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Corresponding author: Hassan G I Ali Hassan.g.el-shrief@agr.sohag.edu.eg **Resistance** Gene Interactions to Acaricides of Different Chemical Classes in Interline Crosses of Two-Spotted Spider Mite *Tetranychus urticae*.

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Abstract

The descriptive homozygous selection of resistance genes of the two-spotted spider mites was to five acaricides (malathion, abamectin, fenpyroximate, bromopropylate and bifenthrin) of different chemical classes. Females were treated by the diagnostic concentrations of these acaricides, which obtained crossing with diheterozygous females. Hybrid females showed no resistance to abamectin, fenpyroximate, brompropylate, and bifenthrin, but the mortality under malathion increased 1.5-2 time compared with homozygous females. Epistatic interaction of resistance genes was manifested due to incompatibility of biochemical processes of protection against poisoning determined by them to toxicants of different chemical classes. The coefficient of correlation R^2 and the regression coefficient β were subjected to present the dependency of Y (dependent variable) upon X (independent variable). On the other hand, the regression coefficient shows that a unit change in X variable will bring change in Y variable. In addition, Euclidean distance analysis distinguish the superiority resistant genes throughout the chemical classes.

Keywords:

Resistance sign, Acaricides, disruptive selection, genotype, twospotted spider mite, Correlation and Regression coefficient.

INTRODUCTION

Tetranychus urticae Koch (Acari: Tetranychidae) is a significant agricultural pest with a worldwide distribution. (Stumpf and Nauen 2002). In Egypt T. urticae, is one of the phytophagous pests, attacking cotton, fruit trees and vegetables. It usually feeds on the leaves, whose epidermis is damaged, resulting in yellow, brown blotch accompanied by dry leaf-fall (Abd El-Moneim, et. al., 2011). It's high reproductive potential and short life cycle, responsible for substantial output losses in horticultural, ornamental, numerous and agricultural crops around the world.

The capacity of T. urticae to quickly develop resistance to many significant acaricides after only a few applications is one of the main issues in controlling this species. (Ali, et.al., 2020). Therefore, it's crucial to switch between acaricides with various modes of action to enable effective resistance management. mechanism-based Several instances of resistance in T. urticae to various chemical acaricide classes have been identified. including mitochondrial electron transport inhibitors (METI) (Stumpf and Nauen, 2001), neurotoxic compounds such as abamectin (Campos et al., 1996: Stumpf and Nauen, 2002), bifenthrin (Van Leeuwen et. al., 2005: Ay, and Oktay GiJrkan, 2005), and malathion (Ali, et.al., 2020).

Analysis of genetic relationship (Euclidean) among genotypes is an important component and play a major role in their effective utilization and serve as a platform for the selection of superior genotypes to be used as parents in hybridization programs in order to identify a desirable segregates for the traits under the concerned study (Salgotra, 2015 and Sandhu and Kumar, 2017). Therefore, in the current study, Euclidean cluster analysis was used to construct a distance matrix for lines and their F1 crosses. This study examined the resistance characteristics of a *T. urticae* strain subjected to five acaricides compounds and indicated the epistatic interaction of alleles of resistance to acaricides of different chemical classes.

MATERIALS AND METHODS

Rearing of two-spotted spider mite

The two-spotted spider mite (*Tetranychus urticae* Koch) were reared continuously on bean plants, *Canavalia ensiformis* L., under laboratory conditions at $25 \pm 1^{\circ}$ C, $70 \pm 5^{\circ}$ RH and a 16h photoperiod.

Acaricides bioassay

The acaricides used were malathion (50%EC), abamectin (1.8 g/L EC) , fenpyroximate (5% EC), bromopropylate (50 g/L EC) and bifenthrin (10 g/L EC).Ten adult females of *T. urticae* of the same family (originated from single female) were placed on a bean leaf disc on water-soaked cotton in a plastic dish (12×25 cm). Acaricides bioassay was conducted using the diagnostic dose by the dipping method. The treated mites on the leaf bean were kept at $25 \pm 1^{\circ}$ C and a 16h photoperiod and mortality was calculated 24 h after treatment. Individual survival mite was considered by touching each mite with a fine brush.

