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Article

Population parameters of *Bactericera cockerelli* in tomato plants treated with menadione

Alberto Roque Enriquez[1]
Mariana Beltrán Beache[2]
Yisa María Ochoa Fuentes[3]
Juan Carlos Delgado Ortiz[4,§]

1 Departamento de Parasitología-Universidad Autónoma Agraria Antonio Narro. Buenavista, Saltillo, Coahuila, México. CP. 25315. (roque-doko@hotmail.com).

2 Departamento de Agronomía-Universidad Autónoma de Aguascalientes. Carretera Jesús María, Posta Zootecnica S/N, Aguascalientes, México. CP. 20920. (beltránmariana89@gmail.com).

3 Universidad Autónoma Agraria Antonio Narro. Buenavista, Saltillo, Coahuila, México. CP. 25315. (yisa8a@yahoo.com).

4 Catedrático Conacyt-Departamento de Parasitología-Universidad Autónoma Agraria Antonio Narro. Buenavista, Saltillo, Coahuila, México. CP. 25315. (moe-788@hotmail.com).

Autor para correspondencia: jdelgado@conacyt.mx.

Abstract

Bactericera cockerelli causes damage to nightshade crops in Mexico, causing millions of pesos in losses to producers; for its control, chemical insecticides have been used, which over time has generated resistance in the insect, causing its control to be more difficult year after year. New alternatives are sought for the control of this pest, among which the use of resistance inducers that are effective and environmentally friendly stands out; menadione sodium bisulfite (MSB) is an effective alternative with low environmental impact that has proven to be an activator of plant defenses and has insecticidal effects. In the present work, tests were carried out to determine the effects of MSB on the survival and development of the insect. Cohorts with *B. cockerelli* eggs were established, using three tomato plants of the Río Grande variety in each of the entomological cages, to test the effect of menadione on the development and survival of the insect *B. cockerelli*. According to the data obtained, no significant differences were found in the days of development of the immature stages of the insect, while in the survival data the MSB treatments had the highest mortalities, of 42 to 80%, demonstrating that MSB causes the mortality of *B. cockerelli* nymphs.

Keywords:

development, survival, tomato psyllid.

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Introduction

In Mexico, nightshade producers face constant problems of pests and diseases in each cycle; among which the insects that vector viruses, bacteria and phytoplasmas stand out, such as the nightshade psyllid *Bactericera cockerelli* (Sulc.) (Hemiptera: Triozidae) (Casteel *et al*., 2006; Almeyda *et al*., 2008; Butler *et al*., 2011). This pest causes direct damage by nymphs sucking the sap out of the plant and injecting toxins, such as yellowing, stunting, leaf deformation, short and thickened internodes, premature senescence, and indirect damage due to the secretion of honeydew, which favors the incidence of fungi and bacteria (Melgoza *et al*., 2018; Berdúo *et al*., 2020; Cerna *et al*., 2021).

Nevertheless, the greatest damage is indirect by the transmission of the pathogen known as BLTVA phytoplasma and the transmission of the bacterium *Candidatus Liberibacter solanacearum*, which cause the disease known as 'permanente del tomate' and cause abnormal plant growth. The symptoms are chlorosis of the edges and curling of the lower leaves, acquiring a brittle structure, causing the plant to stop its growth, the flower clusters dry out causing flower abortion and poor fruit set; it also causes stunting of the plant, shortening of the short internodes, premature abortion of flowers, a purple coloration of the upper leaves of the plant, growth retardation, chlorosis and proliferation of axillary buds, presenting brown vascular ring lesions and necrotic mottling of the tissues (Garzón *et al*., 2004; Hansen *et al*., 2008; Berdúo *et al*., 2020; Cerna *et al*., 2021).

The methods used for the control of *B. cockerelli* are generally based on cultural and mechanical control, biological control, biorational control; among all these methods, the one that stands out the most is the chemical control in the use of insecticides based on chemical active ingredients, focused mainly on the different nymphal and adult stages; it is important to carry out constant sampling so that it is considered a risk for the crop; this is due to the fact that the insect has a high capacity to generate resistance to insecticides; for example, it has been reported that *B. cockerelli* is resistant to endosulfan and imidacloprid in agricultural areas of San Luis Potosí, Aguascalientes and Coahuila-Nuevo León, generating a pressure of selection of the insect (Garzón, 2007; Bújanos, 2015; Cerna *et al*., 2015).

