D5, while the of fragment bands were expressed as species – specific bands. Most of generated by primer L13. Twenty six DNA species- specific bands. The complied occurred in primer I17, while each of primer L12 and L20 gave one them .polymorphism among the eighteen tested aphid species %95 data for the ten primers recoded

Evaluation the Efficiency of the Used Biochemical and Molecular Genetic Techniques in the Aphid Species .4

-:Discrimination

revealed the highest level of polymorphism, it is obvious that each of isozyme and RAPD electrophoresis analysis. It molecular electrophoresis analysis. The electrophoresis study for those different comparing with total protein molecular system were detected, seven of them were systems revealed that 160 different bands pattern in all used species as in some species, while thirty four bands were observed bands in the all tested considered as common in those different molecular systems reflected 95.21% species - specific bands. The electrophoresis studies among the tested species polymorphism.

5. Survey of Biochemical and RAPD-PCR Species – Specific Marker for the Tested Aphid Species .5
specific bands were generated by the ten – It is obvious from obtained data that 26 of DNA fragment species. Each of .distinguish thirteen aphid species out of the eighteen aphid species arbitrary primers which could *Macrosiphum rosae* and *Metopolophium dirhodum*, *Acyrtosiphon pisum*, *Brachycaudus schwartzi*, *Myzus persicae* *sonchi* harbored the species- specific markers with the used primers. In contract, *Doctynotus* didn't generate any the used primer. Primer I17 was the most effective primer highest number of DNA species – specific bands with distinguish five aphid species generated the highest number of species - specific marker. So it could which and *Hyperomyzus*) *lactucae*, *Macrosiphum* (*Sitobion*) *avenae*, *Hyalopterus pruni*) [*Brevicoryne brassicae*, *Nasonovia* species – specific marker, which can *Schizaphis graminum*]. While each of primers L12 and L20 gave one .and *Hyalopterus pruni*, respectively distinguish *Rhopalosiphum maidis* analysis. were observed in each of β - esterase and Acid phosphatase isozyme. Most of obtained isozyme markers species [*Brevicoryne brassicae*, *Brachycaudus schwartzi*, *Rhopalosiphum padi* and ,*elaeagina*, *Nasonovia* (*Hyperomyzus*) *lactucae*, *Macrosiphum rosae* *Capitophorus* protein analysis could distinguish only *Capitophorus elaeagina* by *Hyalopterus pruni*]. On the other hand, Total .protein band with molecular weight 23.789 Kda could distinguish declared that the used biochemical and RAPD – PCR analysis techniques. The previous results species – specific marker weren't observed in each of fifteen aphid species out of eighteen; however any .*pisum*, *Myzus persicae* and *Metopolophium dirhodum* *Acyrtosiphon* be differentiated well by revealed that the fifteen species, with species - specific markers, can. The present study based on only using β - esterase and Acid phosphatase isozyme analyses applying electrophoresis analysis program L12, L13, UBC75 and Z1 which can save ,addition to application of RAPD-PCR analysis with primers B10, I17 in specific makers, – the fifteen aphid differentiated species. So it scored all species time, material and efforts with .which can differentiate among them aphid species out of the hand, a branching molecular key was constructed to identify thirteen. On the other .specific bands – eighteen aphid species based on 26 DNA species

6. Phylogenetic relationships .6
tested aphid species was genetic similarities and phylogenetic relationships among the eighteen. Studying the character analysis, isozyme, totals protein, and RAPD- PCR analysis, as based on obtained data of morphological .well as the combined effect of those techniques characters reflected that the matrix analysis of the eighteen aphid species based on morphological. The proximity between *Brachcaudus helichrysi* and *B. schwartzi*, while the lowest highest similarity value 93.8% was noticed *Acyrtosiphon pisum* and *Chomaphis inculata*, similarity value in morphological character was recorded between depending on that those aphid species can be separated into three main groups which was 42.9%. It declared divided into two sub groups, the first included *Brachcaudus* their morphological characters. The first main group *Doctynotus sonchi*, *B. schwartzi* and *Chomaphis inculata*, and the second sub group included ,*helichrysi* *Rhopalosiphum padi*, *Macrosiphum* (*Sitobion*) *avenae* and ,*Macrosiphum rosae*, *Nasonovia* (*Hyperomyzus*) *lactucae* and *Brevicoryne*. The second main group contains *Rhopalosiphum maidis*, *Hyalopterus pruni* .*Myzus persicae*

two sub groups, the first sub group included *Capitophorus brassicae*, while the third main group divided also into second included *Metopolophium Pleotrichophorus chrysanthemi* and *Pentalonia nigronervosa*. While the ,*elaeagina* .*Acyrtosiphon pisum dirhodum*, *Schizaphis graminum* and aphid species based on combined effect of isozyme, Total protein The proximity matrix analysis for the eighteen was recorded between *Rhopalosiphum* (RAPD-PCR analysis reflected that the highest similarity value (89.9% and similarity in all tested parameters polymorphism (43.9%) was recorded *maidis* and *R. padi*, while the lowest Dendrogram analysis can separate the .between *Brachycaudus helichrysi* and *Pleotrichophorus chrysanthemi* and others. Moreover it showed clearly the gab between Tribe: Macrosiphini Greaminaceae host plant aphid from the eighteen tested aphid species can be Tribe: Aphidini (Sub-tribe: Rhapalosiphina). The study declared that analysis. The first depending on combined effect of isozyme, Total protein and RAPD classify into two main groups sub group includes the Greaminaceae host plants aphid i.e. main group divided into three sub groups, the first *Sitobion*) *avenae* and) *maidis*, *R. padi*, *Schizaphis graminum*, *Hyalopterus pruni*, *Macrosiphum Rhopalosiphum* includes *Acyrtosiphon pisum*, *Brevicoryne brassicae*, *Metopolophium dirhodum*; while the second sub group *Pleotrichophorus chrysanthemi*, *elaeagina* and *Doctynotus sonchi*; the third sub group contains *Capitophorus Myzus persicae*, *Nasonovia (Hyperomyzus) lactucae* and *Pentalonia Macrosiphum rosae*, *Chomaphis inculata* and closely related species *Brachycaudus nigronervosa*. On the other hand, the second main group includes two .*helichrysi* and *B. schwartzi*

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