

# ACARICIDAL ACTIVITY OF PLANT ESSENTIAL OILS AGAINST POULTRY RED MITE (*DERMANYSSUS GALLINAE*)

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**Abstract:** The main objective of the performed experiments was to monitor acaricidal activity of selected plant essential oils against poultry red mites (*Dermanyssus gallinae*) and the second objective was to define the lethal doses (LD<sub>50</sub> a LD<sub>90</sub>) of selected essential oils. Effect of six plant essential oils as acaricides against poultry house-collected red mites was examined using direct contact method – by glass vial bioassay. All used essential oils caused mortality of poultry red mites in all their stages of development. Essential oils derived from clove buds and cinnamon have been shown to be effective acaricides against the poultry red mites at concentrations 0.5 and 0.25 µL/cm<sup>2</sup>, when tested over a 24 h period. The average mortality in the negative control was 2%. Clove bud, lavender and cinnamon essential oils in merit further study as potential poultry red mites control agents.

**Key Words:** natural botanical pesticides, plant essential oils, poultry red mites, *Dermanyssus gallinae*

## INTRODUCTION

The poultry red mite (*Dermanyssus gallinae*) is a huge economic problem in domestic poultry flocks all across the Europe, USA, Japan and China. It is haematophagous ectoparasite of birds (Kim et al. 2004).

Its abundance in poultry houses is a major source of irritation and disturbs egg-laying hens, which are more aggressive feather-pecking themselves and have cannibalistic behaviors. Infestation by this parasite can lead to anemia and in some cases there is causal association with host death syndrome. Infestation can lead to decrease in egg production, egg quality (through shell thinning and spotting) and egg venality (Dohnal 2009). The poultry red mite may also serve as a possible vector of a variety of poultry pathogens. The cost expends on fight with poultry red mite and production losses are calculated on 130 million EUR per year only in the EU (Sparagano et al. 2014).

Poultry red mite's life cycle is very fast and it is becoming increasingly resistant against commercial acaricides, for this reasons the fight with it is very difficult. These problems have highlighted the need for the development of selective poultry red mite control alternatives. It is also necessary to monitor infestation on poultry houses (Sparagano et al. 2014, Mul et al. 2009). Plant essential oils may be an alternative source of materials for mite control because they constitute a rich source of bioactive chemicals and they have little or no harmful effects on non-target organisms (Isman 2006).

## MATERIAL AND METHODS

The acaricidal activity of six plant essential oils against poultry red mites was examined using direct contact method – by glass vial bioassay.

The essential oils and all other chemicals were of reagent grade. There were used six essential oils derived from clove bud, lavender, cinnamon, rosemary, skin of Brazilian oranges and peppermint. The active ingredient in clove bud is eugenol (C<sub>10</sub>H<sub>12</sub>O<sub>2</sub>), in lavender is linalool (C<sub>10</sub>H<sub>18</sub>O),

in cinnamon is cinnamaldehyde ( $C_9H_8O$ ), in rosemary is eukalyptol ( $C_{10}H_{18}O$ ), in skin of Brazilian oranges is limonen ( $C_{10}H_{16}$ ) and in peppermint is menthol ( $C_{10}H_{20}O$ ). Oils were dissolved in methanol or distilled water or Tween 85 with water. Each of the essential oils was applied on two circles of filter paper (Whatman No. 3; 22 mm diameter) at the bottom of the glass vial at concentrations 0.5; 0.25; 0.12; 0.06; 0.03; 0.015  $\mu\text{L}/\text{cm}^2$  in amount of 100  $\mu\text{L}$  solution. The solvent of solution was then evaporated. Always eleven glass vials per each concentration and oil and negative control were used. Negative control contained no essential oil on filter paper.

Colonies of poultry red mites (*Dermanyssus gallinae*) were collected from crevices of poultry houses in the Czech Republic in 2016 and 2017. For trapping these parasites were used plastic containers filled with rolled cardboard attached to the constructions in poultry houses. The plastic containers with collected poultry red mites were closed by lids and transferred to the laboratory. Poultry red mites were tested within two days after collection (Kim et al. 2004). Twenty movable poultry red mites all their stages of development were placed on the filter paper impregnated by essential oil at the bottom of the glass vials. Each glass vial was then closed with lid. The mortality of the poultry red mites in the glass vials was measured 24 h after the treatment. Poultry red mites were considered dead if their appendages did not move when parasites were prodded with a fine pin. Data were processed using software MS Excel, XLSTAT (XLSTAT-Dose) and Unistat 5.1 (Unistat Ltd, ENGLAND) (Hubert et al. 2015).