It has been reported that nightshade producers in Mexico make more than 30 insecticide applications per crop cycle, thus increasing production costs, damage to the environment, damage to farmers' health, and the emergence of resistance to active ingredients due to irrational use (Mayo *et al*., 2018).

Yield losses of up to 93% have been reported in potato crops exposed to the psyllid (Munyaneza *et al*., 2008) and losses of 50 to 80% have been reported in tomatoes (Liu, 2006), which leads producers to abandon production fields (Flores *et al*., 2004).

The overuse of pesticides, the high cost have endangered the soil, environment, plants, animals, and people; it has forced researchers to consider new crop protection strategies. For this reason, it is sought to implement new strategies for the management of the insect, such as biological control organisms, entomopathogenic fungi, insecticides with botanical active ingredients and insecticides of chemical origin that are friendly to the environment and that favor the production of foods with a better quality, with less chemical residues that contaminate the environment and living beings (Bujanos *et al*., 2005; Munyaneza *et al*., 2013; Cerna *et al*., 2015).

Tomato (*Solanum lycopersicum* L.) plants over time have evolved and adapted to new defense mechanisms that are more efficient against insect attacks, although on many occasions these defense mechanisms are not highly effective in counteracting attacks; it is necessary to stimulate these mechanisms (Ortiz *et al*., 2022).

The insect *B. cockerelli* has shown affinity for plants of tomato variety Río Grande (*S. lycopersicum* L.) than for other wild and grafted tomato varieties (Cortez, 2010). Climate changes have caused variations in temperatures, which modifies the behavior of insect populations with an alteration of voltinism and may be more beneficial for multivoltine species, generating changes in the geographical distribution of insects (Useche *et al*., 2019).

The free selection of the insect is related to the morphometry of adults and nymphs of *B. cockerelli*, which can be affected by the tomato varieties in which it develops (Vargas *et al*., 2014). Plants have natural resistance mechanisms that are activated when they are threatened by various physical or biological factors.

Antibiosis is an antagonism regulated by metabolites (specific or non-specific), enzymes, volatile compounds, and other toxic substances, which generates biocontrol of plants against pests and pathogens that reduce their growth or survival (Fravel, 1988). Insects choose an alternate host plant in less acceptance due to morphological characteristics (thick epidermal layers, presence of wax, trichome density) and chemical characteristics (repellent phytochemicals, toxic), altering the behavior of insects, this interaction between plants and insects is known as antixenosis (Díaz *et al*., 2014).

With all of the above, menadione sodium bisulfite (MSB) is a derivative of vitamin K3 or provitamin that has insecticidal properties, it is a viable strategy due to its composition since it activates the natural defense mechanisms of plants and the subsequent induction of resistance to pathogen and pest attacks, in addition to having antifeedant properties. MSB is a systemic, biodegradable, non-toxic and environmentally innocuous compound, it is not phytotoxic to plants, animals and humans (Borges, 2010; Borges, 2011; Borges, 2012; Ortiz *et al*., 2022).

In relation to the described characteristics of MSB, different authors report positive effects when using the active ingredient as an inducer of resistance to various insects; for example, Carrillo *et al*. (2016) report the use of MSB against the mollusk pest in tomato plants, it is a useful tool for the control of snail infestations, highlighting that it can be used in low concentrations and is compatible with biological pest control, and tomato plants that were treated with MSB had a more noticeable repellency than plants treated with water alone.

Borges (2012) used low doses of MSB, showing an inhibitory effect for one of the most serious citrus diseases known as huanglongbing (HLB), which was also able to control the vectors psyllids *Trioza erytreae* and *Diaphorina citri*. Therefore, the present research aimed to know the effects of MSB on the development of the nymphal stages of the insect *B. cockerelli*.

Materials and methods

The present work was carried out under greenhouse conditions in the Department of Parasitology of the Antonio Narro Autonomous Agrarian University (UAAAN, for its acronym in Spanish), located in Saltillo, Coahuila, Mexico.

