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Research Article

# Chemical control optimization of *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae) in gerbera commercial greenhouses

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**Abstract:** Whiteflies are becoming a very serious menace and have shown resistance to many synthetic insecticides since early 1980s. The greenhouse white fly, *Trialeurodes vaporariorum* (Westwood) is one of the most significant pests in many horticultural and greenhouse crops worldwide. Currently, it is controlled by chemical pesticides. In current study, the best conditions for chemical control of *T. vaporariorum* on gerbera applying Confidor®, Palizin® and Proteus® were investigated. The experiment was performed in a commercial greenhouse in Pakdasht, Tehran. The same size and not yet flowering gerbera plants in pots were selected for further analysis. Before the experiment, the numbers of nymph and adults were counted in order to evaluate application effect on their population. Each pot was covered by insect-proof net, separately. Selected factors included: the pesticide in three levels, pesticide dose in three levels (0.5, 1 and 1.5 ml/l), application time in three levels (at 8, 12 and 16 O'clock) and replications in a month in three levels (2, 4 and 6 times). The experiment was conducted on the basis of Response Surface Method with central composite design to optimize the chemical control condition. Data were analyzed using Design Expert 10 software. The mortality percentage model for adults was predicted. Based on the predicted model, the optimum conditions for controlling greenhouse whitefly in gerbera commercial greenhouses were obtained. Optimal conditions with the less replications, which was two, were predicted with the dose of 0.5 to 0.7 ml/l up to the time at 10 or dose of 1.5 ml/l at the time 16 using Proteus®.

**Keywords:** Gerbera, Confidor®, Palizin®, Proteus®, *Trialeurodes vaporariorum*

## Introduction

Whiteflies are one of the most crucial key pests of many crops with a global distribution (Zabel *et al.*, 2001; Anderson *et al.*, 2004; Lapidot *et al.*, 2014), which attack more than 500 species of food, fiber and ornamental plants such as gerbera and cause crop losses that total to hundreds of millions of dollars (Gerling and Mayer, 1996). These insect pests damage the plants directly and indirectly. In direct damage they extract plant sap which leads to lessen plant's vitality, productivity and causes plant damage (Alegbejo and Banwo, 2005; Colvin *et al.*, 2006; Prijovic *et al.*, 2013). In indirect damage they excrete sticky honeydew, which leads to growth of sooty mold fungi, and this affect the process of the plant physiology (Bi *et al.*, 2002), also they can transmit plants' viruses (Morales and Jones, 2004). If white flies leave uncontrolled large

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populations of them, they may develop in greenhouse crops during the production season (Martin *et al*., 2005) and may have a significant effect on plant growth and yield (Smith, 2009).

The benefits of crops grown for food and fiber are clear; however, the actual merits of horticultural crops are less apparent, which is interesting since consumers are constantly being exposed to ornamental plants, whether it is in interior spaces, botanical gardens, conservatories, zoos, garden centers, retail distributors or public gardens. In addition to the visual aesthetics of ornamental plants, they may have a profound effect on the observers or occupants either visually (Kim and Mattson, 2002), aromatically or by purifying the air (Wood *et al.*, 2002).

So to control ornamental arthropod pests, pesticides are commonly applied when populations reach to the economic injury level (Pedigo, 2002). Though, in the case of ornamentals, the entire plant is sold (except cut flowers) for its aesthetics; therefore, ornamental producers have a lower tolerance or more restrictive threshold for these pests in order to minimize the level of plant damage. Occasionally the tolerance threshold is near zero, as is the case with arthropods that vector plant viruses, which can reduce market ability and sale ability. As such, the use of pesticides is required in order to prevent or minimize plant damage, viral transmission and complete crop losses (Bethke and Cloyd, 2009). Furthermore, a single application of a systemic insecticide may provide control of several different phloem feeding insect pests like aphids and whiteflies (Gianessi, 1993).

Among the most economically important whiteflies, the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae), is a key cosmopolitan and highly polyphagous pest of different crops (Vet *et al.*, 1980; Johnson *et al.*, 1992). Adults and larvae cause damage to causing chlorosis and overall weakening of their host plants by sucking the phloem sap, encouraging the growth of sooty molds on leaves reduces plant photosynthesis, and transmitting some plant viruses specially the genera Crinivirus and Torradovirus (Coffin and Coutts, 1995; Guzman *et al.*, 1997; Wisler *et al.*, 1998; Brødsgaard and Albajes, 1999; Martin *et al.*, 2000; Navas-Castillo *et al.*, 2011; Navas-Castillo *et al*., 2014). Adults can transmit some plant viruses like beet pseudo yellows virus (BPYV), tomato yellow leaf curl virus (TYLCV), tomato chlorosis virus (ToCV), tomato infectious chlorosis virus (TICV) and bean golden mosaic virus (BGMV) (Liu *et al.*, 1993; Wisler *et al.*, 1998; Bi *et al.*, 2002; Wintermantel *et al.*, 2009; Karatolos *et al.*, 2010). In addition, *T. vaporariorum* dwells on the undersurface of plant foliage, not easily reached by conventional spraying equipment (Dittrich *et al*., 1990).

Whitefly management is generally based on application of foliar insecticide when pest levels are low. Once population levels are high, management is very difficult (Sorensen *et al.*, 1994; McLeod, 2006).

The use of pesticides may be the only feasible and cost effective strategy to deal with arthropod pests in many ornamental production systems. Pesticides provide a range of benefits to ornamental producers (Hudson *et al.*, 1996), allowing them to control or manage several arthropod pests. Also control of *T. vaporariorum* populations worldwide is mainly dependent on repeated applications of conventional insecticides, like organophosphates, carbamates, and pyrethroids. Although effective, their repeated use for decades has disrupted natural biological control systems and led to resurgence of this insect (Dittrich *et al.*, 1990), sometimes resulted in the development of resistance (Dittrich *et al.*, 1990; Omer *et al.*, 1993) to first generation insecticides (organophosphates, carbamates, pyrethroids), as well as to neonicotinoids and other newer insecticides (Gorman *et al.*, 2002; Gorman *et al.*, 2007; Gorman *et al.*, 2010; Karatolos *et al.*, 2010; Whalon *et al.*, 2012) had undesirable effects on none-target invertebrates (Pisa *et al.*, 2015) and human health concerns (Cimino *et al.*, 2017).

