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Optimization of the Use of Acaricides Fenazaquin, Propargite, and Fenpyroximate against *Tetranychus Urticae*

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ABSTRACT

Beans are widely cultivated worldwide and are consumed as an important food source. *Tetranychus urticae* is one of the most important pests of beans. In this study, optimization of acaricide use was carried out using a central composite design and response surface statistical method using Design Expert 7.00 software to reduce the use of chemical pesticides and investigate the best conditions for maximum mortality and adult mites. The process input variables were considered in the temperature range of 25–30 °C, relative humidity of 55–65%, and application doses of 125-1500, 900-2300, and 69–2330 µl/l for the acaricides fenpyroximate, propargite, and fenazaquin, respectively. The bean leaf disc method was used for bioassay tests. The results of variance analysis confirm the statistical significance of the linear model and the factors of dose and relative humidity with the best maximum mortality point of the adult ticks of the acaricide fenpiroximate at H: 57.03% and D: 14.1045 µl/ 1000 ml H_2O, M: about 22%, and the significance of the quadratic model 2FI, the interaction effects of temperature and relative humidity for the acaricide propargite with the lowest dose, T: 26.01 ºC and H: 62.97%, M: about 16%, and the significance of the linear model combined with the relative humidity factor for the use of the acaricide fenazaquin with the best maximum mortality point of the adult ticks at H: 62.97%, D: 527.30 µl / 1000 ml H_2O, M: about 9% which are the most favorable conditions for the use of the above three acaricides.

Keywords: *Tetranychus urticae*, Beans, Acaricides, Chemical pesticides, Optimization.

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INTRODUCTION

Green plants, as the first level of energy production, are always attacked by a wide range of herbivores. *Tartanychus urticae* Koch is one of the most important agricultural pests [1, 2]. This mite can grow and spread on more than 1100 plant species [3, 4]. *Tartanychus urticae* starts to weave webs on the lower surface of the leaf and by feeding and sucking the cell sap, it destroys plant cells and leads to the appearance of yellow spots on the leaf surface. In this case, the chloroplasts of the leaves are gradually destroyed by the increase in the population of mites, feeding on the plant, the stomata of the leaf surface are closed and the reduction of transpiration leads to a decrease in production in the host plant [5-7].

The high growth and reproduction rate, short generation time, and high fertility and reproduction rate of *Tartanychus urticae* allow it to rapidly increase its population under appropriate conditions and cause increased damage and injury to the host plant [8, 9]. Due to the rapid increase in the population of *Tartanychus urticae*, farmers are forced to use acaricides in some cases. Therefore, it is necessary to pay attention to issues such as the use of specific and effective acaricides, the preservation of natural enemies, the prevention of resistance in the pest population, and chemical control is carried out with great care [10]. The most important challenge in the use of chemical pesticides is the persistence of these toxins in nature and plants. Excessive use of pesticides and their persistence in nature is one of the important sources of environmental pollution that negatively affects the health of living organisms, including humans [11-13].

Protecting the environment and its resources is one of the most important agricultural issues in the world, and therefore, modern agriculture is moving in a direction that creates the least risk from pesticides and achieves the highest yield in the crop [14, 15]. The acaricide fenpyroximate, with the trade name Ourtus, with a 5% suspension formulation, is a contact acaricide with a rapid impact effect on larvae, nymphs, and adult mites from the pyrazole group. Propargite, with the trade name Omite, with a 57% emulsifiable formulation, is from the sulfite group, which is a contact acaricide with a long-lasting and non-systemic effect that partially penetrates the outer layer of the leaf and, of course, remains mostly on the leaf surface. Fenazaquin acaricide, under the trade name Pride, is formulated as a 20% suspension from the quinazoline group. Its mode of action is an electron transport inhibitor that has a contact and digestive effect on larval, pupal, and adult tick stages and is used with a low dosage and a long-term effect on active stages and tick eggs [16].

