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Short Communication

Insecticidal effects of thymol on *Mahanarva spectabilis* (Hemiptera: Cercopidae) in two evaluation methodologies

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Abstract. The objective of this study was to develop and evaluate an alternative, less laborious and reliable method for investigating the insecticidal effect of thymol on *Mahanarva spectabilis* (Hemiptera: Cercopidae). Two methods for evaluating the effects of thymol on *M. spectabilis* (conventional and alternative) were tested for optimum survival rates. The conventional method proved to be the most suitable for investigating the insecticidal effect of thymol on *M. spectabilis*. Thymol concentrations of 1.2, 2.5, and 5.0% resulted in the highest mortality rates of eggs, nymphs and adults, respectively. Thus, the study determined a methodology to evaluate the effects of insecticide products on spittlebugs, which indicated that thymol may be used effectively for the control of *M. spectabilis*.

Keywords: Spittlebugs, integrated pest management (IPM), bioinsecticides.

Introduction

Among the pest species that occur in Brazil, the spittlebug *Mahanarva spectabilis* (Distant) is a limiting pest in the production of forage grasses (Auad *et al*., 2007). The main current control strategies for this insect pest have some limitations, for example, the long time between the discovery of resistant forages and the release of a cultivar (Auad and Resende, 2018), and the fact that chemical control is economically and ecologically impracticable (Ferrufino and Lapointe, 1989; Valério 2005). Therefore, alternative measures to control *M. spectabilis* are required.

Naturally occurring plant products have been used to protect agricultural crops against pest insects for many years in various parts of the world (Kpatinvoh *et al*., 2017). These products are attractive alternatives to the synthetic chemical insecticides used in pest management, due to the fact that they supposedly pose little threat to the environment or to human health (Isman, 2006).

Studies have shown the potential of pure thymol in the control of several insect pests of agricultural importance (Tak and Isman, 2017; Oliveira *et al*., 2018). The method used to evaluate the effects of pesticides on any insect can have an effect on the final results obtained in the study (Studebaker and Kring 2003). The only study on the potential control of spittlebugs with bioinsecticides was performed by Garcia *et al*. (2006), who used the spray application method in plants. After spraying, individual *Mahanarva fimbriolata* (Stål) spittlebugs were released onto the plants. However, this method is very laborious, and due to the lack of indication of bioinsecticides for the control of spittlebugs, the study is aimed to develop and evaluate a reliable and less laborious method for investigating the insecticidal effect