The neonicotinoid group of insecticides is generally one of the most effective insecticides





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against whiteflies (Omer *et al*., 1992). Neonicotinoid insecticides are compounds acting agonistically on insect nicotinic acetylcholine receptors (nAChR). They are particularly important to agriculture because of their activity against hemipteran insects (like aphids, whiteflies, and plant-hoppers), many coleopteran and some lepidopteran pest species (Nauen *et al.*, 2003; Iwasa *et al.*, 2004). Confidor® (imidacloprid) is one of the neonicotinoid insecticides which good control of sucking insects has been observed by that (Saleem and Khan, 2001; Khattak, 2004). Elbert *et al.* (1998) however suggested that imidacloprid can control whiteflies' resistant to conventional insecticides and soil treatments have no adverse effects on beneficial organisms, so it fits well into resistant management strategies and can be considered as a significant component of integrated whitefly management. Sood *et al.* (2006) described that *T. vaporariorum* rapidly acquires resistance to some neonicotinoid insecticide like imidacloprid. The ongoing introduction of new compounds (e.g., acetamiprid, thiamethoxam, thiacloprid, dinotefuran, and clothianidin), unless carefully regulated and coordinated, seems bound to increase exposure to neonicotinoids and to enhance conditions favoring appearance of resistant phenotypes (Nauen and Denholm, 2005). In order to postpone the development of resistance in greenhouse whitefly against a particular insecticide, it is essential to find an alternative approach which is both effective and environmentally compatible (Avery *et al.*, 2015). Safavi and Bakhshaei (2017) considered both lethal and sublethal effects of Calypso on *T. vaporariorum*. They showed that all concentrations of insecticides have caused mortality on *T. vaporariorum* eggs, nymphs and adults in experimental conditions. However, nymphs were considerably more susceptible to Calypso compared to eggs and adults.

Botanical insecticides have been recommended as suitable alternatives to synthetic chemical insecticides for pest management because they pose little threat to the environment or to human health (Isman, 2006). Palizin®, which is coconut soap, is recommended for the control of some pests especially sucking insects. Experiments have demonstrated that insecticidal soap can decrease plant pest populations and provide effective control (Moore *et al.*, 1979). Insecticidal soaps can cause high mortality rates in a variety of soft bodied insect pests such as aphids, whiteflies, leaf hoppers, thrips, and scale insects (Baniameri, 2008).

Optimization is typically used to improve the system performance and provide the best possible response. Optimization was carried out using multivariate statistical techniques. Response Surface Methodology (RSM) is the most crucial multivariate techniques used in analytical optimization. This can be well used when a response or a set of favorite responses is affected by multiple variables. The goal is to simultaneously optimize the levels of these variables in order to achieve the best performance of the system (Bezerra *et al.*, 2008). RSM is one of the most useful statistical optimization methods in biological and chemical process (Vishwanatha *et al*., 2010). In recent years, it has been applied for optimization in several different research fields (Morshedi and Akbarian, 2014). Many agricultural experiments involve responses to the explanatory variables that are binary or counts in nature and which can therefore be modelled within the generalized linear model framework (Myers *et al.*, 2004). RSM is a collection of mathematical and statistical techniques for empirical model building. Fundamentally, RSM consists of central composite design (CCD), Box-Behnken design, one factor design, D-optimal design, user-defined design and the historical data design. CCD and Box-Behnken design are the most frequently used statistical methods. For one numeric variable, CCD has 5 levels ($-\alpha$, $-1$, 0, +1, $+\alpha$) while the Box-Behnken design only has 3 levels ($-1$, 0, +1) (Bezerra *et al.,* 2008). RSM also provides an experimental model that predicts the association and interaction between a series of experimental variables and observed results, and then provides optimized conditions (Zheng *et al.*, 2008).

The present study was carried out to study the efficacy of chemical applications conditions of some common pesticides. Additionally, since there is not much research on the optimum conditions for chemical control of white flies, the optimum conditions for applications were determined so that the least amount of application is used to have the greatest impact on the greenhouse whitefly mortality rate.

## Materials and Methods

### Greenhouse experiment

The experiment was carried out in a commercial greenhouse of Gerbera in Pakdasht, Iran in April and May 2017. The average greenhouse temperature at the time of the experiment was 18 °C at 8 am, 22 °C at 12 noon and 21 °C at 4 pm. The relative humidity was 35-45%, while the photoperiod was 14:10 h L: D.

In order to simulate the normal greenhouse conditions and to obtain a more accurate and realistic result, artificial infestation was not performed. The pots were placed at a height of 100 cm on platforms. There were three rows holding 15 pots each. Plants were naturally infested with greenhouse whiteflies and the same size, not yet flowering gerbera plants in pots were selected for further analysis. Each pot was considered as a treatment and was covered by net. Pesticides were applied at the labeled rate in the morning, at noon and late afternoon by directing the sprays slightly above the plant canopy while walking through the compartment. During application, ventilation fans were off and the vents were closed and remained so overnight.

Before and after the experiment, the number of nymph and adults was counted. To determine the relative populations of pest on the plant, the number of nymph and adults behind the leaves were counted using a 5 × 5 cm frame. The frame was randomly placed on the back and middle of 5 leaves located at different directions of each pot and considered as the demographic model for each pot to determine the pest population. The mean initial population prior to treatment was about 5.7 nymph and 12 adults for each treatment. The nymph and adults were also counted two days after each treatment application based on the previous model. Thus, counting the mortality of whiteflies was performed randomly, using the same frame in each replication.