Agricultural pesticide residues in fields may hurt farmers' health, and the long-term effects of pesticides can cause damage to liver and kidney tissue [17]. Achieving the best combination of factors affecting the process has always been one of the challenges for researchers. Optimization means using each input in the right amount and at the right time. Optimization refers to improving the performance of a product to achieve maximum benefit from it [18].

Managing the selection and optimal use of inputs and pesticides is one of the ways that reduce pesticide consumption. Given the adverse effects of pesticides and the resulting savings, the importance of optimizing the use of pesticides is becoming increasingly clear. One of the most important measures to increase the effectiveness and optimize the use of pesticides, including acaricides, is to use them at the appropriate dose and under appropriate temperature and relative humidity conditions [19, 20].

The response surface methodology is one of the optimization methods. This method began with the works of Box and Wilson in 1951 [21, 22]. Beans are among the most important legumes that are widely cultivated in the world and are consumed as an important source of nutrition. Beans contain 20 to 25% protein, which can be a good substitute for animal protein [23, 24]. Given the importance of legumes in various agricultural and food aspects, and given that no study has been conducted on optimizing the use of acaricides such as fenpyroximate, fenazaquin, and propargite against spider mites on beans, this study aimed to determine the optimal conditions for using the above three acaricides to control the spider mite *Tetranychus urticae* on cowpeas at different doses, temperatures, and humidity levels in laboratory conditions using the response surface methodology. This study aims to investigate the increase in mortality and the reduction in pesticide consumption.

MATERIALS AND METHODS

Bean plant cultivation

In this study, all laboratory studies were conducted using bean leaf discs with the scientific name *Vigna unguiculata* L. Bean seeds were soaked in a damp cotton cloth for 48 hours at a temperature of 27 °C and a light-to-dark ratio of 16 to 8 hours. After germination, 3 seeds were sown in each pot in a triangular shape in plastic pots with a diameter of 9 cm and a height of 10 cm containing a perlite substrate, and its surface was covered with a layer of sawdust [25]. The pots were placed in a growth chamber at a temperature of 25 ± 2 °C. The watering period of the pots was adjusted twice a week and macro and micro fertilizers were used as the plant went through the growth stages. The 3- to 4-leaf stage of the plant was used for experiments and the establishment of the *Tartanychus urticae* mite.

Tartanychus urticae mite cultivation

The population of *Tartanychus urticae* was established using bean plants. For this purpose, leaves infested with spider mites were collected from greenhouses and transferred to the laboratory. Using a 0.3 cm thin brush, mites were separated from the back of the leaves and placed on bean leaf discs that were placed in a plastic Petri dish containing cotton soaked in water to provide humidity. To prevent mites from escaping, a 3 mm high barrier was created around the leaves with cotton. The discs containing mites were transferred to several pots and placed at a temperature of 25 ± 2 °C and a relative humidity of 55 ± 5%. To increase the population of *Tartanychus urticae*, healthy pots were first separated from infected pots and the dried leaves of the infected pots containing spider mites were placed on healthy bean plants and reared for at least two generations in the laboratory [10]. During cultivation, mites, by feeding on leaf chlorophyll and growth and development, caused yellowing, drying, and browning of the leaf surface, and the destruction of the entire bean plant. Therefore, to maintain the colony of *Tartanychus urticae*, pots containing infected plants were replaced with healthy, uninfected pots every week.

Age matching of ticks

To prepare a population of the same age of *Tartanychus urticae* mites to begin the experiments, the first 3-4 infected leaves were separated from the bean plant. To obtain a population of the same age of adult female mites, 60 adult female mites were separated from the infected pots using a small 0.3 cm brush and with the help of a stereomicroscope and placed on a bean leaf disc surrounded by wet cotton, with its back surface facing up and its upper surface on wet cotton in a plastic Petri dish with an opening diameter of 8 cm and a height of 1 cm. To create proper ventilation inside the Petri dish, part of the Petri dish lid was cut and covered with a double-layered silk mesh [26]. The adult female mites were given 24 hours to lay eggs. Then, the adult females were removed and the Petri dish containing the eggs laid by the adult mites was kept in a germinator at a temperature of 25 ± 2 °C, relative humidity of 55 + 5%, and a light-to-dark ratio of 16 to 8 hours until reaching maturity.

