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Melanization rates of the parasitoid *Anagyrus* sp. nr. *pseudococci*, (Hymenoptera, Encyrtidae) by Parasitized against five mealybug species (Hemiptera: Pseudococcidae).

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Abstract \

This experiment was aimed to study melanization rates of the parasitoid *Anagyrus* sp. nr. *pseudococci*, (Hymenoptera, Encyrtidae) when this parasitoid was parasitized on five hosts of melybug species (*Planococcus ficus*, *Planococcus citri*, *Pseudococcus calceolariae*, *Pseudococcus viburni*, *Phenococcus peruvianus*) the results showed from the highest number to the lowest number of all eggs (nun melenized eggs + melenized eggs) in the hosts of mealybugs *P. citri* 63, *P. fcus* 52, *P. calceolaria* 27, *P. peruvianus* 27 and *P.viburni*19 gradually, from all numbers of eggs, the melanized eggs were arranged from the highest number to the lowest number regarding the sum of all eggs in each host were gradually. Also, the sum of the larvae from the highest number to the lowest number were arranged (nun melenized larvae + melenized larvae) in the hosts of mealybugs *P. ficus* 37, *P. citri* 35, *P. peruvianus* 25, *P. viburni* 20 and *P. calceolaria* 20, whereas, the highest number to the lowest regarding the sum of all eggs in each host were *P. viburni* 18, *P. citri* 7, *P. calceolaria* 2, *P. ficus* 2 and *P. peruvianus* 1.

Keywords: Melanization rates, *Anagyrus* sp. *near pseudococci*, Mealybug species, parasitoid, eggs, Larvae.

Introduction \

mealybugs belong to soft scale insect, they belong to Pseudococcidae family, order Hemiptera [1,8]. These species of insects are important pest which effect of many kinds of plants including vegetables, fruit trees. The problem of these pest when they present in new origin which their parasitoids or predators absent, they cause damage in national production [7,9,11,12].

Anagyrus sp. nr. pseudococci parasitizes on mealybug species, lately, in many countries have started in using this parasitoid in biological control program to reduce the damage of this pests. [14] has registered *A. sp. nr pseudococci* in Italy (Sicily), morphologically, its similar to *Anagyrus pseudococci*, except, the first funicle segment of antenna (in *A. pseudococci* half is black and other half is white, whereas, *A. sp. nr. pseudococci* is completely black).

In this study, we aimed to present the of melanization rates of the parasitoid *A. sp. nr. pseudococci* against five mealybug species when the eggs and larvae of parasitoid covered by melanin which secreted by mealybugs.

Material and methods \

Rearing of Mealybugs: Mealybugs were collected from two regions in Portugal, (Mainland and Azores) from two host plants, sweet orange and Grapevine, these mealybugs were reared on sprouted potatoes (*Solanum tuberosum* L.) several generations (six generations). Third instars of each 13 species were isolated on sprouted potatoes within ventilated plastic boxes seven days before the beginning of the experiments to standardize age, physiological state and obtain pre-reproductive adult females. Isolated mealybugs were kept at controlled conditions (25.0±0.5oC, 55-65% RH, in the dark).

Parasitoid rearing: The parasitoid was collected from their host *P. citri* in citrus orchards in silves region (Portugal). To start a colony in laboratory, about 30 individuals of parasitoid were used. The parasitoid was rearing in plastic box on *P. citri* under laboratory conditions (25.0±0.5ºC, 55-65% RH) to obtain several generations.

Experiments: Under laboratory conditions (19-22ºC and 55-65% RH) the experiments were conducted at 12PM. In each of 22 replicates, one adult parasitoid female was exposed to 10 pre-reproductive adult mealybug females in a Petri-dish (9 cm diameter), and observed 30 min.

Dissection of mealybugs: At the end of each experiment, the mealybugs were kept in the same Petri-dish was used 7days. After that, the mealybugs were immersed in clarification solution consisting of 1-part glacial acetic acid and 1 part chloral-phenol and then dissected to count the number of melanisations of the

parasitoid *A. sp. nr. pseudococci* on five mealybug species when the eggs and larvae of parasitoid covered by melanin which secreted by mealybugs.

Statistical analysis: The number of all eggs and larvae of the parasitoid were counted, also the number of melanized eggs and melanized larvae were counted in five mealybug species. All data were carried out using IBM SPSS 25.0 for Windows (IBM Corporation, Armonk, New York, USA).