### Experimental design

The Response Surface Methodology (RSM) was applied to evaluate the interaction of various variables and then used to find the optimum greenhouse conditions that affect the response, namely the most adult mortality. RSM is a set of statistical and mathematical techniques that are useful for the design of experiments, the development of models and the assessment of the effects of variables in which the response of interest is influenced by a number of variables and the objective is to optimize this response (Baş and Boyacı, 2007).

### Central composite design

Central composite designs (CCD) were carried out in order to identify optimum parameter levels of pesticide application condition for *T. vaporariorum* nymph and adults in gerbera commercial greenhouses. The independent variables that were investigated were: the pesticide (including Confidor®, Proteus® and Palizin®), pesticide doses (0.5, 1 and 1.5 ml/l), pesticide application time (at 8, 12 and 16 O'clock) and replications per month (2, 4 and 6 times) (Table 1). The experiment consisted of 45 experimental units (Table 2).

**Table 1** Experimental range and levels of the independent variables.

| Pesticide | Pesticide dose | Application time | Replications in a month |
|---|---|---|---|
| Confidor® | 0.5 | 8 | 2 |
| Proteus® | 1.0 | 12 | 4 |
| Palizin® | 1.5 | 16 | 6 |

**Table 2** Forty five experimental units and treatments.

| Std | Run | Factor 1A: dose | Factor 2B: time | Factor 3C: repeat per month | Factor 4D: type |
|---|---|---|---|---|---|
| 1 | 32 | 1.5 | 16 | 2 | Confidor® |
| 2 | 29 | 1.5 | 8 | 6 | Confidor® |
| 3 | 30 | 0.5 | 16 | 6 | Confidor® |
| 4 | 1 | 0.5 | 8 | 2 | Confidor® |
| 5 | 37 | 0.5 | 12 | 4 | Confidor® |
| 6 | 17 | 1.5 | 12 | 4 | Confidor® |
| 7 | 26 | 1.0 | 8 | 4 | Confidor® |
| 8 | 25 | 1.0 | 16 | 4 | Confidor® |
| 9 | 5 | 1.0 | 12 | 2 | Confidor® |
| 10 | 35 | 1.0 | 12 | 6 | Confidor® |
| 11 | 6 | 1.0 | 12 | 4 | Confidor® |
| 12 | 44 | 1.0 | 12 | 4 | Confidor® |
| 13 | 33 | 1.0 | 12 | 4 | Confidor® |
| 14 | 18 | 1.0 | 12 | 4 | Confidor® |
| 15 | 20 | 1.0 | 12 | 4 | Confidor® |
| 16 | 14 | 1.5 | 16 | 2 | Palizin® |
| 17 | 4 | 1.5 | 8 | 6 | Palizin® |
| 18 | 8 | 0.5 | 16 | 6 | Palizin® |
| 19 | 31 | 0.5 | 8 | 2 | Palizin® |
| 20 | 41 | 0.5 | 12 | 4 | Palizin® |
| 21 | 36 | 1.5 | 12 | 4 | Palizin® |
| 22 | 16 | 1.0 | 8 | 4 | Palizin® |
| 23 | 9 | 1.0 | 16 | 4 | Palizin® |
| 24 | 3 | 1.0 | 12 | 2 | Palizin® |
| 25 | 43 | 1.0 | 12 | 6 | Palizin® |
| 26 | 34 | 1.0 | 12 | 4 | Palizin® |
| 27 | 12 | 1.0 | 12 | 4 | Palizin® |
| 28 | 39 | 1.0 | 12 | 4 | Palizin® |
| 29 | 45 | 1.0 | 12 | 4 | Palizin® |
| 30 | 15 | 1.0 | 12 | 4 | Palizin® |
| 31 | 13 | 1.5 | 16 | 2 | Proteus® |
| 32 | 21 | 1.5 | 8 | 6 | Proteus® |
| 33 | 42 | 0.5 | 16 | 6 | Proteus® |
| 34 | 27 | 0.5 | 8 | 2 | Proteus® |
| 35 | 24 | 0.5 | 12 | 4 | Proteus® |
| 36 | 19 | 1.5 | 12 | 4 | Proteus® |
| 37 | 40 | 1.0 | 8 | 4 | Proteus® |
| 38 | 28 | 1.0 | 16 | 4 | Proteus® |
| 39 | 2 | 1.0 | 12 | 2 | Proteus® |
| 40 | 7 | 1.0 | 12 | 6 | Proteus® |
| 41 | 10 | 1.0 | 12 | 4 | Proteus® |
| 42 | 11 | 1.0 | 12 | 4 | Proteus® |
| 43 | 23 | 1.0 | 12 | 4 | Proteus® |
| 44 | 22 | 1.0 | 12 | 4 | Proteus® |
| 45 | 38 | 1.0 | 12 | 4 | Proteus® |

### Statistical data analysis

Design Expert 10 software (StatSoft, Tulsa, OK, USA) was used for the experimental design, data analysis and linear model building. Contour plots were generated to understand the interaction of different factors.

### Optimal levels for increasing mortality percentage

The numerical optimization process was performed in order to find the highest percentage of total insect mortality (nymph and adults) in the case of the lowest amount of pesticide dose and replications. Additionally, the graphical optimization was used to achieve the insects' mortality between 60 and 100 percent with the highest confidence.

## Results

The numbers of nymph and adults of *T. vaporariorum* were recorded before and after each experiment and the whitefly mortality was counted. Sampling proceeded until two days after the last application for each treatment. The statistical analysis based on the Table 3 showed each factor effect and their interactions on nymph and adult population.

