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Research Article

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Comparative Aspects of the Formation of Resistant Populations of the Twospotted Spider Mite Tetranychus Urticae Koch (Acariformes, Tetranychidae) to Two Groups of Avermectin Preparations

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ABSTRACT

The comparative rate of development of resistance to insectoacaricides based on abamectin (vertimec) and aversectin C (phytopharm-M and phytoverm) of the twospotted spider mite Tetranychus urticae Koch was studied under laboratory conditions by the method of artificial selection. The production population («Mullinsky hothouse factory», Perm) with initial 2-fold resistance to these drugs was systematically treated by them in sublethal concentrations during the evolution of 72 generations of mites. 50 treatments were performed, as a result of which the level of resistance to the vertimec increased by 38.6 times, to phytophythm-M and phytophythm in 63.3 and 102.2 times, respectively. The process of formation of resistance passed the stages characteristic for pesticides with their continuous application.

Keywords: *twospotted spider mite Tetranychus urticae, toxicity, resistance, insectoacaricides, abamectin, aversectin C.*

INTRODUCTION

At the present time, insectoacaricides based on avermectins synthesized by actinomyces Streptomyces avermitilis are widely used to protect plants from herbivorous mites. According to their chemical structure, they are macrocyclic lactones. Depending on the composition of the active substance, the preparations can be divided into two groups: bicomponent and multicomponent. For bicomponent drugs (vertimec, dainamec, abac), an avermectin-containing substance is one homologous pair of avermectins (B_{1a} and B_{1b}) isolated from the synthesized complex [1]. On the basis of a fundamentally different scientific idea, a multicomponent active substance containing four homologous pairs of avermectins - A1, A2, B1, B2 was developed. Purified natural complex, which includes a strict ratio of avermectins of groups B and A, the accumulative and synergistic effect of which provides a high insecticocaricidal effect, was named aversectin C. Based on aversectin C, Russian researchers have developed Fitoverm preparations and have established promising directions for their use in the protected soil of Russia against the main pests of vegetable and ornamental plants (spider mites, aphids, thripses), [2, 3].

In the struggle against herbivorous mites in the sheltered ground vertimac, preparations of the brand Phytoverm (phytoverm, phytoverm-M) are used for more than 20 years. We can say that they are still leading among a relatively small number of assortment of insectoacaricides based on organophosphorous compounds, Biphenthrin, Bacillus thuringiensis, allowed for use in the territory of the Russian Federation. As a rule, prolonged unilateral use of one type of pesticide consistently leads to a decrease in its effectiveness. For today, both in our country and abroad, there are data on a decrease in the effectiveness of avermectins.

The formation of resistance of herbivorous mites against the background of avermectins treatments is widely wrote about in the world literature, mainly they relate to preparations based on abamectin. Immediately after the appearance of abamectin in the pesticide market (1987), it was believed that, due to the complicated composition of the active substance (a complex of closely related molecules), such a reaction is either not realized, or is extremely slow, and more likely in a heterogeneous population [4]. However, after 10 years of use in different regions of the world, there have been reports of the formation of mites' resistance to abamectin. Thus, a monitoring of the sensitivity to abamectin of spider mites selected on ornamental plants in California revealed a spread of toxicological resistance indicators (RI) values from 1 to 658-fold. Later, resistance analysis of spider mites selected in hothouses (in California, Florida, the Canary Islands and in the Netherlands) showed the variation of the resistance of mites to abamectin from 0.5 to 175-fold levels [5, 6].

In the states of Washington and Oregon, after 7-8 years of using abamectin, mites formed an 80-fold resistance to it [7]. In Mexico, spider mites on fruit plants treated with acaricides about 30 times per season, have formed populations resistant to abamectin: RI = 233 x [8]. In Brazil, due to a decrease in the effectiveness of avermectin preparations against spider mites, large-scale studies to study the specific features of the formation of phytophagous resistance to them (speed of evolution, cross-resistance, stability) are underway. It was noted that field populations of twospotted spider mite collected from fruit plant and chrysanthemums treated with abamectin and milbemectin as a result of artificial selection in the laboratory acquired, respectively, 342 and 409-fold resistance values literally after 5-6 treatments [9, 10].

In our initial studies, sensitivity to aversectin C in different populations of the twospotted spider mite Tetranychus urticae Koch was studied in detail. There were significant interpopulation differences in this feature, which are most apparent in the phytophagan in recent years [11-13].