Breeding of *B. cockerelli*

The colony was established under greenhouse conditions; it was kept inside an entomological cage (60 cm × 60 cm × 70 cm) on 30-day-old tomato plants of the Río Grande variety, at a temperature of 25 ±2 °C with photoperiod 14:10 h (light/dark) (Levy *et al*., 2013).

Establishment of the experiment

To assess the effect of MSB on the development of *B. cockerelli*, the following treatments were evaluated: 55 ppm, 80 ppm, 100 ppm, and 500 ppm, which were determined according to what was reported by Borges (2012) and Ortiz *et al*. (2022), they were sprayed in four applications for each treatment; the first application was made before placing the insects for oviposition and later applications were made at eight-day intervals (the application was directed to leaves free of *B. cockerelli* nymphs).

To determine the parameters (development period, survival, preoviposition, incubation period, immature stage period, total development period, and total life cycle length), cohorts were established with eggs of *B. cockerelli* on three 30-day-old tomato plants of the 'Río Grande' variety.

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An entomological trap was used to release the adults (five females and five males); the female was left to oviposit for a period of 24 h, then the adults were removed. With a magnifying glass of (10x), 30 eggs per plant were counted and selected for daily counts, recording hatching and survival until emergence of the adult.

Data collection criteria

The differentiation of insect stages and development was determined by observing the morphological characteristics and behavior of *B. cockerelli* on infested plants. *B. cockerelli* consists of five nymphal stages, one of its most relevant main characteristics in nymphs is that their body is flattened, elliptical in shape.

Characteristics of the different instars

First stage, the division of head, thorax and abdomen is not evident, they are yellow-orange, similar to the immature thrips, but more rounded; second stage, they become pale green, divisions are more evident; third stage, the nymphs are light green, red and prominent eyes can be seen, vestigial or rudimentary wings less than half of the body length; fourth stage, their coloration is still light green and the antennae are well formed with visible censor setae; fifth stage, the segmentation of antennae and legs, as well as the wing packages, is well defined, the nymphs are located on the undersides of the leaves, but can be found throughout the plant (Castillo *et al*., 2021).

Statistical analysis

Data obtained in the development period, survival, preoviposition (PO), incubation period (IP), immature stage period (ISP), total development period (TDP) and total life cycle length (TLC), calculated with the sum of TDP and PO, were used to perform an analysis of variance. When the P test showed significant differences between the treatments under study, Tukey's test (α= 0.05) was applied for the separation of the means with SAS 9.1.

Results and discussion

In Table 1, regarding PO, the control presented the first oviposition at four days, while in the treatments it occurred on the fifth day. In the IP, the treatments of 80 and 500 ppm showed fewer days for egg hatching and the treatments of 55, 100, and the control took one more day for hatching. In the case of TDP, the treatment of 55 ppm needed 26 days of development and the treatment of 500 ppm 29 days, the rest of the treatments together with the control had a very even behavior, taking 28 days for their development.

Table 1. Development period of *Bactericera cockerelli* at each stage of development.

	Period in days (means ±SD)*				
	PO^{1}	**IP**^{2}	**TDP**^{3}	**ISP**^{4}	**TLC**^{5}
Control	4 ±1a	6 ±1a	28 ±1a	22.66 ±6.51a	33 ±1a
55 ppm	5 ±1a	6 ±1a	26.66 ±1.52a	22.33 ±0.57a	31.66 ±1.52a
80 ppm	5 ±1a	5.33 ±0.57a	28.33 ±0.57a	23.33 ±0.57a	33.33 ±0.57a
100 ppm	5 ±1a	6.33 ±1.52a	28 ±0a	23.66 ±0.57a	33.33 ±1a
500 ppm	5 ±1a	5.66 ±0.57a	29 ±1a	23.66 ±0.57a	33 ±1a
p-value	0.5801	0.7818	0.1277	0.7239	0.0339

SD= standard deviation; *= means in the same columns with different letters are statistically different (α= 0.05). 1= preoviposition; 2= incubation period; 3= total development period; 4= immature stage period; 5= total life cycle.

For the ISP, the control and 55 ppm showed a lower development in the immature stage, with 22.66 and 22.33 days, the treatments with higher doses presented a lethargy of one day in the cycle. In the case of TLC, the treatment of 55 ppm obtained the shortest life cycle, with 31.66 days, the rest of the treatments needed two more days (33.3 days) to complete their cycle.