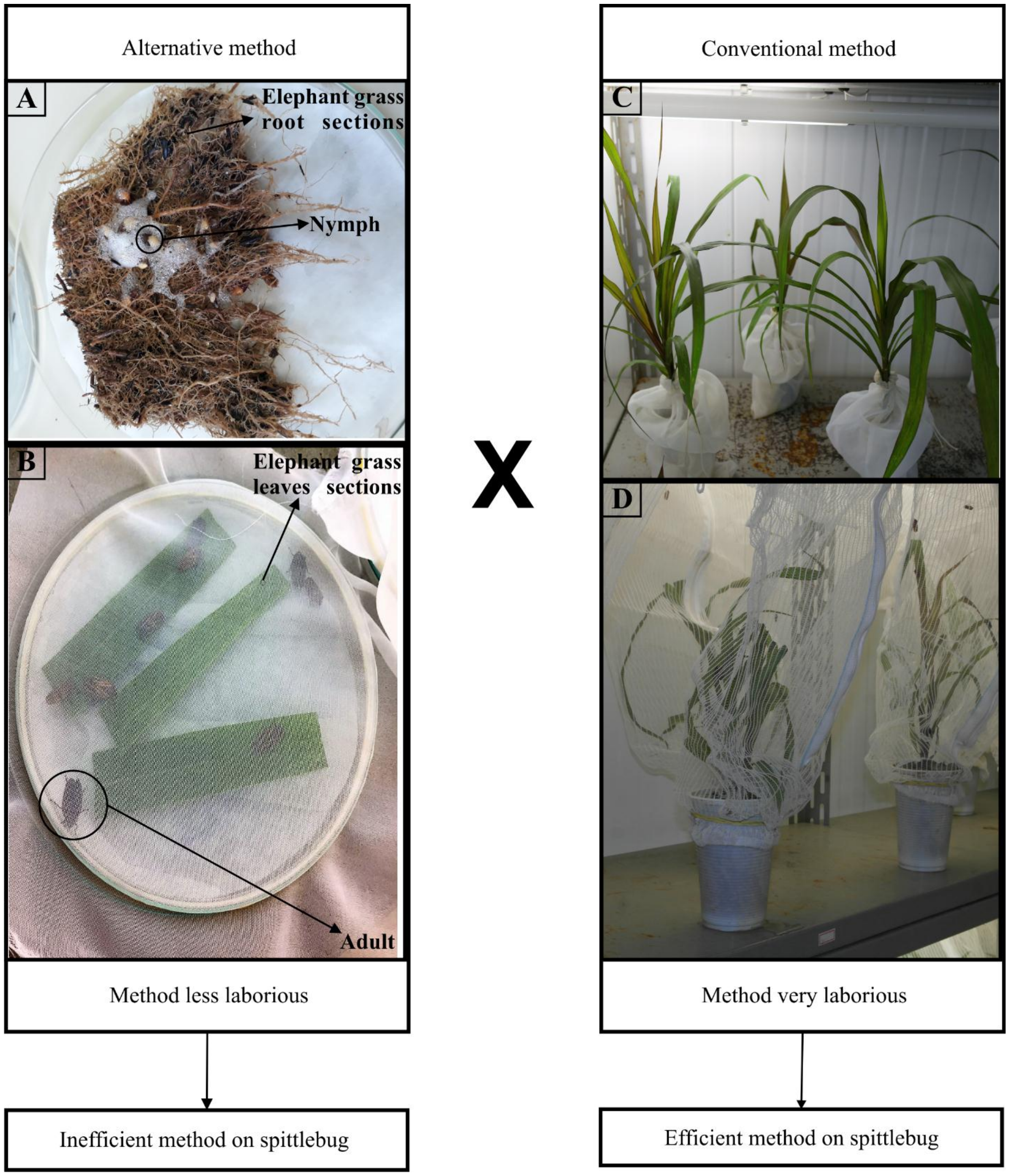

Figure 1. Methodologies for evaluating the effects of thymol on *M. spectabilis* used in this study. Alternative method: Plates lined with filter paper, containing sections of elephant grass roots and *M. spectabilis* nymphs (A); plates containing elephant grass leaf sections and *M. spectabilis* (B). Conventional method: Vases containing *M. spectabilis* nymphs (C) and adults (D).

of thymol on *M. spectabilis*.

METHODOLOGY

Maharva spectabilis were collected in the experimental field of Embrapa Cattle Dairy in Coronel Pacheco, MG, Brazil, and transported to the Laboratory of Entomology in Juiz de Fora, MG, Brazil. Elephant grass plants (*Pennisetum purpureum*) were propagated in plastic pots (500 mL), containing soil and manure in a 1:1 ratio. The plants were kept in a greenhouse and irrigated daily, until they were used in the experiments (after 60 days). Thymol crystals and dimethyl sulfoxide (DMSO) were purchased in standard chemical form from Sigma-Aldrich®. The concentrations used in this study were based on previous studies by Novato *et al*. (2015), Senra *et al*. (2013), and preliminary tests.