In the protected ground of Russia, the twospotted spider mite T. urticae is a widespread and most dangerous pest. The range of pesticides that are allowed to be used against mites in the country is extremely limited, which causes serious concern for plant protection specialists due to resistance to avermectins and often forces them to use unpopular measures: increase concentrations of used pesticides or multiplicity of treatments. But this solves the problem only for a short time. In this situation, resistance to pesticides prevents the effective control of pests, which means that it is necessary to improve non-resistant systems of their rational use on the basis of studying the characteristics of resistance formation.

Therefore, it seemed expedient to study the relative rate of development of resistance of the twospotted spider mite T. urticae to avermectin preparations used in Russia's agricultural production: vertemec, 18 g/l (abamectin), phytoverm (emulsion concentrate), 2 g/l (aversectin C) and phytoverm -M, 2 g/l (aversectin C).

MATERIALS AND METHODS

In this study, we used the population of the twospotted spider mite T. urticae from the cucumber culture («Mullinsky hothouse plant», Perm). In comparison with similar populations from other hothouse farms, it was characterized by the widest range of reactions to the effects of avermectins [12].

Mites in the laboratory were kept in isolated glass boxes on young plants of Saksa bush beans at a temperature of 22 ± 3 ° C, relative humidity of air 55-70%, light day 18 hours.

The initial population was divided into three subpopulation lines, each of which was treated with the appropriate preparations (vertimec, phytophythm-M and phytophythm). As the effectiveness decreased, the norm with the next treatment of the preparations was increased, determining the level of resistance of the mite.

The selective treatments were carried out as follows: the bean plants infested with a spider mite were immersed for 3 seconds in an aqueous solution of the preparation, after which the surviving individuals were allowed to migrate to the untreated plants. The concentrations of insecticacaricides for initial treatment were selected in such a way as to cause the death of at least 85% of individuals: for phytoverm - 0.3 μg a.s./ml; for phytoverma-M - 0.06 m μg a.s./ml; for the vertimec - 0.2 μg a.s./ml. Effects of acaricides were subjected to all phases of evolution of mites simultaneously, but they were not taken into account for the results of the experiment. The efficacy (percent of female deaths) was determined on 2-3 isolated bean plants 48 hours after treatment with the preparation studied. Control was performed by infected plants, which were immersed in water for 3 seconds.

The selective treatments were repeated as the number of mites was restored to a conditionally initial level (approximately 2,000 movable individuals per 100 leaves).

The level of resistance of the treated mites was determined every 8-10 generations. Toxicological tests were performed in 4 replicates. Each isolated plant (in the phase of two true leaves) was inhabited by at least 100 females. To determine the median lethal concentration of preparations, the plants were immersed, one at a time, according to the standard procedure, in solutions of preparations with 5-7 concentrations for 3 seconds. The experimental data were statistically processed by the probit method [14]. Criteria for the development of resistance were the resistance values (RI_{50} and RI_{95}), established from the LC_{50} / LC_{50} and LC_{95} / LC_{95} ratios in the selected line and the parent (original) population. A 10-fold or greater value of these relations was considered true resistance, levels less than 10-fold - increased tolerance.

RESULTS AND DISCUSSION

The change in the effectiveness of avermectin preparations with prolonged use is shown in Fig. 1, from which it follows that under the conditions of laboratory experiment as a whole, there are similar patterns of gradual formation of resistance in twospotted spider mites to phytoverm and phytoverm-M on the one hand and vertimec, on the other. The decrease in the effectiveness of preparations at first could be overcome by increasing their concentration. All increases in concentrations for preparations were carried out synchronously.

The process of development of resistance in experimental mite lines to avermectin preparations during the evolution of 72 generations of mite passed the stages typical for pesticides in their continuous application in pest control [15].

Stage 1. The initial period was characterized by a slow elimination of sensitive individuals and the concentration of individuals with nonspecific tolerance. 11 selective treatments for 18 generations of mites were carried out. During the treatment, the concentration of the preparations was gradually increased after each reduction in mortality in the previous treatment. So, already in the 3rd treatment the concentration of preparations (μg a.s./ml) was increased in the following order:

- Phytoverm from 0.3 to 0.4 (in 1.3 times);
- Phytoverm-M from 0.06 to 0.07 (1.2 times);
- Vertimec from 0.2 to 0.3 (1.5 times).