## RESULTS AND DISCUSSION

### Influence of selected essential oils on mortality poultry red mites

Comparison of the acaricidal activity of six plant essential oils at a dose of 0.5; 0.25; 0.12; 0.06; 0.03; 0.015  $\mu\text{L}/\text{cm}^2$  with solvents of methanol, distilled water and Tween 85 with water, against poultry red mites to all their stages of development is shown in Table 1–3. Out of used essential oils, three gave more than 90% mortality against poultry red mites (clove bud, lavender and cinnamon with all solvents at concentration 0.5  $\mu\text{L}/\text{cm}^2$ ), it was measured after 24 h.

Rosemary essential oil with distilled water at all concentrations caused less than 50% mortality, therefore this oil was tested with methanol and Tween 85 with water only at the highest concentration (0.5  $\mu\text{L}/\text{cm}^2$ ). Essential oils derived from skin of Brazilian oranges and peppermint were tested only with distilled water at concentration 0.5  $\mu\text{L}/\text{cm}^2$  because of low mortality of poultry red mites. For this reason rosemary, skin of Brazilian oranges and peppermint essential oils were not tested at other concentrations with methanol and water and Tween 85. The average mortality in controls was 2%.

Many essential oils are known to possess various bioefficacies such as ovicidal, repellent, antifeeding and biocidal activities against various arthropod pests. Essential oils are volatile, so their environmental performance is limited (Pavela 2011). Chemical differences between seemingly similar oils can result from variations in a number of factors, including environmental conditions, harvesting regimens, and extraction protocols. This problem might be resolved by isolating active components from plant products and developing them for use as acaricides; geraniol and several forms of cinnamaldehyde are toxic to poultry red mites (Isman 2006). As a benefit of plant pesticides would be that the numerous active compounds in essential oils would make development of pest resistance to any essential oil-based product extremely difficult (Miresmailli et al. 2006).

### Influence of essential oils diluted with distilled water on mortality of poultry red mites

Statistically significant the most effective essential oil with dissolved water as solvent was clove bud, which caused 99% mortality of poultry red mites at concentrations of 0.5 and 0.25  $\mu\text{L}/\text{cm}^2$  and more than 95% mortality at concentration of 0.12  $\mu\text{L}/\text{cm}^2$  and at 74.5% mortality still at concentration 0.06  $\mu\text{L}/\text{cm}^2$ , which was statistically significantly the highest mortality at the same concentration from all tested oils ( $P < 0.001$ ). At concentrations 0.12 and 0.06  $\mu\text{L}/\text{cm}^2$  the mortality was significantly the highest at using clove bud essential oil ( $P < 0.001$ ).

Lavender and cinnamon caused higher than 99% mortality only at concentrations 0.25 and 0.5  $\mu\text{L}/\text{cm}^2$ . At these concentrations, there were significant difference ( $P < 0.001$ ) between the efficiency of these three oils in comparison with the efficiency of rosemary, skin of Brazilian oranges and peppermint essential oils.

Excepting clove bud all oils caused less than 50% mortality at concentrations 0.12; 0.06 and 0.03  $\mu\text{L}/\text{cm}^2$  (here clove bud too). At these concentrations, there were significant differences between the oils ( $P < 0.001$ , see Table 1).

Essential oil derived from skin of Brazilian oranges caused 55.5% mortality at concentration of 0.5  $\mu\text{L}/\text{cm}^2$ , other concentrations caused lower than 50% mortality. Rosemary as well as peppermint caused less than 50% mortality even at the highest concentration (see Table 1).

*Table 1 Influence of essential oils with distilled water to mortality poultry red mites*