Determining the range of independent variables of the acaricides fenpyroximate, fenazaquin, and propargite

Based on the biology of the pest, the best and most desirable relative humidity and temperature (independent variables) were determined to be 55-60% and 25-30 °C, respectively. To determine the lethal concentrations that cause 25 to 75% mortality, the acaricides were bioassayed, which included concentrations of 125-1500 μL per liter of distilled water for fenpyroximate, 900-2300 μL per liter of water for propargite, and 69-2330 μL per liter of water for fenazaquin, as the minimum and maximum lethality. Bioassay data analysis for the lethal dose ratios for the tested acaricides and their ranges was performed using POLO-PC software. The most important issue in response surface studies is the interaction between factors. In the present study, the effects of independent variables including X1 temperature, X2 humidity, and X3 dose of application were evaluated **(Table 1)**. Based on the number of variables, statistical design tables were determined for each acaricide with 14 experiments and six replications at the central point to calculate the repeatability of the process **(Table 2)**. The optimization of the three-point overhead crane consumption was carried out using Design Expert 7.00 software and the Response Surface Method with Central Composite Design (CCD).

Table 1. Independent process variables and their values for the experiments.

Levels based on application codes			Sign	Independent variables
		Fenpyroximit		
-1	0	1	-	-
26	28	29	X1	Temperature levels (°C)
57	60	63	X2	Humidity levels (%)
415	730	1045	X3	Dose levels (µl/l)
		Propargite		
-1	0	1	-	-
26	28	29	X1	Temperature levels (°C)
57	60	63	X2	Humidity levels (%)
527	1200	1872	X3	Dose levels (µl/l)
		Fenazaquin		
-1	0	1	-	-
26	28	29	X1	Temperature levels (°C)
57	60	63	X2	Humidity levels (%)
1184	1600	2016	X3	Dose levels (µl/l)

RESULTS AND DISCUSSION

Selecting the appropriate model: Lack of fitness test, coefficient of determination (R^2), and adjusted coefficient of determination (R^2adjusted) are used to check the correctness of the model. The significance of the lack of fit test for a model indicates that the points are not well located around the model and the model cannot be used to predict the values of the function variables. As a result, when the lack of fit test becomes insignificant, it can be seen that the model can be well fitted to the data under test. The adjusted coefficient of determination confirms that the model can estimate the answers well and the coefficient of determination is the ratio of the changes described by the model to the total changes and is a measure of the degree of goodness of fit [10]. According to **Table 3**, the lack of fit test is not significant for all three pesticides, which indicates that the model shows the data trend well. The coefficient of determination and the adjusted coefficient of determination are given in **Table 3**. Comparison between the regression models of all three acaricides showed that the linear model was statistically significantly different from the other models ($P < 0.0001$). The significance index of the lack of fit test for a model indicates that the points are not well located around the model and the model cannot be used to predict the values of the function variables. Therefore, with the lack of fit test being insignificant, it can be seen that the model can be well fitted to the data under study.

Table 2. Test pattern.

Dosage	Humidity	Temperature	Dosage	Humidity	Temperature
			Fenpyroximit		
730	60	28	1045	57	26
730	60	28	1260	60	26
415	63	26	200	60	28
1045	63	26	730	60	28
415	57	26	730	55	28
730	60	28	415	63	29
730	60	28	730	60	28
730	60	30	730	60	25
730	65	28	1045	63	29
1045	57	29	415	57	29
			Propargite		
1184	63	29	1600	60	30

1184	63	26	2016	63	29
11600	60	28	1600	60	28
11600	60	28	1600	60	28
900	60	28	2016	55	29
2016	57	26	1600	55	28
1184	57	26	1600	60	25
1600	60	28	1600	65	28
1600	60	28	1184	57	29
2016	63	26	2300	60	28
			Fenazaquin		
1200	60	28	527	60	26
1872	57	26	1200	60	25
2330	57	30	1871	63	26
1200	60	28	527	57	26
1200	60	28	527	57	26
1200	60	28	1200	65	28
527	63	30	527	30	57
1200	60	28	1200	60	30
1200	60	28	1871	57	29
1872	63	30	1200	55	28

Table 3. Response surface variance analysis table for the linear model of mortality of adult female ticks treated with the acaricides fenpyroximate, propargite, and fenazaquin.