As a result, the toxicological evaluation of the 11th generation mites showed a slight decrease in the effectiveness of avermectin preparations associated with the manifestation of tolerance in the experimental lines (Table 2).

Taking into account the fact that the mortality in the experimental lines decreased to 70%, for the subsequent (7th treatment) the concentration of phytoverm and vertimec was further increased to 0.5 μg a.s./ml, but the resistance level (RI_{50}) of the mite to all preparations in the 18th generation did not differ from that in the 11th generation, and was practically the same in all variants of the experiment (to the phytoverm - 3.5x, to the phytoverm- M - 2.6x and to the vertimec - 2.6x).

The results of the laboratory experience obtained during the first stage can be approximated in some ways to processes occurring in production conditions, where initially, during the first 5 years (2001-2004) of the use of avermectins, the sensitivity of spider mites to a number of farms was practically at the level of the laboratory population [16].

It is obvious that in the initial period of tolerance avermectins can be used in the fighting system until the moment when a noticeable decrease in their effectiveness is recorded.

Stage 2. A long period of secretive accumulation of individuals with a reduced sensitivity, theoretically characterized by maximum heterogeneity of the lines selected. During this period, selective treatments with phytoverm, phytoverm-M and vertimec were carried out, respectively, in concentrations of 0.12, 1.0 and 0.8 μg a.s./ml.

In the variant with phytoverm and vertimec, the mortality of mites ranged from 90-80%, and only at the 31st treatment it decreased to 70%. In the variant with phytoverm-M, the mortality before the 16th treatment inclusive was also high, but at 17-21 treatments it fell sharply to 40%.

Thus, during this period only the line processed by the phytoverm-M entered: the probit method of the sensitivity of mites in the 27th generation registered an 18.8-fold resistance level.

For phytoverm and vertimec, it remained within 0.8x and 1.5x, respectively (Table 2). So, up to the 27th generation inclusively, the lines treated with these preparations were still in the first stage of resistance formation.

In the future, in connection with the gradual decrease in the effectiveness of the preparations, we again had to increase concentrations (μg a.s./ml) in the following ratio:

- Phytoverm-M 0.5 - 1.0 (i.e. increased by 16.7 times);
- Phytoverm 2.0 - 3.0 - 5.0 (i.e. increased by 16.7 times);
- Vertimec 2.0 - 3.0 - 6.0 (i.e. increased by 30.0 times).

As can be seen from Fig. 1, the mortality of mites in experiments was highly variable, nevertheless, there was a tendency to decrease it. At the same time, not only preparation concentrations increased, but also the frequency of treatments increased to 2.6 in each month, against 1.1 and 1.3 in the two previous stages.

The toxicological analysis indicates a progressive increase in the resistance level of all experimental lines in the 36th generation (up to 43.5x to phytoverm-M, up to 13x to phytoverm and up to 16x to vertimec).

In general, this period of resistance growth was very typical for the populations of spider mites in the protected ground of Russia. Rare reports of a significant decrease in the effectiveness of avermectin preparations in many farms have appeared since 2006-2007 [16]. For today, monitoring data indicate an almost universally progressive development of resistance of spider mites to avermectins, in particular, to the vertimec, resistance values in T. urticae in floricultural plants, often reach «extremely» high levels: more than 3,000-fold [17] .

Further intensification of regular use of avermectins will invariably lead to the further formation of resistant populations and minimize all efforts to resist the loss of pest sensitivity.

On the basis of the above data, it is obvious that already during this period it is necessary to completely exclude the handling of the pest by the phytoverm-M. The most effective method of inhibition of resistance is the rotation of preparations of other species of origin, a different mechanism and mode of action.

Stage 3. The period of a sharp increase in the frequency of homozygosity of the experimental lines, characterized by a spasmodic increase in resistance. In the 46th generation (25 treatments), a sharp jump in the resistance level of the mites (with a subsequent decrease) to phytoverm-M and phytoverm (up to 132.6x and 81.2x, respectively) was recorded (Table 2).

On the contrary, resistance to the vertimec remained at almost the same level up to the 59th generation (RI50 - 16.0x-18.7x), i.e. these data indicate that the line remained in the second stage of development of stability.