| Essential oil + ddH <sub>2</sub> O | Average mortality poultry red mites $\pm$ SE (%) |                              |                              |                             |                              |
|------------------------------------|--------------------------------------------------|------------------------------|------------------------------|-----------------------------|------------------------------|
|                                    | Dose ( $\mu\text{L}/\text{cm}^2$ )               |                              |                              |                             |                              |
|                                    | 0.5                                              | 0.25                         | 0.12                         | 0.06                        | 0.03                         |
| Clove bud                          | 99.5 $\pm$ 0.5 <sup>b</sup>                      | 100.0 $\pm$ 0.0 <sup>b</sup> | 98.2 $\pm$ 1.0 <sup>c</sup>  | 74.5 $\pm$ 7.3 <sup>b</sup> | 34.5 $\pm$ 7.0 <sup>c</sup>  |
| Lavender                           | 99.5 $\pm$ 0.5 <sup>b</sup>                      | 99.5 $\pm$ 0.2 <sup>b</sup>  | 25.0 $\pm$ 6.1 <sup>ab</sup> | 17.5 $\pm$ 2.8 <sup>a</sup> | 9.0 $\pm$ 2.1 <sup>ab</sup>  |
| Cinnamon                           | 100.0 $\pm$ 0.0 <sup>b</sup>                     | 99.5 $\pm$ 0.5 <sup>b</sup>  | 30.5 $\pm$ 3.4 <sup>b</sup>  | 18.0 $\pm$ 2.5 <sup>a</sup> | 23.3 $\pm$ 5.1 <sup>bc</sup> |
| Rosemary                           | 45.0 $\pm$ 4.8 <sup>a</sup>                      | 34.5 $\pm$ 8.1 <sup>a</sup>  | 10.4 $\pm$ 2.1 <sup>a</sup>  | 3.5 $\pm$ 1.0 <sup>a</sup>  | 16.6 $\pm$ 4.2 <sup>ab</sup> |
| Skin of Brazilian oranges          | 55.5 $\pm$ 12.2 <sup>a</sup>                     | 25.0 $\pm$ 3.9 <sup>a</sup>  | 19.8 $\pm$ 3.4 <sup>ab</sup> | 4.2 $\pm$ 1.5 <sup>a</sup>  | 0.0 $\pm$ 0.0 <sup>a</sup>   |
| Peppermint                         | 46.6 $\pm$ 8.3 <sup>a</sup>                      | 37.6 $\pm$ 7.0 <sup>a</sup>  | 27.1 $\pm$ 6.6 <sup>ab</sup> | 12.5 $\pm$ 2.2 <sup>a</sup> | 7.2 $\pm$ 2.1 <sup>ab</sup>  |
| P                                  | $P < 0.001$                                      | $P < 0.001$                  | $P < 0.001$                  | $P < 0.001$                 | $P < 0.001$                  |

*a, b – between the values marked with different letters is statistically significant difference between the essential oils at specific concentration; SE – standard error.*

### **Influence of essential oils diluted with methanol on mortality of poultry red mites**

Significantly the most effective essential oil with methanol as solvent was clove bud, which caused 99% mortality of poultry red mites at concentrations 0.5; 0.25 and 0.12  $\mu\text{L}/\text{cm}^2$  and more than 90% mortality at concentration 0.06  $\mu\text{L}/\text{cm}^2$  and 73.5% mortality at concentration 0.03  $\mu\text{L}/\text{cm}^2$ .

Lavender and cinnamon essential oils caused more than 90% mortality at concentrations 0.5  $\mu\text{L}/\text{cm}^2$  and 0.25  $\mu\text{L}/\text{cm}^2$ . The lavender caused 69.5% mortality at concentration 0.03  $\mu\text{L}/\text{cm}^2$  and cinnamon more than 50% mortality at concentration 0.06  $\mu\text{L}/\text{cm}^2$ .

At concentrations 0.25; 0.12; 0.06 and 0.03  $\mu\text{L}/\text{cm}^2$  there was no significant difference between the efficiency of clove bud and lavender. At concentration 0.015  $\mu\text{L}/\text{cm}^2$  there was significant difference in the efficiency of clove bud and lavender oils ( $P < 0.05$ ) (see Table 2).

Rosemary essential oil caused only 14.4% mortality at concentration 0.5  $\mu\text{L}/\text{cm}^2$ , for this reason it was not tested at other concentrations. At concentration 0.5  $\mu\text{L}/\text{cm}^2$  there was significant difference ( $P < 0.001$ ) between the efficiency of clove bud, lavender and cinnamon considering to the efficiency of rosemary oil.

*Table 2 Influence of essential oils with methanol to mortality poultry red mites*