Based on the results obtained in this survey, the $R^2$ value, $R^2$adj and Pred $R^2$ in whitefly adults were 0.80, 0.77 and 0.44, respectively, while they were 0.76, 0.64 and 0.24 in nymph, respectively. R-squared ($R^2$) is a statistical measure of how close the data are to the fitted regression line. In general, the higher the R-squared, the better the model fits the data. Therefore, the high value of $R^2$, in this analysis and its proximity to other values, both the variability of errors and the normalization of the residuals, indicates the significance prediction of the model. Based on the ANOVA analysis, type and replication had significant effects ($P < 0.05$) on adult mortality percentage after the application, whereas type of the pesticide had significant effect ($P < 0.05$) on nymph mortality percentage. Also in nymph population the interactions between dose and time of the application (AB); dose and type of the pesticide (AD); dose, time and the number of replication (ABC); dose, replication and type of the pesticide (ACD) had significant effect on nymph population (Table 3). Other factors had no significant effects on greenhouse whitefly mortality. Fig. 1 shows adult's mortality in the case of using different replications and different pesticides in a special condition (dose 1, time 12). Fig.1a-c showed that using Confidor® or Proteus® will not make a significant difference in adult mortality percentage even in different replications. The highest pest population was observed when using Palizin® such that there was a high difference in using this pesticide compared with the others. Neither of the two factors, including dosage and application, had a significant effect on the insect population during the use of Confidor®, while the replication time had a significant effect (Fig. 1d). In Palizin® usage, changing in dosage and application time did not change the greenhouse whitefly adults' mortality, whereas replication time had a significant effect on the pest mortality percentage (Fig. 1e). Palizin® resulted in no more than 60 percent mortality. In Proteus® usage changing the pesticide dosage and time of the application had no significant effects on adult whitefly mortality percentage, though the replication time had a significant effect (Fig. 1f).

Different types of pesticides had a significant effect on nymph population (Fig. 2). Confidor® and Proteus® were not significantly different in nymph mortality, whereas they were significantly different from Palizin® (Fig. 2). Also types of the pesticide had significant interaction with dosage. Moreover different doses of pesticides cause different mortality in nymph population at different times. Significant interactions were also observed in dose, time and replication with the P-value about 0.0096, and dose, replication and type of the application with the p-value about 0.0003 (Table 1, Fig. 3).

**Table 3** Analysis of variance of *Trialeurodes vaporariorum* for response model.

| Source | Sum of Squares | | df | | Mean Squares | | F Value | | P-value | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Adult | Nymph | Adult | Nymph | Adult | Nymph | Adult | Nymph | Adult | Nymph |
| Model | 14899.00 | 6839.27 | 5 | 15 | 2979.80 | 455.95 | 32.15 | 6.11 | < 0.0001 | < 0.0001 |
| A | 97.16 | 185.19 | 1 | 1 | 97.16 | 185.19 | 1.05 | 2.48 | 0.3122 | 0.1264 |
| B | 6.52 | 104.17 | 1 | 1 | 6.52 | 104.17 | 0.070 | 1.40 | 0.7922 | 0.2474 |
| C | 645.16 | 66.00 | 1 | 1 | 645.16 | 66.00 | 6.96 | 0.88 | 0.0119 | 0.3551 |
| D | 14150.15 | 2341.45 | 2 | 2 | 7075.08 | 1170.73 | 76.34 | 15.699 | < 0.0001 | < 0.0001 |
| AB | - | 573.56 | - | 1 | - | 573.56 | - | 7.69 | - | 0.0098 |
| AC | - | 195.48 | - | 1 | - | 195.48 | - | 2.62 | - | 0.1168 |
| AD | - | 567.81 | - | 2 | - | 283.90 | - | 3.80 | - | 0.0346 |
| BC | - | 31.90 | - | 1 | - | 31.90 | - | 0.43 | - | 0.5186 |
| CD | - | 38.23 | - | 2 | - | 19.11 | - | 0.26 | - | 0.7758 |
| ABC | - | 577.22 | - | 1 | - | 577.22 | - | 7.73 | - | 0.0096 |
| ACD | - | 1649.74 | - | 2 | - | 824.87 | - | 11.05 | - | 0.0003 |
| Residual | 3614.59 | 2089.68 | 39 | 28 | 92.68 | 74.63 | - | - | - | - |
| Lack of Fit | 2983.54 | 868.29 | 27 | 16 | 110.50 | 54.27 | 2.10 | 0.53 | 0.0885 | 0.8805 |
| Pure Error | 631.05 | 1221.39 | 12 | 12 | 52.59 | 101.78 | - | - | - | - |
| Cor Total | 18513.60 | 8928.95 | 44 | 43 | - | - | - | - | - | - |

Adult: Std. Dev. = 9.63, Mean = 83.07, C.V.= 11.59, $R^2$ = 0.80, Adj, $R^2$ = 0.77, Adequate Precision = 15.45.
Nymph: Std. Dev. = 8.64, Mean = 90.50, C.V.= 9.55, $R^2$ = 0.76, Adj, $R^2$ = 0.64, Adequate Precision = 10.717.
A: Dose; B: Time; C: Repeat; D: Type.