Source	Sum of Squares	Df	Mean Squares	F–value	P–value	Significant
			Fenpyroximite			
Model	900.06	3	300.02	19.76	0.001	Significant
(A) Temperature	0.03	1	0.030	003–E1.953	0.9653	Non-significant
(B) Humidity	283.95	1	283.95	18.70	0.0005	Significant
(C) Dosage	616.08	1	616.08	40.58	0.0001	Significant
Residual	165.92	6	27.65	-	-	-
Lack of Fit	106.94	11	9.72	0.36	0.9280	Non-significant
Pure Error	136	5	27.20	-	-	-
R^2	0.7874	-	-	-	-	-
R^2 adjusted	0.7624	-	-	-	-	-
			Propargite			
Model	492.90	6	820.82	3.93	0.0001	Significant
(A) Temperature	79.74	1	79.74	0.38	0.5476	Non-significant
(B) Humidity	712.38	1	712.38	3.41	0.0878	Non-significant
(C) Dosage	244.77	1	244.77	1.17	0.8929	Non-significant
Residual	2718.30	13	209.10	-	-	-
Lack of Fit	1400.97	8	175.12	0.66	0.711	Non-significant
Pure Error	1317.33	5	263.47	-	-	-
R^2	0.6443	-	-	-	-	-
R^2 adjusted	0.4802	-	-	-	-	-
			Fenazaquin			
Model	799.17	3	266.39	5.10	0.0001	Significant
(A) Temperature	1361	1	13.61	0.26	0.6165	Non-significant
(B) Humidity	785.07	1	785.07	15.04	0.0013	Significant
(C) Dosage	0.47	1	0.47	9.920E-003	0.9252	Non-significant

Residual	835.01	16	52.19	-	-	-
Lack of Fit	501.71	11	45.16	0.68	0.7219	Non-significant
Pure Error	333.33	5	66.67	-	-	-
R^2	0.6890	-	-	-	-	-
R^2 adjusted	0.59	-	-	-	-	-

Among the different variables, the variable with the largest sum of squares is selected as the most influential variable [27]. Therefore, the closer the R2 value is to one, the greater the power of the fitted model in describing the response changes as a function of the independent variables. In the case of the acaricide fenpyroximate, the results of the analysis of variance showed that the linear effects of humidity and the dose used had a significant effect on the mortality rate of adult female mites, but the independent variable of temperature was considered insignificant by the proposed model. Therefore, considering the variables that have a significant effect, the Eq. 1 fitted to the dependent variable of the response is as follows:

$$Y^{-0.35} = + 0.23 - 5.95\ X_2 - 8.812\ X_3 \qquad (1)$$

In this equation, $Y^{-0.35}$ is the predicted response, $\beta_0 = 0.23$ is the fixed coefficient, $\beta_1 = 5.95$ and $\beta_2 = 8.812$ is the linear effects, X_2 is the independent variable of humidity, and X_3 is the independent variable of dose consumed.

In the case of propargite acaricide, the results of variance analysis showed that linear effects related to temperature, humidity, and dose consumed have a significant effect on the percentage of mortality of adult female mites. The percentage of mortality of adult female mites in the case of propargite is given in Eq. 2:

$$Y^{0.69} = + 12.19 + 0.35A + 1.17B - 0.73C + 3.06AB \qquad (2)$$

$Y^{0.69}$ is the predicted response, $\beta_0 = 12.19$ is the fixed coefficient, $\beta_1 = 0.35$, $\beta_2 = 1.17$, and $\beta_3 = 0.73$ is the linear effect coefficient, A is the temperature, B is the relative humidity, C is the acaricide dose, $\beta_2\beta_3$=3.06 is the interaction effects of temperature and relative humidity in the model.