Results \

According to the table (1), the results were presented from the highest number to the lowest number of all eggs (nun melenized eggs + melenized eggs) in the hosts of mealybugs *P. citri* 63, *P. ficus* 52, *P. calceolaria* 27, *P. peruvianus* 27 and *P. viburni* 19 gradually, whereas, the melanized eggs were arranged from the highest number to the lowest number regarding the sum of all eggs in each host were *Ps.viburni* 17(image 1), *P. citri* 35(image2), *P. calceolaria* 13(image3), *P..ficus* 19(image4), *P. peruvianus* 4(image5) gradually. the results were presented from the highest number to the lowest number of all larvae(nun melenized larvae + melenized larvae) in the hosts of mealybugs *P. ficus* 37, *P .citri* 35, *P. peruvianus 25, P.viburni* 20 and *P. calceolaria* 20, on other hand, the highest number to the lowest regarding the sum of all eggs in each host were *P. viburni* 18(image1), *P .citri* 7(image2), *P. calceolaria* 2(image3), *P. ficus* 2(image4) and *P. peruvianus* 1(image5).

Table 1. Presents from the highest number to the lowest number of all eggs and larvae compering to the highest and lowest number of melanized eggs and larvae for five hosts of mealybugs.

	SUM of N̈° eggs	SUM of melanized egg	SUM of N̈° larvae	SUM of melanized larvae
P. citri	63 a	35 a	35 a	7 b
P. calceolaria	27 c	13 c	20 c	2 c
P. ficus	52 b	19 b	37 a	2 c
P. viburni	19 d	17 b	20 c	18 a
P. peruvianus	27 c	4 d	25 b	1 c
Grand Total	188	88	137	30
Average	37.6	17.6	27.4	6

Mean in columns following by the same letter were no statistically different at P<0.05 (Duncans multiple range test).

The melinized eggs of the parasitoid accoding to the mealybug hosts

P. viburni, *P. citri,* *P. calceolaria,* *P. ficus,* *P. peruvianus*

The melinized larvae of the parasitoid accoding to the mealybug hosts

P. viburni, *P. citri,* *P. calceolaria,* *P. ficus,* *P. peruvianus*

All these images have been taken by microscope USB connected to lab top.

Discussion \

The host mealybugs present the reaction against the parasitoid attack through defense behavior or immune response [2,3,4,8]. the immune response appears as encapsulation or Melanization reactions[10], this reaction happens against strange body which has been inserted inside the body, that what happened in this study when the parasitoid laid her eggs inside the host, this reaction agrees with [13] when the three mealybug species(*Planococcus ficus* (Signorel), *Planococcus citri* (Risso) and *Pseudococcus calceolariae* (Maskell) were shown to encapsulate the eggs, in this study was the same reaction against the eggs or larvae of the parasitoid. also, this reaction has been registered by [5] when the defense reaction of the host mealybug *Phenacoccus manihoti* (hemiptera: Pseudococcidae) against the parasitoid *Epidinocarsis lopezi* (De santis), in the same context,[10] *Maconellicoccus hirsutus* Green presented celluar defense reaction against the parasitoid *Anagyrus kamali* Moursi,

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Anagyrus sp, nr, pseudococci (Hymrnoptera, Encyrtidae) معدل الميلانين لطفيل حينما يتطفل على خمس أنواع من البق الدقيقي (Hemiptera: Pseudococcidae)

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المستخلص /

هدف هذه التجربة لدراسة معدل الميلانين لطفيل *Anagyrus* sp. nr. *pseudococci*, (Hymenoptera,Encyrtidae) حينما تطفل على خمس أنواع من العوائل للبق الدقيقي (*Planococcus ficus, Planococcus citri, Pseudococcus calceolariae, Pseudococcus viburni, Phenococcus peruvianus*) أظهرت النتائج من أعلى عدد إلى أقل عدد من البيوض(البيوض الغير مغطاه بالميلانين + البيوض المغطاه بالميلانين) التي وجدت داخل عوائل البق الدقيقي *P. citri* 63, *P. ficus* 52, *P.calceolaria* 27, *P. peruvianus* 27 and *P. viburni* 19 على التوالي، من المجموع الكلي لأعداد تلك البيوض، كانت أعداد البيوض المغطاة بالميلانين من أعلى عدد إلى أقل عدد مع الأخذ بعين الأعتبار المجموع الكلي لأعداد البيوض في كل عائل كانت 4 *P. peruvianus*, 19 *P. ficus*, 13 *P. calceolaria*, 35 *P. citri*, 17 *P. viburni* على التوالي. أيضا، مجموع اليرقات من أعلى عدد إلى أقل عدد (اليرقات الغير مغطاه بالميلانين + اليرقات المغطاة بالميلانين) كانت *P. ficus* 37, *P. citri* 35, *P. peruvianus* 25, *P.viburni* 20 *and P. calceolaria* 20، في حين اليرقات المغطاة بالميلانين من أعلى عدد إلى أقل عدد أيضا مع مع الأخذ بعين الأعتبار المجموع الكلي لليرقات لكل عائل كانت *P. viburni* 18, *P. citri* 7, *P.calceolaria* 2, *P. ficus* 2 *and P. peruvianus*1 على التوالي.

الكلمات الدالة : معدل الميلانين، طفيل *Anagyrus sp. near pseudococci*، البق الدقيقي، الطفيل، البيض، اليرقات.