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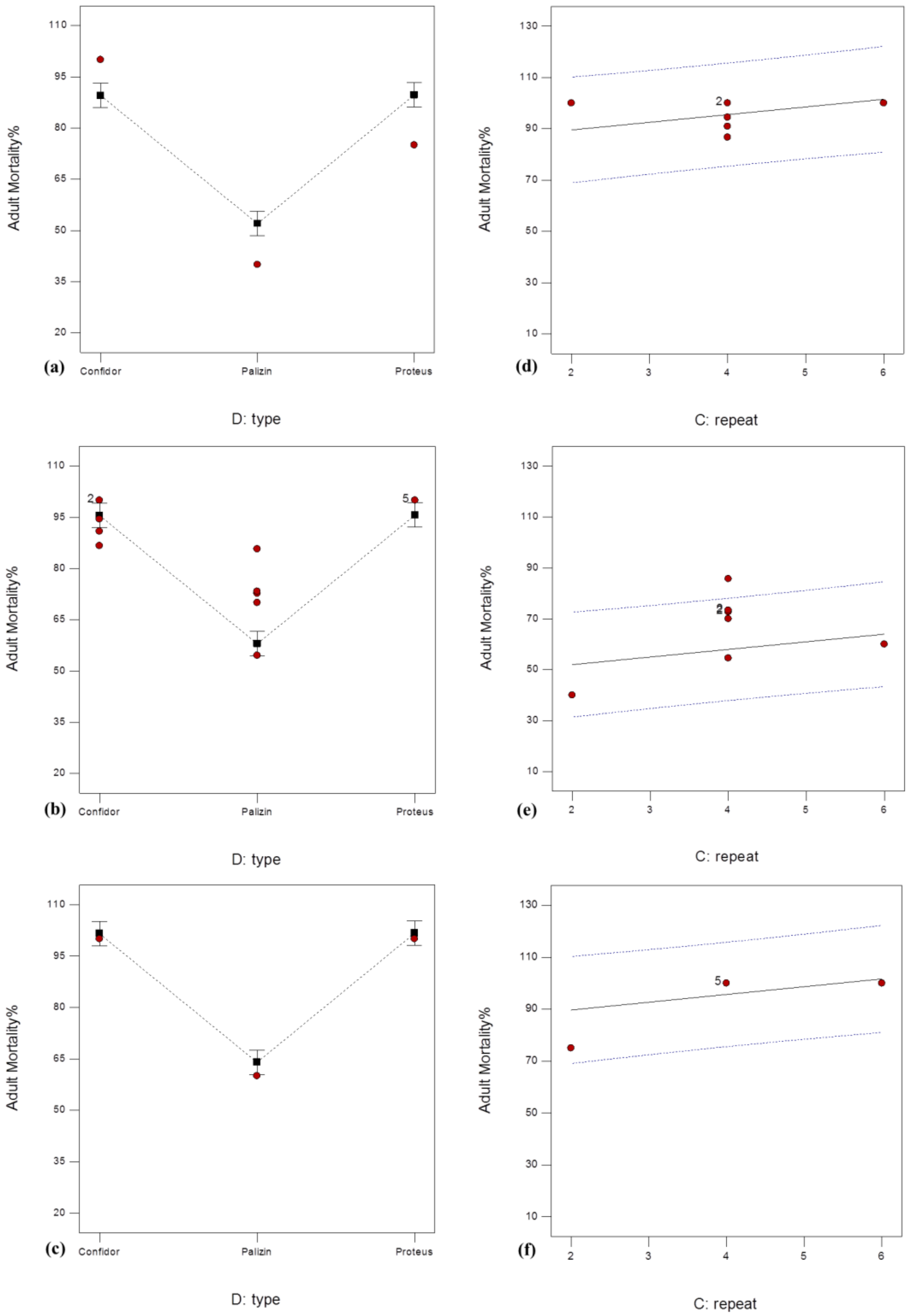


**Figure 1** Type and repeat as the significant factors in *Trialeurodes vaporariorum* adults' mortality percent; type comparison in dose 1, time 12 (a) repeat 2, (b) repeat 4 and (c) repeat 6; repeat comparison in dose 1, time 12, (d) Confidor®, (e) Palizin® and (f) Proteus®.





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Consequently, the Proteus® with dose 0.5, and two applications in a month at 16 o'clock was the optimum condition to control of *T. vaporariorum* adults and nymph population for getting the maximum mortality percent. In optimum condition the adult mortality will be about 87.98%, while the nymph mortality will be about 100% with the desirability about 0.91 (Figs. 4a and 4b). Also, the best condition for achieving maximum mortality percent with 2 replications was using dose 0.5 to 0.7 up to time 10 and dose 1.5 at time 16 (Fig. 4c).

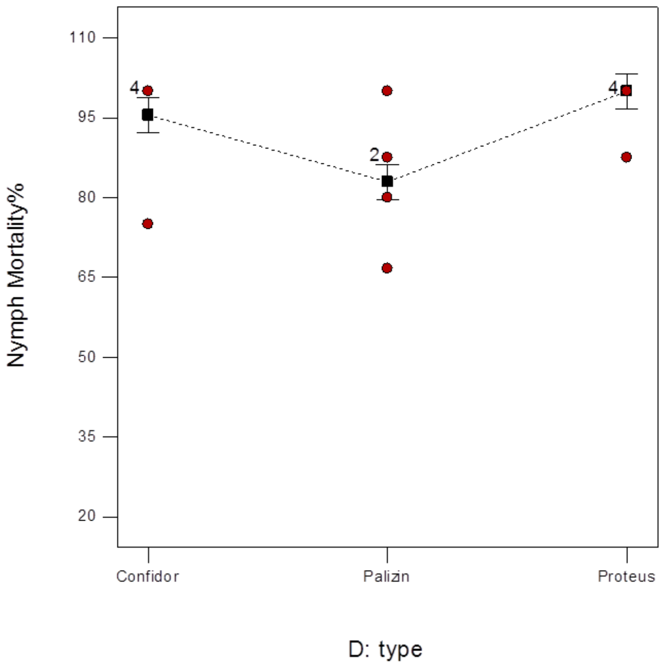


**Figure 2** Different types of pesticides as the significant factor in *Trialeurodes vaporariorum* nymph mortality percent, in dose 1, time 12 and repeat 4.