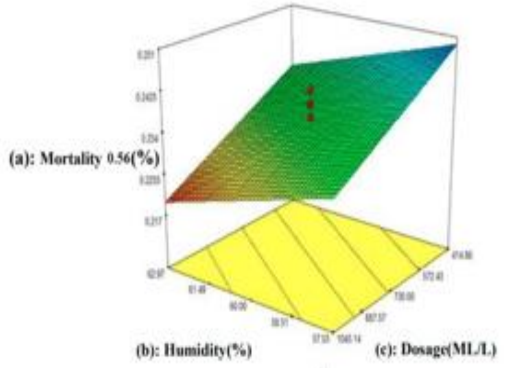
In the case of fenazaquin, the results of variance analysis showed that the linear effects of relative humidity on the percentage of adult female tick mortality are significant.

The percentage of adult female tick mortality in the case of equine fenazaquin is presented as Eq. 3:

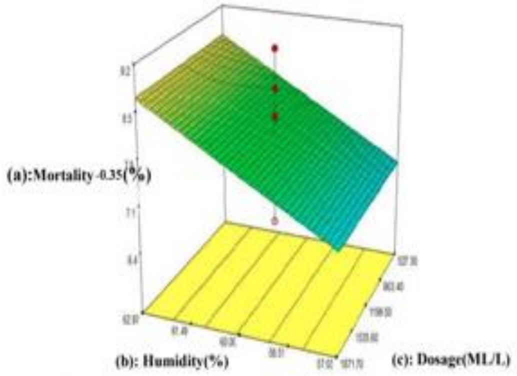
$$Y^{0.56} = 9.4 + 1.11X \qquad (3)$$

$Y^{0.56}$ is the predicted response, $\beta_0 = 9.4$ is a fixed coefficient, and X is the independent variable of relative humidity in the model.

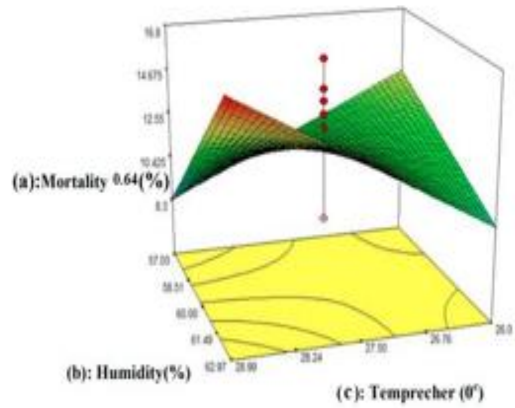
Figure 1 shows the interaction effects of variables on tick mortality. The highest mortality rate of adult female ticks in the case of the acaricide fenpiroximate is related to the simultaneous effect of two factors: humidity and consumption when the acaricide conditions are at the consumption dose of 1045 microliters in 1000 microliters of distilled water and relative humidity of 57%. The three factors of consumption dose, humidity, and temperature in the acaricide fenpiroximate were examined, the temperature factor in the range of 25-30 °C did not have a statistically significant difference in the mortality rate of adult female ticks. However, since the growth and development of arthropods are affected by various environmental factors, especially temperature, it is essential to know the low and high-temperature ranges for the growth and development of spider mites. Temperature is one of the most important abiotic factors that affect the mobility and dynamics of the tick population by affecting the growth and development rate. Temperature is also an important factor in the development and reproduction of spider mites [28]. Therefore, increasing the temperature from the range of 25-30 °C increases the rate of reproduction and causes the increase of spider mites. With increasing temperature, the rate of metabolism in the tick's body increases, and as a result, the amount of acaricide used to control this pest will also increase, which will result in high mortality in spider mites. Reducing the temperature from the range of 25-30 °C in spider mites causes a decrease in the rate of metabolism and slowing down of motor activity and feeding in the body, which leads to death [29]. The results are consistent with the research of Riahi *et al.* [30] who reported a temperature of 27 to 30 °C as the best temperature for the growth and development of spider mites. The independent effect of the acaricide fenpiroximate on the mortality rate of adult female mites without considering other variables has linearly increased mortality in mites. The significance of the linear effect of the factor in the consumption range of 200-1260 microliters confirms that the reason for the increase in the percentage of mortality of adult female mites by the acaricide fenpiroximate is probably because this acaricide is from the pyrazole group, related to tin-containing insecticides, and plays the role of metabolic inhibitors and prevents the storage or disruption of the production of adenosine triphosphate energy, in other words, prevents cell respiration. Since toxins that prevent electron transfer prevent oxidative phosphorylation, as a result, energy will not be stored in the arthropod body and causes the death of the arthropod. The present results are consistent with the results obtained by Ghibi and Taheri [31], who investigated the effect of increasing concentrations of the acaricide Envidor on fig mites.