In the 72th generation of mites, as a result of the last, 50th selective treatment, an increase in the level of resistance of the mite was recorded in all variants of the experiment: with the vertimec (up to 38.8x), with phytoverm-M and phytoverm (respectively, up to 63.3x and 102.2x). The experimental line, processed by the vertimec, passed to the third stage of development of resistance only in the 72nd generation.

Based on the results obtained, it can be concluded that the rate of formation of resistance of twospotted spider mites to selective preparations is different: during the period under study, it developed most rapidly to phytoverm-M and phytoverm.

It should be noted that compared to other chemical insectoacaricides, the rate of resistance formation to all three preparations studied was much slower. For example, according to Zilbermints et al. [18], the resistance to the insectoacaricides Bi-58 (dimethoate), kelthane (dicofol), neorone (bromopropylate), omite (propargite), etc. widely used against plant-eating mites reaches a maximum value (up to 1000-fold) during the evolution of 9-25 generations of mite.

In terms of scientific provision of effective control against spider mites, it is necessary to develop preventive measures (including taking into account the possibilities [19-21]), which allow us to be guided in the choice of methods, and among the means to make the most effective use of the range of pesticides. Undoubtedly, avermectins outperform many groups of pesticides in terms of biological effectiveness, sanitary and hygienic and environmental standards. And one of the significant properties of avermectin preparations is the slow development of resistance to spider mites, which can provide their long-term use, but under conditions of timely detection of the level of resistance and decision-making with regard to the pest resistance indicators.

The toxicological characteristics of the preparations obtained for each local population of mites in the protected ground will allow them to optimize the pattern of their use, to prevent or overcome resistance.

CONCLUSION

In laboratory conditions, the comparative rate of development of resistance to insecto-acaricides based on abetectin (vertimec) and aversectin C (phytoverm-M and phytoverm) of the twospotted spider mite Tetranychus urticae Koch from the production population («Mullinsky hothouse factory», Perm) was studied.

During the evolution of 72 generations of mites (about 2 years), 50 treatments were performed, as a result of which the level of resistance to the vertemec increased by 38.6 times, to phytoverm-M and phytoverm - by 63.3 and 102.2 times, respectively.

Most rapidly, resistance developed to preparations based on aversectin C (phytoverm-M and phytoverm) compared with the vertimec (abamectin).

The process of formation of resistance passed all the stages typical for pesticides in their continuous application.

It has been established that the most vulnerable, from a practical point of view, is the second period of growth of T. urticae resistance to avermectins. This process can be interrupted by introducing into the system of fighting alternation of preparation of a different origin, with a different mechanism and mode of action.