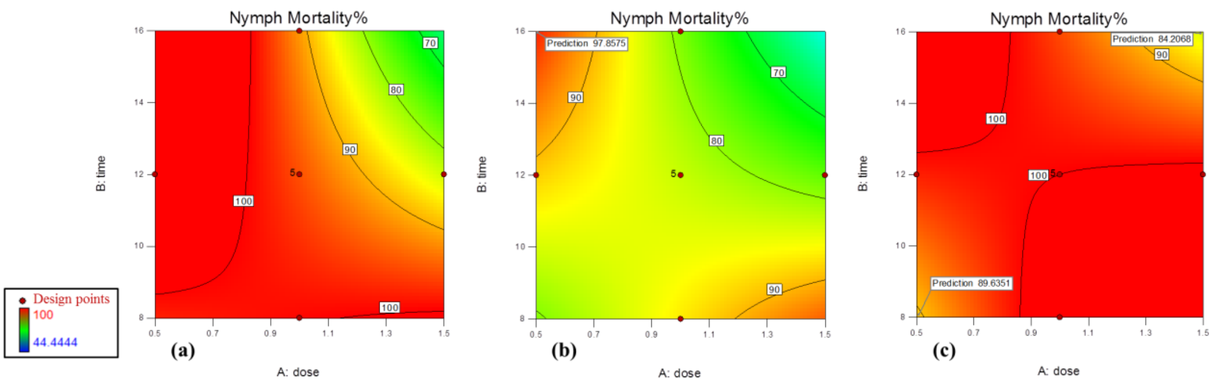


**Figure 3** Contour plots of interaction factors of dose and time in repeat 4 in *Trialeurodes vaporariorum* nymph mortality percent, using (a) Confidor®, (b) Palizin®, the flag (prediction sign) shows the most nymph mortality percent and (c) Proteus® the flag (prediction sign) shows the least nymph mortality percent.

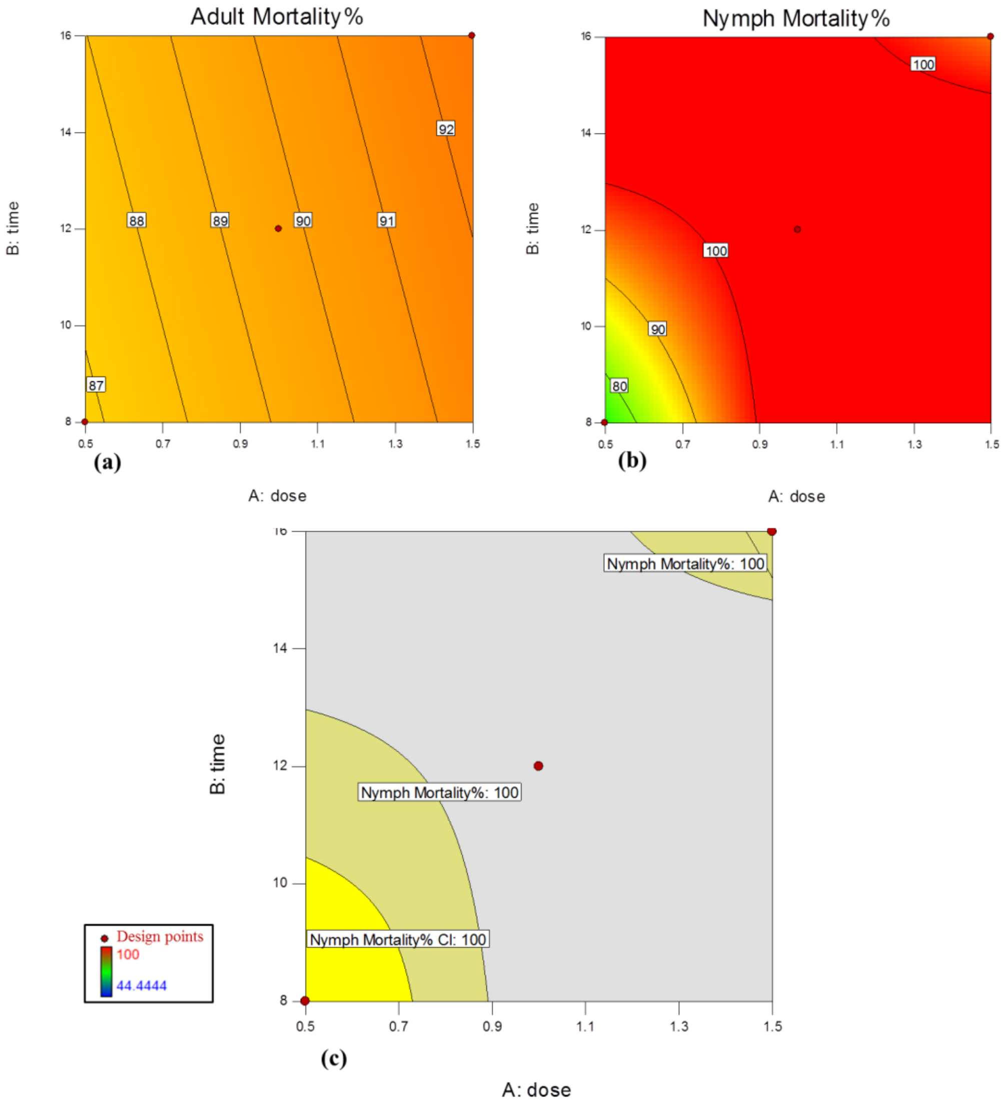


**Figure 4** Graphical optimal levels for increasing mortality percentage of *Trialeurodes vaporariorum* between 60 and 100 percent using Proteus® with two replications. Numerical optimization process with highest percentage of (a) adult mortality (b) nymph mortality using lowest amount of pesticide dose and replications by Proteus®. (c) Overlay plot of chemical application optimal level to achieve *T. vaporariorum* mortality between 60 and 100 percent using Proteus®.