The conventional method consisted of packing the insects in groups of 10 into plastic pots (500 ml) containing an elephant grass plant. The plastic pots containing the plants were wrapped in a cage made of voile fabric to prevent the escape of *M. spectabilis* nymphs and adults (Figure 1). The alternative method consisted of placing *M. spectabilis* nymphs and adults in groups of 10 in Petri dishes (9 cm), lined with filter paper moistened with distilled water, containing sections of elephant grass leaves as a food source for adults and root sections as a food source for nymphs (Figure 1). In order to evaluate the reliability of the two evaluation methods used, the mortality rates of *M. spectabilis* submitted to each methodology were compared. The experimental design used in the bioassays was completely randomized, with 10 replicates for each methodology. The method that resulted in higher survival rates of the insects up to 48 h after treatment application was considered the better of the two methods. The data were analyzed using analysis of variance (ANOVA). Results were considered significant at $p < 0.05$.

After the selection of the preferred evaluation method, 10 µL of thymol solutions at concentrations of 2.5, 3.75, 5.0, 7.5, and 10.0% was used. A 1.0% DMSO solution was used as control. Solutions were applied to the dorsal region of *M. spectabilis* nymphs and adults (using a V3-Plus micropipette, 5-10 µL). After application, nymphs and adults were kept room at 25 ± 2 ºC with a 12:12 h light: dark cycle, and at a relative humidity of 70 ± 10%.

Maharva spectabilis eggs were obtained according to Auad and Resende (2018). In the egg test, 340 µL (20 µL/egg) of the thymol solutions were applied in concentrations of 0.3, 0.6, 0.9, 1.2, and 1.5%, and 1.0% DMSO was used as the control. Solutions were applied to groups of 17 eggs at an advanced embryonic stage (S4), arranged in Petri dishes that contained filter paper that was moistened daily. The concentrations were selected based on preliminary tests, in which the lowest concentration used in the tests using nymphs and adults resulted in 100% egg unviability. The Petri dishes containing the eggs were also kept in an air-conditioned room as described above.

The insecticidal effect of thymol on eggs, nymphs, and adults was evaluated at 24 and 48 h after application. The experimental design used in the bioassays was completely randomized, with 10 replicates for each concentration. Control efficiency was calculated using Abbott's formula (1925). The data were transformed by the square root of (x + 0.5) for ANOVA, and the means were compared by the Tukey test (with significance set at $p < 0.05$). The analyses were conducted using SAS software version 9.2 (SAS Institute, Cary, North Carolina, 2008).

RESULTS

No significant difference in nymph survival rates was found when comparing the two methodologies used ($F = 0.217$; $df = 1$; $p = 0.6470$). However, adult survival was significantly higher in the tests using the conventional method (70%) as compared to the alternative method (0%) ($F = 39.44$; $df = 1$; $p = 0,0001$). Thus, the tests to evaluate the effects of thymol on *M. spectabilis* nymphs and adults were performed using the conventional method.

In the tests to evaluate the effect of thymol on *M. spectabilis*, using the conventional method, nymph survival rates were significantly lower for all thymol concentrations used as compared to the control at 24 ($F = 38.65$, $df = 5$, $p = 0.0001$) and 48 h ($F = 50.50$, $df = 5$, $p = 0.0001$) after treatment application. All thymol concentrations resulted in efficiency above 70% and 80% after 24 h and 48 h, respectively (Figure 2A, B).

The adult survival of *M. spectabilis* was significantly lower at thymol concentrations of 3.75, 5.0, 7.5, and 10.0%, as compared to the control at 24 ($F = 41.45$, $df = 5$, $p = 0.0001$) and 48 h ($F = 30.55$, $df = 5$, $p = 0.0001$) after treatment application. When using thymol at concentrations above 5.0%, the efficacy rates were 94% and 97% after 24 and 48 h, respectively. At a concentration of 2.5%, thymol was not efficient for adult control (Figure 2C, D). When using a 5.0% thymol solution, a state of agitation followed by paralysis and death in *M. spectabilis* nymphs and adults was observed.