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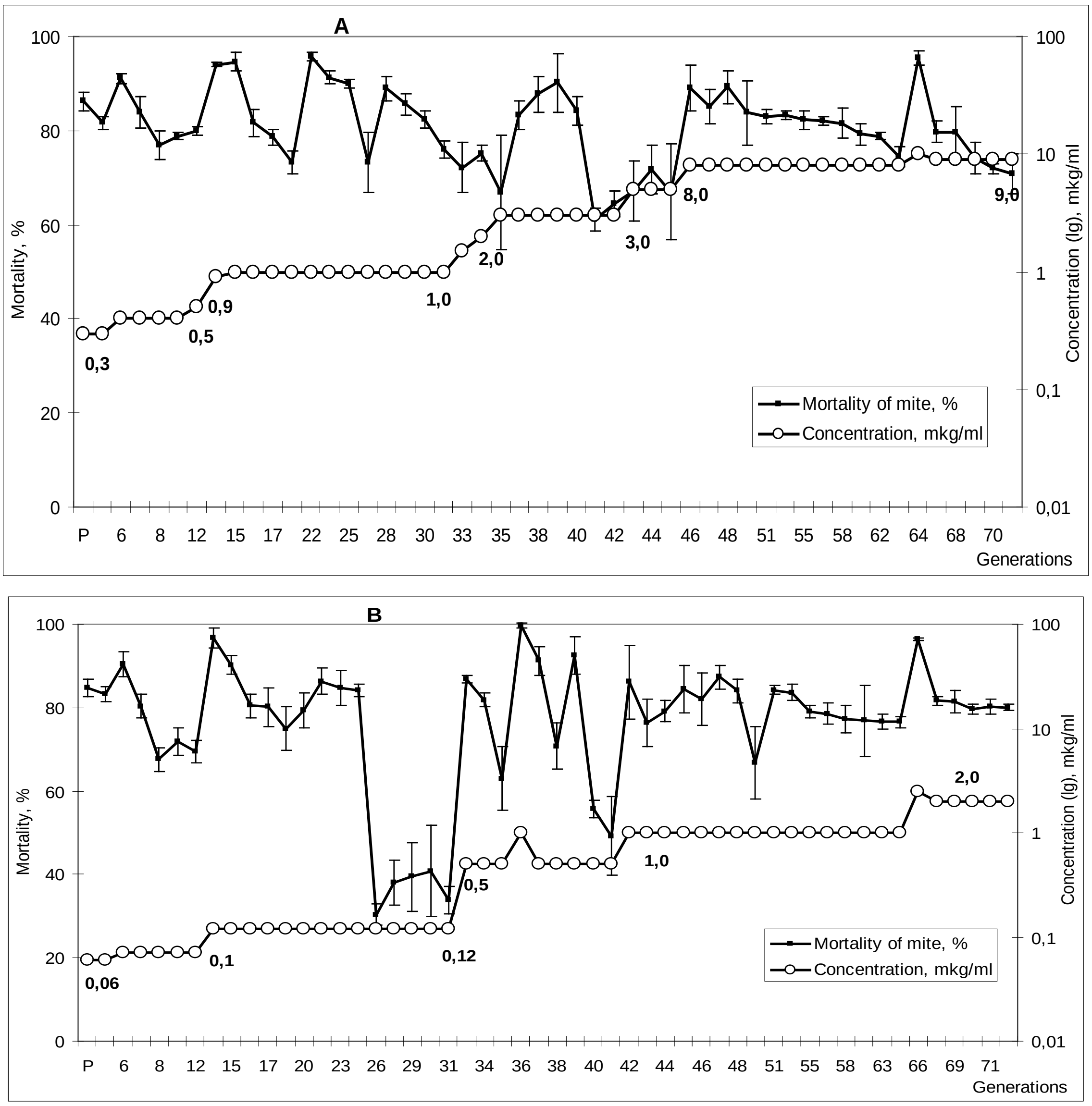

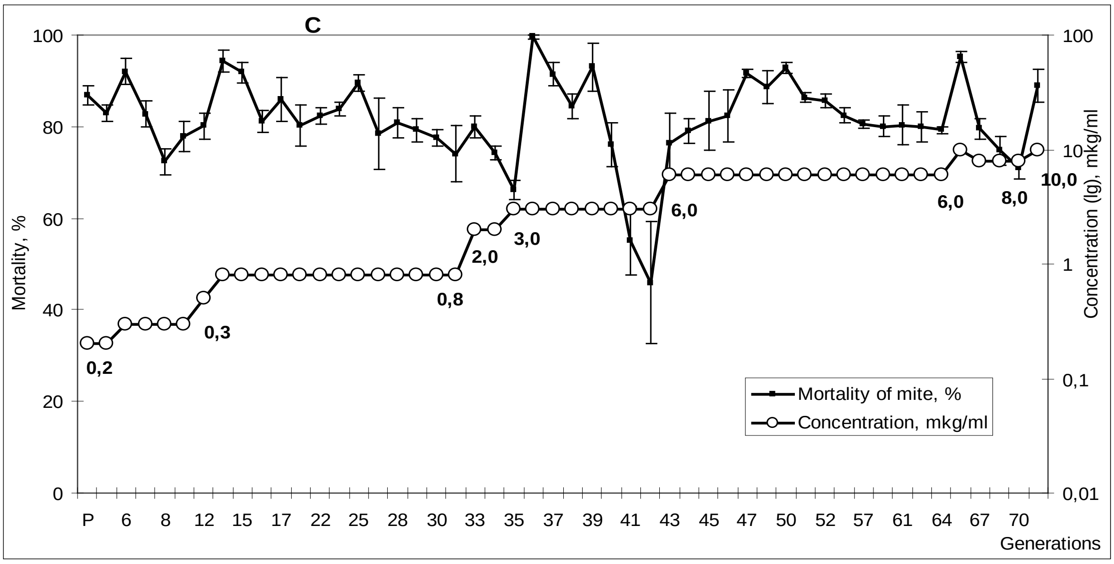

Fig. 1. The dynamics of the population mortality of the twospotted spider mite Tetranychus urticae during the periodic treatments with Phytoverm (A), Phytoverm-M (B) and Vertimec (C) in increasing concentrations (μg/ml by a.s.)