a)



b)



c)

Figure 1. Three-dimensional curve; (a) and (b) the effect of two factors: humidity and the consumption of fenpyroximate and fenazaquin, and (c) temperature and humidity (propargite) on the mortality and pupation rate of adult mites.

Data from the mortality of adult female mites with the acaricide Propargite showed that temperature has an increasing effect on the mortality rate of adult female mites, such that at a temperature of 26 °C and a relative humidity of 63%, the mortality rate reaches 15-17%. While at a temperature of 29 °C and a relative humidity of 57%, the mortality rate of mites reaches less than 8%. An increase in temperature in the range of 26-28 °C at a humidity of 57% causes an increase in mortality. From a temperature of 29-28 °C at a relative humidity of 57%, the mortality rate of adult female mites decreases. As shown in **Figure 1c**, the interaction effect of the two independent variables of temperature and humidity on the mortality rate of adult female mites is directly related to temperature and inversely related to relative humidity. Temperature is one of the vital factors in the mobility and dynamics of the tick population. Temperature affects the time and rate of growth [32]. As temperature increases, the rate of physiological and biochemical processes in the tick body increases, causing the life cycle of the tick to decrease and leading to an increase in the tick population.

The effect of humidity on the percentage of adult female mites mortality with the acaricide fenazaquin indicates that the relative humidity factor is significant and with increasing humidity, the mortality rate of adult female mites increases in an upward direction. Air humidity increases the effectiveness of many pesticides and prevents the rapid drying of toxins on the surface of the host leaf, but a decrease in relative humidity and air dryness causes the evaporation of water from the leaves along with the toxins and the toxin particles remain in crystalline form on the leaf surface, and less absorption occurs and the leaf will not be able to absorb it. The effect of the two factors of temperature and the dose of the acaricide fenazaquin on the mortality of mites is statistically insignificant. If the dose of the acaricide fenazaquin exceeds the range of 69-2330 microliters in 1000 ml of distilled water, the transfer of electrons along the chain is not possible due to its electron-inhibiting properties. Increasing the dose of the acaricide fenazaquin increases the metabolism and fuel consumption in the arthropod body, which requires a lot of energy consumption. Mitochondria are the center of energy production in cells, so electrons in the mitochondrial electron transport chain are unable to transfer from low oxidation potential levels to higher oxidation levels, and molecular oxygen cannot receive two electrons and two protons to produce water required for metabolic activities, disrupting the arthropod.

CONCLUSION

In this study, optimization of acaricide use was carried out using a central composite design and response surface statistical method using Design Expert 7.00 software to reduce the use of chemical pesticides and investigate the best conditions for maximum mortality and adult mites. The results of this study indicate the useful efficiency of the response surface methodology in optimizing the acaricides fenpyroximate, propargite, and fenazaquin. Therefore, effective control operations against spider mites under temperature and humidity conditions and laboratory dosages, considering the highest mortality rate in mites, fenpyroximate, propargite, and fenazaquin in the present study are recommended to farmers and greenhouse growers as first, second, and third priorities, respectively.

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