Cerna *et al*. (2018) determined the population parameters of *B. cockerelli* from three field populations in northeastern Mexico under controlled conditions of 25 ±2 °C, the results showed that the population of San Luis Potosí was the longest-lived colony to complete its life cycle with 31.75 days, while the population of Coahuila-Nuevo León recorded a development of 24.25 days. Abdullah (2008) reported the study of population parameters of *B. cockerelli* in a commercial greenhouse at 26-27 °C, reporting an average oviposition of 6.9 days, incubation period of 6.7 days, immature stage period of 21.9 days, with a total insect development of 28 days and the total life cycle of the insect was 34.7 days, data that are very similar to those reported in this study as the development of the insect was not affected by MSB treatments, compared to the control.

The optimal climate for the development of *B. cockerelli* ranges between 25 and 28 °C so that it has a development of 19 to 21 days to complete its life cycle, while at low temperature, like 5 °C, there was a drastic reduction, at 15 °C the life cycle of the insect showed a duration of more than 40 days and the population decreases at 32 °C and ceases its reproduction at 35 °C (Tran *et al*., 2012; Cabrera *et al*., 2022).

Table 2 shows the survival of *B. cockerelli*, where significant differences can be observed between the treatments; the treatment of 100 ppm had the lowest egg hatching, with 58.89% viability, the treatments of 80 and 500 ppm report a similar behavior in terms of hatching and the treatment of 55 ppm has the highest hatching, with 85%, only below the control (100%). For the first nymphal stage, the treatment of 100 ppm presented 47.78% survival, while the treatments of 80 and 500 ppm reported a similar behavior, the treatment of 55 ppm showed the highest hatching among the treatments, with 74.44%; for the instar 2, the treatments of 80 and 500 ppm had a hatching of 60%, being the highest, at 55 ppm it showed a hatching of 53.33% and the lowest hatching was reported in the treatment of 100 ppm, with 47.78%.

Table 2. Survival for the immature stages of *Bactericera cockerelli*.

	Percentage of survival (mean ± SD)*					
Stage	**55 ppm**	**80 ppm**	**100 ppm**	**500 ppm**	**Control**	***p*-value**
Egg	85.56 ±25.01a	78.89 ±28.34a	58.89 ±10.71a	75.46 ±16.44a	100 ±0a	0.001
1st instar	74.44 ±21.17a	70 ±6.67a	47.78 ±8.39a	70 ±17.32b	96.66 ±3.33a	0.0129
2nd instar	53.33 ±5.77b	60 ±10b	42.22 ±6.93b	60 ±16.67b	88.89 ±6.9a	0.0026
3rd instar	50 ±3.33bc	54.44 ±8.38b	33.33 ±3.33c	54.44 ±11.7b	88.89 ±6.94a	0.0001
4th instar	45.55 ±3.85b	51.11 ±8.39b	33.33 ±3.33b	35.55 ±5.09b	76.66 ±17.64a	0.0014
5th instar	41.11 ±5.09b	46.66 ±6.66b	26.67 ±10b	30 ±3.33b	73.33 ±20.27a	0.0027
Adult	37.77 ±5.09b	22.22 ±20.36b	16.66 ±14.53b	14.44 ±12.61b	72.22 ±21.43a	0.0067

SD= standard deviation; *= means in the same rows with different letters are statistically different (α= 0.05).

For instar 3, the treatments of 55, 80 and 500 ppm showed a very similar behavior, with a hatching percentage of 50 to 54%, and the treatment of 100 ppm presented the lowest hatching, with 33.33%. In instars 4 and 5, the treatments of 55 and 80 ppm reported a very similar behavior, the treatments with the lowest hatching were 100 and 500 ppm, showing 33.33 and 35.55% in the fourth stage, and for the fifth instar, the lowest survival rate was shown by the treatment of 500 ppm, with 80% survival, less than the control.

Several authors highlight that the highest mortality of nymphs is in the early stages, in this work progressive death occurred in all stages, this response is attributed to the effects generated by MSB, the first attribute is due to the effect of induction of resistance that it generates in plants, activating several defense pathways in the metabolism of plants, the second is due to the antifeedant effect that MSB generates on insects, causing imminent death in all nymphal stages.