The viability of *M. spectabilis* eggs was significantly lower at thymol concentrations of 0.9, 1.2, and 1.5%, as compared to the control at 24 h ($F = 29.14$, $df = 5$, $p = 0.0001$) after treatment application. The viability of *M. spectabilis* eggs was significantly lower at all thymol concentrations used when compared to the control at 48 h ($F = 26.84$; $df = 5$; $p = 0.0001$) after treatment application. When using the 1.5% thymol solution, 81% of eggs were not viable at 24 h after treatment application, and this increased to 88% at 48 h after treatment application (Figure 2E, F).

DISCUSSION

The conventional method, although more laborious, resulted in low adult mortality of *M. spectabilis*, when compared to the alternative method used in this study. This indicates the efficiency and reliability of the conventional method in tests evaluating the effects of thymol and/or other chemical compounds on spittlebugs. Garcia *et al*. (2006) successfully employed a similar method using plant pots to verify the insecticidal activity of Neem-based formulations on *M. fimbriolata*. The inefficiency of the alternative method using Petri dishes, evidenced by the significant mortality of adults, suggests that the jumping behavior of adults may also have affected this result. For the nymphs, the alternative method was effective, showing that the spittlebugs were able to use the elephant grass root sections as a sufficient source of nutrients. The alternative method was efficient for studying the effect of insecticides on nymphs of *Orius insidiosus* (Say) (Studebaker and Kring, 2003) and the effects of thymol on larvae of *Diaphania hyalinata* (Linnaeus) (Melo *et al*., 2018). It should be noted that the