## Discussion

The greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) is one of the most serious pests of many horticultural and greenhouse crops worldwide (Helgesen and Tauber, 1974; Zabel *et al.*, 2001). To the extent that one of the extreme technical challenges in potted plants and cut plant production is the regulation of *T. vaporariorum*. Large populations of this pest can consume high quantities of plant phloem sap which can result in the reduction of plant growth and yield (Johnson *et al*., 1992). Control of the greenhouse whitefly is very hard and for a long time has been accomplished by variable success. The use of synthetic insecticides is the primary mode of its control; however, indiscriminate and injudicious use of pesticides has posed a major problem of developing insecticidal resistance both in the greenhouses and fields. This pest had already been reported to have developed resistance to pyrethroids, carbamates and organophosphate like dimethoate (Elhag and Horn, 1984; Omer *et al.*, 1992; Sanderson and Roush, 1992; Zheng and Gao, 1995; Rufinger *et al.*, 1999; Gorman *et al.*, 2002; Sood *et al.*, 2003; Yorulmaz and Ay, 2009; Karatolos *et al.*, 2010; Karatolos *et al.*, 2012). Other disadvantages of chemical pesticides are side effects on natural enemies, rapid photo degradation and inadequate distribution within the crop canopy (Thoeming *et al.*, 2003). But chemical control is still a key component of integrated pest management systems (Ganesan *et al*., 2007). The effectiveness of the chemical insecticides declines as resistance builds up. Nevertheless, the 'non-effectiveness of an insecticide can often be ascribed to bad application technology and to localization of the pest (Gorman *et al*., 2007). To overcome these difficulties, the pesticide application condition could help. In this study, the efficacies of three insecticides conditions were compared with regard to their different application strategies. The chemical control treatments consisted of different monthly pesticides spray applications of Confidor®, Proteus® or Palizin®.

Palizin® is coconut soap (65 ± 5%) manufactured by Kimiasabzavar Company, Iran. Kabiri and Amiri-Besheli (2012) reported that Palizin® provides a physical and chemical barrier against insect pests and shows considerable potential for effective control of them in certain agricultural crops.

RSM is a powerful statistical technique for investigating the interactive effects between several factors at diverse levels (Pang *et al.*, 2011). Originally, RSM was developed to model experimental responses (Box and Draper, 1987), and then migrated into the modelling of numerical experiments. The RSM is equipped with statistical tools to regulate the significance of a factor over a response. The evaluation of factors using the RSM uses experimental design in order to distribute the selected variables within the boundaries of the design. To provide some context, there is good commercial software available to help with designing and analyzing response-surface experiments. The most popular is Design-Expert (Stat-Ease, 2017), which provides for generating Box-Behnken and central-composite designs, fitting first- and second-order response surfaces, and visualizing them. This program generally exceeds RSM's capabilities like more types of designs, provisions for mixture experiments, etc. The central composite design (CCD) allows the allocation of pesticides' type, replication time, and number of applications to evaluate their effect on the adult population of whiteflies. The application of RSM to design optimization is aimed at reducing the cost of expensive analysis methods and their related numerical noise. Venter *et al*. (1996) have discussed the advantages of using RSM for design optimization applications.

R-squared is the percentage of the response variable variation that is explained by a linear model. $R^2$ value was 0.80 in whitefly adult mortality analysis while it was 0.76 in nymph mortality analysis. These high values showed the significance prediction of the model. Adjusted R-squared is a measure of the amount of variation around the mean described by the model, adjusted for the number of terms in the model. The adj-$R^2$ decreases as the number of terms in the model increases if those additional terms don't add value to the model. It was 0.77 for the adult mortality analysis while it was 0.64 in nymph mortality. The Adjusted R-squared and Predicted R-squared should be within approximately 0.20 of each other to be in reasonable agreement. In this analysis the pred-$R^2$ confirmed the adj-$R^2$. The results of this experiment showed that just type of the pesticide and replications had a significant effect on the mortality of *T. vaporariorum* adults in gerbera greenhouses (Figure 1). So that, based on the predicted model, changing the dose or application time will not make any significant change in greenhouse whitefly adult percentage. Also, no significant interaction was observed between factors in adult's mortality percentage. Whereas the pesticide type is crucial to nymph mortality and makes a significant difference. Also interaction among different factors had a significant effect on the nymph population e.g. in the interaction between dose and type of the pesticide, with increasing the dose of the Palizin® and Proteus®, the nymph mortality will be increased. Whereas there was no significant difference in nymph mortality with the dose change in Confidor®. The interaction factors between dose and time in different types of pesticides are shown in Fig. 3a-c. In using Confidor® with the dose and time increasing, the nymph mortality will decrease (Fig. 3a). While in using Palizin® the most nymph mortality percentage was observed using the lowest dose (0.5 l/h) and latest time (16 o'clock) with the mortality about 97.85 percent or in the earliest time (8 o'clock) and highest dose (1.5 l/h) with 94.45 percent nymph mortality (Fig. 3b). In using Proteus® all the time there would be high mortality of nymph except in the lowest dose (0.5 l/h) and earliest time (8 o'clock) with the nymph mortality about 89.63 percent or latest time (16 o'clock) and highest dose (1.5 l/h) with the mortality about 84.20 percent (Fig. 3c).

The results from this case study suggested that significant factors and their optimum settings for controlling *T. vaporariorum* in Gerbera greenhouses were Proteus® with dose 0.5 applied twice per month at 16 o'clock. Also based on the optimization analysis, using Confidor® with dose 0.5 applied twice per month at 16 o'clock can cause maximum adult mortality (87.82%) with desirability about 0.94. Although the mortality percent in using Confidor® is a bit less than Proteus®, it is not significant. As Proteus® is more expensive than Confidor® in Iran, we suggest using Confidor® to control of greenhouse whitefly adults in gerbera greenhouses with the above mentioned conditions. As pesticides have detrimental effects on the environment and pest resistance the optimization was done to get the maximum pest mortality with less pesticide use. In this case, the best condition with 2 replications will be predicted with the dose 0.5 to 0.7 up to the time 10 and dose 1.5 at time 16 while using Proteus® (Fig. 4(c)). When using Confidor®, time of the application should be at 11 with dose 1.5, at 14 with dose 1.1 to 1.4 and at time 16 with dose 1.1 to 1.3 (Fig. 5a). Use of Palizin® will not give a good result in either adults or nymph mortality (Fig. 5b).

Current research used RSM and CCD to evaluate the effects of application conditions on *T. vaporariorum* in gerbera commercial greenhouses. Based on the developed models, it was found that type of the pesticide and repetition in the month were correlated with the whitefly adults' control. However, type of the pesticide showed a significant effect on the whitefly nymph control. The application of response surface methodology combined with the CCD can be used as effective methods for investigating the optimum application conditions on gerbera whitefly control. The factors considered in this study are important in influencing whitefly control.