Selection for resistance

Five lines of gravid females were selected for resistant against five acaricides and disruptive selection was conducted using the diagnostic concentrations of each acaricide ($LC_{95} \times 2$ for the mites of a susceptible strain). In each line, the mites were bred in families from single female after an inbred cross. The families derived from individual female were kept on the bean leaf discs placed on moistened cotton wool in crystallizers at a long day photoperiod. Mite families were treated with the diagnostic dose of the selected acaricide using the dipping methods. Survivors of 2-3 families which showed less susceptibility after 24 h were used to initiate the next generation. In each generation, ten females only were taken from each the two or three, families and used for the acaricide bioassay and the remaining were kept to produce the next generation. The LC_{95} values were calculated by the prophet's method according to Litchfield and Wilcoxon (Belenkiy, 1959).

Statistical analysis

Correlation and regression coefficient (Kozak *et. al.*, 2012 and Al-Salim *et. al.*, 2017) among two-spotted spider mite lines and their F1 crosses toward five chemical classes were calculated to investigate the correlation effects from cross to cross on the expected proportion of lines for the mortality rate %. The correlation and regression coefficients were statistical analysed using (MS Excel) computer software. In addition, Euclidean cluster analysis dendogram was performed by the statistical analysis package PAST-3.

RESULTS AND DISCUSSION

Haplo-diploid species of arthropods include the two-spotted spider. Only haploid males are produced by virgin females, while diploid and haploid females are produced by impregnated females. Males who were resistant to certain acaricides were produced by females who were heterozygous for such resistance. (Oku and van den Beuken, 2017). Via diagnostic doses of selective acaricides, families with the lowest mortality rates were found using disruptive selection for the presence or absence of a trait of resistance to five acaricides of two-spotted spider mite lines. In the fig (1) shows the arithmetic mean values of the percentage of mortality used for crossing females from these families.

Reciprocal crossing of males with deutonymphs from these families resulted in a generation of diheterozygous mites with genome-compatible alleles of resistance to all different acaricides. All combinations of the R- gene to malathion with R-genes to other acaricides were individually evaluated on the half of the hybrid females of each family that contained the R-genes against acaricides of two different chemical groups. In comparison to parent females homozygous for the R-gene malathion, the mean mortality rate increased by 1.5 to 2 times when the diagnostic concentration of malathion impacted hybrid females. (Fig. 2). The largest number of hybrid families when mites were treated with a diagnostic concentration of malathion turned out to have a mortality rate of females of 30 and 70%, compared with the mortality of females in parental families - 20-40% According to the indicator of female mortality attributable to abamectin, fenpyroximate, brompropylate, and bifenthrin diagnostic concentrations (Fig. 2).

Epistatic interaction of alleles of resistance to acaricides of various chemical classes combined in the mite genome occurs at the stage of their regulation of biochemical processes that counteract the development of the pathogenesis of poisoning (Sundukov, et. al., 2017). When malathion induces the expression of the resistance gene to this toxicant, the transport functions of the plasma membrane of cells are normalized with the participation of the carboxylesterase isozyme, the increased synthesis of which is encoded by this gene (Bass, and Field, 2011).

The third step of gene expression, phenotypic expression, involves epistatic interactions between R-genes to acaricides using various methods (Sundukov, *et. al.*, 2017). Due to the nature of the dominant-recessive relationship between the biochemical reactions regulated by the genes, the combination of alleles for resistance to malathion with alleles for resistance to other acaricides, abamectin, fenpyroximate, brompropylate, and bifenthrin was suppressed but not completely. The presence of different chemical classes of acaricide resistance alleles in the genome of arthropods prevents their expression to each of the toxicants.

For the two-spotted spider mite lines the correlation coefficient was 0.359 while the regression coefficient was 5.302. On the other hand, the correlation coefficient and the regression coefficient for their crosses were 0.274 and 5.173, respectively as showed in Fig. 3 and 4. The correlation and regression coefficient values suggested that Mortality rate % is the most important trait that plays principal role and have a direct influence in management throughout chemical classes. Thus, variability for this character among different lines is a good sign, because of regression predicts the value of the dependent variable based on the independent variable to explain variation in the Y variable based on changes in the X variable (Ashraf, et al., 2014 and Prajapat et. al., 2020). Regression is a method for examining the relationships between qualities, and regression analysis is a statistical method for determining whether there is a linear relationship between the dependent variable and the independent variables (Kozak et. al., 2012 and Al-Salim et. al., 2017). Kose et. al., 2018, determine the best indirect selection criteria for genetic improvement of in spring safflower in terms of oil yield emphasized that seed weight had positive and significant regression coefficient.