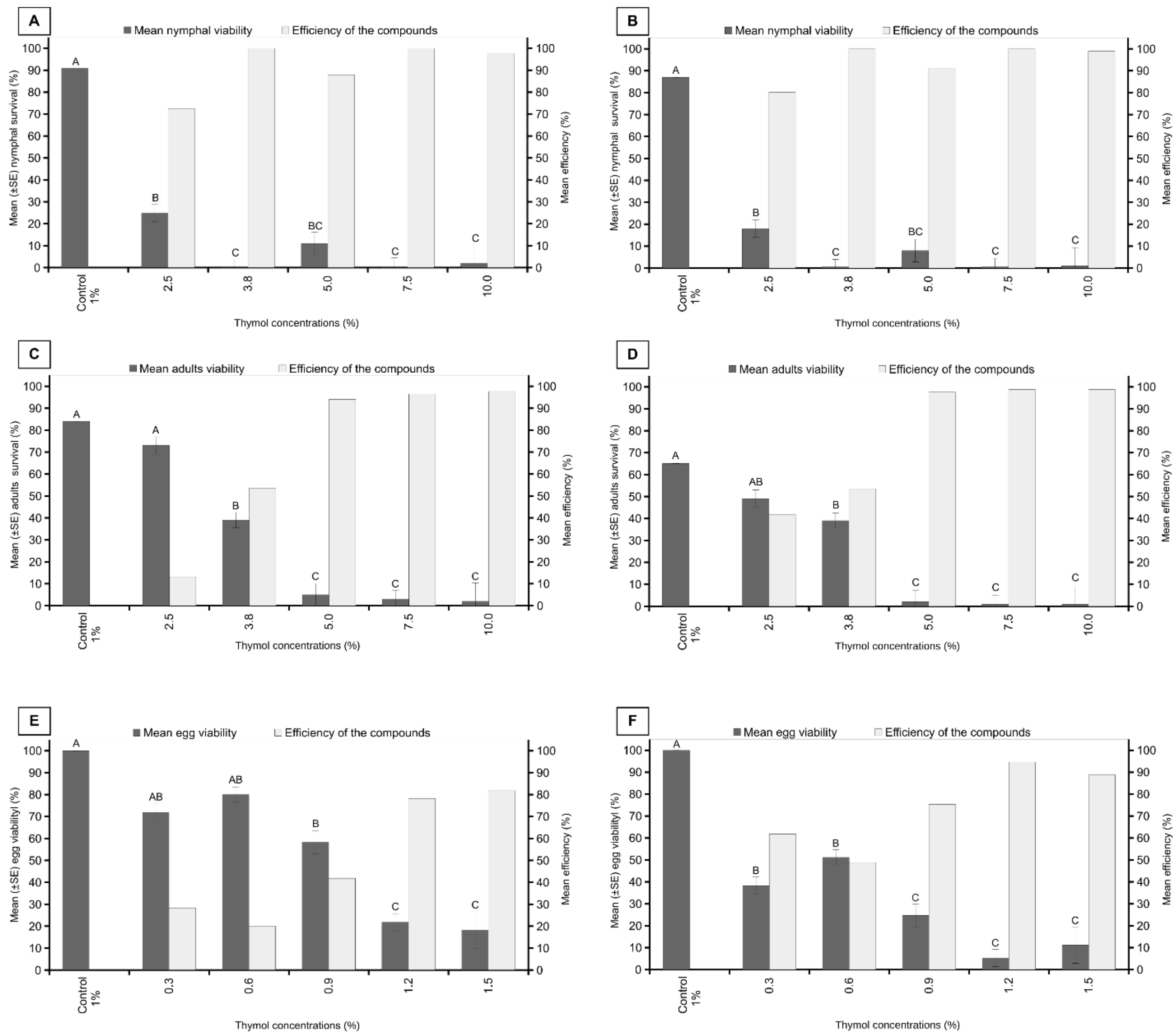

Figure 2. Insecticidal activity of thymol against *M. spectabilis* nymphs 24h (A) and 48 h (B); adults at 24h (C) and 48 h (D), and eggs at 24 (E) and 48 h (F) after application. Different letters in the columns represent significant differences between the treatments by the Tukey test ($p < 0.05$). Control efficiency was calculated using Abbott's formula (1925).

behavior of the insects is related to the efficiency of the method used, and the inefficiency of the alternative method described here in studies on adult insects limits its indication for tests with bioinsecticides, which seek to control both nymphs and adults.

The relationship between thymol concentrations and insect mortality obtained in this study was also observed by Ismail (2018) in tests used to evaluate the toxic effects of thymol on *Megaselia scalaris* (Loew). In addition, the agitation behavior exhibited by *M. spectabilis* has also been reported by Hummelbrunner and Isman (2001) in thymol toxicity tests on *Spodoptera litura* (Fabricius). These researchers attributed this effect to the action of thymol in the octopaminergic system, which is exclusive to insects, and of considerable interest as a target location for control agents.

Thymol has already been shown to have an ovicidal effect, as reported in studies on *Rhodnius prolixus* (Stal) (Figueiredo *et al*., 2017) and Nezara viridula (Linnaeus) (Werdin González *et al*., 2011). The egg shell is described as a barrier against insecticides; however, it contains some areas that could allow the penetration of insecticides, aeropiles, and micropiles (Campbell *et al*., 2016). Caperucci and Camargo-Mathias (2006) described the presence of numerous small pores in the exochorion of eggs of *M. fimbriolata*, which probably facilitate the oxygenation of the internal structures of the eggs. These structures may explain the high rate of unviability observed in thymol treatments of *M. spectabilis* eggs, requiring 10 times lower concentrations to promote embryo mortality than those applied to nymphs and adults. This result was also observed by Kovaříková *et al*. (2017), when evaluating the effects of botanical insecticides on *Aleyrodes proletella* (Linnaeus) eggs and nymphs. These researchers attributed this difference to the serosa layer that protects the nymphs. This study suggest that, in addition to the presence of pores in eggshell, this difference may occur because of the foam secreted by the nymphs of spittlebugs, which may function as a partial elimination mechanism for the irritant.

CONCLUSION

The conventional method, although more laborious, is the most efficient way to evaluate the insecticidal activity of thymol and/or other chemical compounds on adults and nymphs of *M. spectabilis*. Furthermore, thymol is recommended as an active substance with a potential use in combination with other control methods in integrated pest management (IPM) programs.

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