The optimization process identified the optimum conditions for controlling *T. vaporariorum* in gerbera greenhouses. The analysis showed that the predicted optimum levels of the factors on gerbera whitefly control were as follows; pesticide type: Proteus®, dose: 0.5 to 0.7, application time at 10 and replication: two times per month, or pesticide type: Proteus®, dose: 1.5, application time at 16 and replication: two times per month. The current study recommends other researchers to adopt this approach in the gerbera greenhouses with different pesticides. Finally, the method applied in this research has been shown to be effective in saving farmers the cost of farm inputs because of less number of insecticide applications. Also, future studies can show how other pesticides can be more effectively used in *T. vaporariorum* integrated pest management programs on gerbera or other plants.

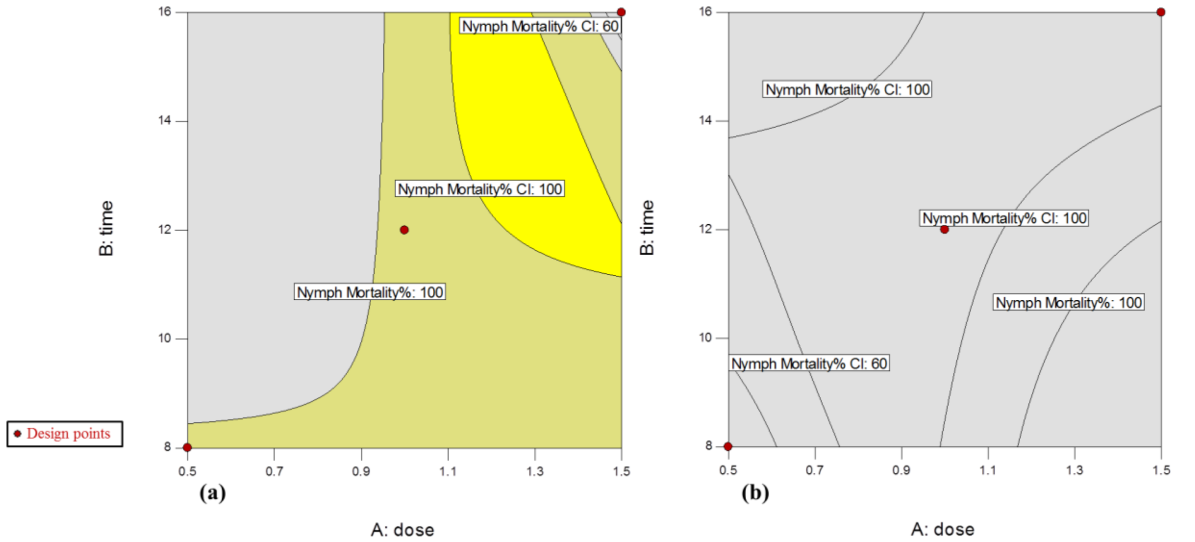


**Figure 5** Overlay plot of graphical optimal level to achieve *Trialeurodes vaporariorum* mortality between 60 and 100 percent using two replications by (a) Confidor® and (b) Palizin®.

## Conflict of Interests

The authors declare that they have no conflict of interest.

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# بهینه‌سازی کنترل شیمیایی *Trialeurodes vaporariorum* (Westwood) در گلخانه‌های تجاری ژربرا

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**چکیده:** سفیدبالک‌ها از اوایل دهه ۱۹۸۰ به یک تهدید جدی تبدیل شده و در برابر بسیاری از حشره‌کش‌های شیمیایی مقاومت نشان داده‌اند. سفیدبالک گلخانه *Trialeurodes vaporariorum* (Westwood) یکی از مهم‌ترین آفات در بسیاری از محصولات باغی و گلخانه‌ای در سراسر جهان است. در حال حاضر، این آفت توسط سموم شیمیایی کنترل می‌شود. در این تحقیق، بهترین شرایط برای کنترل شیمیایی *T. vaporariorum* روی ژربرا توسط کنفیدور®، پالیزین® و پروتئوس® مورد بررسی قرار گرفت. این آزمایش در یک گلخانه تجاری در پاکدشت، تهران انجام شد. گلدان‌های هم‌اندازه و بدون گل ژربرا برای تجزیه و تحلیل انتخاب شد. قبل از آزمایش، تعداد پوره‌ها و حشرات کامل سفیدبالک گلخانه برای ارزیابی اثر کاربرد سموم بر جمعیت آنها شمارش شد. هر گلدان به‌طور جداگانه توسط توری ضدحشره پوشانده شد. فاکتورهای انتخاب شده شامل: سموم استفاده شده در سه سطح، دز سموم در سه سطح (۰/۵، ۱ و ۱/۵ میلی‌لیتر در لیتر)، زمان استفاده سموم در سه سطح (ساعت ۸، ۱۲ و ۱۶) و تکرار سمپاشی در یک ماه در سه سطح (۲ ، ۴ و ۶ بار) بودند. این آزمایش براساس روش سطح پاسخ (Response Surface) با طرح مرکب مرکزی جهت بهینه‌سازی شرایط کنترل شیمیایی انجام شد. تجزیه و تحلیل داده‌ها با استفاده از نرم‌افزار Design Expert 10 انجام شد. مدل درصد مرگ‌ومیر حشرات کامل پیش‌بینی گردید. براساس مدل پیش‌بینی شده، شرایط بهینه برای کنترل سفیدبالک گلخانه در گلخانه‌های تجاری ژربرا به‌دست آمد. شرایط بهینه با کم‌ترین تکرارسمپاشی، که دو تکرار در ماه بود، با دز ۰/۵ تا ۰/۷ تا ساعت ۱۰ یا دز ۱/۵ در ساعت ۱۶ با استفاده از سم پروتئوس® پیش‌بینی شد.

**واژگان کلیدی:** ژربرا، کنفیدور®، پالیزین® و پروتئوس®، *Trialeurodes vaporariorum*





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