According to the Euclidean cluster analysis dendrogram, the two-spotted spider mite lines and their F1crosses were classified into two main group against the studied five chemical classes Fig. 5 and 6 The first group of the twospotted spider mite lines include R-Abamectin, **R**-Fenpyroximate **R-Bromopropylate** and chemical classes. Meanwhile, the second group **R**-Bifenthrin **R**-Malathion contains and chemical classes. On the other hand, the first group for their F1 crosses includes, R-Malathion chemical class while the second

group content the others chemical classes. Euclidean dendogram cluster analysis is a suitable solution to group and select desirable lines for selection which can be exploited in future breeding programs (Salgotra, 2015 and Sandhu and Kumar, 2017).

The reflects correlation coefficient the response of mortality rate % with its counterpart. In addition, provides a good index corresponding predict the change. to Regression is a method for examining the relationships between qualities, and regression analysis is a statistical method for determining whether there is a linear relationship between the dependent variable and the independent variables (Kozak et al., 2012 and Al-Salim et al., 2017). Kose et al. (2018) determine the best indirect selection criteria for genetic improvement of in spring safflower in terms of oil yield emphasized that seed weight had positive and significant regression coefficient.

CONCLUSION

Resistance gene interactions of different chemical classes acaricides was not showed to abamectin, fenpyroximate, brompropylate, bifenthrin, and it was increased 1.5-2 time to malathion acaricide as compared with homozygous females. Correlation and regression coefficient and Euclidean dendrogram cluster analysis have approved effective in selection criteria for creating new ecological lines.



Fig. 1. Mortality rate of the two-spotted spider mite homozygous for resistance genes in response to different acaricides.



Fig.2. Mortality rate of interlinear hybrid females of the two-spotted spider mite with various combinations of resistance alleles in the genome to several acaricides.



Fig. 3. Scatter diagram with phenotypic coefficient of correlations and regression for Mortality rate % for the studied twospotted spider mitelines throughout the studied five chemical classes.



Fig. 4. Scatter diagram with phenotypic coefficient of correlations and regression for Mortality rate % for the studied twospotted spider mite lines throughout the studied five chemical classes.



Fig. 5. Euclidean dendogram cluster analysis for Mortality rate % for the studied two-spotted spider mite lines throughout the studied five chemical classes.



Fig. 6. Euclidean dendogram cluster analysis for Mortality rate % for the studied two-spotted spider mitelines F1 crosses throughout the studied five chemical classes.

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تفاعلات المقاومة الجينية المتداخلة لمجموعات كيميائية مختلفة من المبيدات الأكاروسية في أكاروس العنكبوت ذو البقعتين Tetranychus محسن جبر الله إسماعيل¹، تمارا أستر اخانافا²، هيثم محي الدين عبدالرحمن³ ¹قسم وقاية النبات – كلية الزراعة – جامعة سوهاج - مصر. ²معهد التكنولوجيا الزراعية - جامعة الصداقة – روسيا. الاتحادية ⁸قسم الوراثة – كاية الزراعة جامعة سوهاج - مصر.

الملخص العربي

تم اختيار السلالات الحاملة لجين المقاومة في أكاروس العنكبوت ذي البقعتين في خمسة مبيدات أكاروسية وهي (مالاثيون ، أبامكتين ، فينبيروكسميت ، بروموبروبيلات، وبيفنثرين) من مجموعات كيميائية مختلفة. تم معاملة الإناث من خلال التراكيز التشخيصية لكل من هذه المبيدات. لم تظهر الإناث الهجينة أي مقاومة للأبامكتين والفينبير وكسميت والبر ومبر وبيل والبيفينثرين ، في حين زاد معدل الوفيات تحت تأثير مبيد الملاثيون بما يعادل 1.5-2 مرة مقارنة بالإناث متماثلة الزيجوات. ويرجع التفاعل المعرفي لجينات المقاومة بسبب عدم توافق العمليات البيوكيميائية لمقاومة التأثير السام التي تحددها هذه \mathbb{R}^2 الجينات للمبيدات المختلفة. تعرض معامل الارتباط ومعامل الانحدار β لتقديم تبعية Y (متغير تابع) على X (متغير مستقل). من ناحية أخرى ، يوضح معامل الانحدار أن تغيير الوحدة في متغير X سيؤدي إلى تغيير في متغير Y. بالإضافة إلى ذلك ، يميز تحليل الشجرة الوراثية بين الجينات المقاومة للتفوق في جميع المجموعات الكيميائية المختلفة

الكلمات المفتاحية: معدل المقاومة ، المبيدات الأكاروسية ، النمط الوراثي ، أكاروس العنكبوت ذو البقعتين ، معامل الارتباط والانحدار.