Survival rates are consistent with what was reported in studies on the development stage of *B. cockerelli* by Cerna *et al*. (2018), who report that the population of Coahuila-Nuevo recorded 6-13% survival until adulthood. This agrees with Vargas *et al*. (2020); Abdullah (2008), where it was mentioned that high mortality rates are recorded in the immature stages among the first three nymphal stages, also that mortality rates for all stages were below 50% for all stages and adults.

It is worth mentioning that in this study, mortalities similar to the mortality reported by Ortiz *et al*. (2022) are obtained, since in *in vitro* bioassays with nymphs of the fourth and fifth instars of *B. cockerelli*, mortalities of 53-85% were recorded in the dose range of 50-500 ppm.

Table 3 shows the comparison between the treatments for each nymphal stage, in which the development time in the different stages of development can be appreciated. There are significant differences in the egg stage, the control required four days to hatch, while the treatments at 55 and 100 ppm lasted 5.33 and 5.66 days and the treatments at 80 and 500 ppm took six days. For instar 1, treatments of 55, 100, 500 and the control required four days and at 80 ppm it took only three days. In instar 2, the development varied from 3.33 days to 4.66 days, showing no significant difference.

Table 3. Development time for immature stages of *Bactericera cockerelli*.

	Days of development (means ± SD)					
Stages	**55 ppm**	**80 ppm**	**100 ppm**	**500 ppm**	**Control**	***p*-value**
Egg	5.33 ±0.57ab	6.66 ±1.15a	5.66 ±0.57ab	6 ±1ab	4 ±1b	0.0435
1st instar	4 ±0a	3 ±1a	4.33 ±0.57a	4.33 ±0.57a	4 ±1a	0.02287
2nd instar	4 ±0a	4.66 ±1.15a	3.33 ±0.57a	3.33 ±0.57a	4 ±0a	0.1264
3rd instar	4 ±0a	4.33 ±1.52a	5.33 ±1.52a	4 ±0a	5 ±1a	0.4651
4th instar	7 ±0b	8.33 ±0.57a	7.66 ±0.57ab	8.33 ±0.57a	7 ±0b	0.007
5th instar	8.33 ±1.54a	8.33 ±0.57a	7.66 ±0.57a	9 ±1a	7 ±0a	0.0791

SD= standard deviation; *= means in the same rows with different letters are statistically different (α= 0.05).

For instar 3, it should be noted that the treatment of 100 ppm required an additional day, while the other concentrations had a similar behavior in days. The treatments of 80 and 500 ppm required 8.33 days to develop as instar 4, while in the development time for instar 5, the 500 ppm dose required nine days to reach the adult stage, two days more compared to the control.

For the development time of the immature stages, Vargas *et al*. (2020) no significant differences were reported between the cycles of the instars. In a study of estimates and thermal requirements for the life cycle of *B. cockerelli*, Tran *et al*. (2012) report that the development of eggs, nymphs and adults is affected at temperatures of 8 °C. Cerna *et al*. (2018) report a stage of development similar to the one recorded in this work, where they mention that the development time between the stages for the population of Coahuila-Nuevo León ranges from three to four days in the first three stages and for eggs it ranges from five to six days, differing a little in stages 4 and 5, where they report five days for development and hatching, in these research findings, an increase of more than two days to those reported was recorded.

Carrillo *et al*. (2016) report that MSB has antifeedant properties in mollusks by reducing the growth rate by 50%. Borges (2010; 2012) referred to MSB as a biostimulant of the natural mechanisms of plants and that they originate by themselves the development of antifeedant properties against the attacks of pathogens and pests. Ortiz *et al*. (2022) in a study carried out with *B. cockerelli* nymphs, report that MSB has an insecticidal effect on *B. cockerelli*.

Conclusions

The application of MSB did not have a significant effect on the preoviposition, incubation period, total development period, immature stage period and total life cycle of *Bactericera cockerelli*, although we can highlight that MSB does influence some of the parameters. However, survival in the different stages of the insect was drastically affected, demonstrating that MSB has a positive effect on the mortalities of *B. cockerelli* nymphs, highlighting the treatment of 500 ppm, which presented mortalities above 85%, compared to the control. Likewise, the application of MSB shows an increase in the development time of the egg stage, as well as the fourth and fifth nymphal instars.

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