| Essential oil + CH <sub>3</sub> OH | Average mortality poultry red mites $\pm$ SE (%) |                             |                              |                               |                             |
|------------------------------------|--------------------------------------------------|-----------------------------|------------------------------|-------------------------------|-----------------------------|
|                                    | Dose ( $\mu\text{L}/\text{cm}^2$ )               |                             |                              |                               |                             |
|                                    | 0.5                                              | 0.25                        | 0.12                         | 0.06                          | 0.03                        |
| Clove bud                          | 100.0 $\pm$ 0.0 <sup>b</sup>                     | 99.5 $\pm$ 0.3 <sup>a</sup> | 99.1 $\pm$ 0.6 <sup>b</sup>  | 91.6 $\pm$ 3.1 <sup>b</sup>   | 73.5 $\pm$ 9.8 <sup>a</sup> |
| Lavender                           | 98.2 $\pm$ 0.8 <sup>b</sup>                      | 91.4 $\pm$ 5.1 <sup>a</sup> | 85.5 $\pm$ 4.5 <sup>ab</sup> | 69.5 $\pm$ 10.9 <sup>ab</sup> | 69.5 $\pm$ 9.7 <sup>a</sup> |
| Cinnamon                           | 99.7 $\pm$ 0.2 <sup>b</sup>                      | 99.5 $\pm$ 0.2 <sup>a</sup> | 75.6 $\pm$ 6.8 <sup>a</sup>  | 56.5 $\pm$ 8.5 <sup>a</sup>   | -                           |
| Rosemary                           | 14.4 $\pm$ 2.6 <sup>a</sup>                      | -                           | -                            | -                             | -                           |
| P                                  | $P < 0.001$                                      | $P > 0.05$                  | $P < 0.01$                   | $P < 0.05$                    | $P > 0.05$                  |

*a, b – between the values marked with different letters is statistically significant difference between the essential oils at specific concentration; SE – standard error.*

### **Influence of essential oils diluted with Tween 85 with water on mortality of poultry red mites**

The most effective essential oils with Tween 85 with water as solvent were clove bud and cinnamon, which caused more than 99% mortality of poultry red mites at concentrations 0.5; 0.25; 0.12 and 0.06  $\mu\text{L}/\text{cm}^2$ . Cinnamon caused more than 90% mortality and clove bud caused 81.8%

mortality at concentration  $0.03 \mu\text{L}/\text{cm}^2$ , there was no significant difference ( $P < 0.05$ ) between these two oils. Both oils were significantly more effective considering to the efficiency of lavender at concentrations 0.25; 0.12; 0.06 and  $0.03 \mu\text{L}/\text{cm}^2$ .

Lavender essential oil caused more than 99% mortality at concentration  $0.5 \mu\text{L}/\text{cm}^2$ . At concentration  $0.5 \mu\text{L}/\text{cm}^2$  there was no significant difference between the efficiency of lavender, cinnamon and clove bud. Lavender caused less than 50% mortality at other concentrations.

Rosemary essential oil caused only 22.1% mortality at concentration  $0.5 \mu\text{L}/\text{cm}^2$ , for this reason it was not tested at other concentrations. At concentration  $0.5 \mu\text{L}/\text{cm}^2$  was activity of this oil significantly the lowest ( $P < 0.001$ ).

At concentration  $0.15 \mu\text{L}/\text{cm}^2$  there was significant difference between the efficiency of clove bud and lavender, however both caused less than 10% mortality (see Table 3).

*Table 3 Influence of essential oils with Tween 85 with water to mortality poultry red mites*

| Essential oil +<br>Tween 85 | Average mortality poultry red mites $\pm$ SE (%) |                   |                   |                   |                  |                 |
|-----------------------------|--------------------------------------------------|-------------------|-------------------|-------------------|------------------|-----------------|
|                             | Dose ( $\mu\text{L}/\text{cm}^2$ )               |                   |                   |                   |                  |                 |
|                             | 0.5                                              | 0.25              | 0.12              | 0.06              | 0.03             | 0.015           |
| Clove bud                   | $100.0 \pm 0.0^b$                                | $100.0 \pm 0.0^b$ | $100.0 \pm 0.0^b$ | $100.0 \pm 0.0^b$ | $81.8 \pm 7.0^b$ | $8.6 \pm 1.2^b$ |
| Lavender                    | $100.0 \pm 0.0^b$                                | $38.6 \pm 7.5^a$  | $19.5 \pm 2.3^a$  | $2.3 \pm 1.2^a$   | $10.9 \pm 2.9^a$ | $3.6 \pm 1.9^a$ |
| Cinnamon                    | $100.0 \pm 0.0^b$                                | $100.0 \pm 0.0^b$ | $100.0 \pm 0.0^b$ | $99.4 \pm 0.5^b$  | $97.5 \pm 1.9^b$ | -               |
| Rosemary                    | $22.1 \pm 5.7^a$                                 | -                 | -                 | -                 | -                | -               |
| P                           | $P < 0.001$                                      | $P < 0.001$       | $P < 0.001$       | $P < 0.001$       | $P < 0.001$      | $P < 0.05$      |

*a, b – between the values marked with different letters is the statistically significant difference between the essential oils at specific concentration; SE – standard error.*

### Lethal doses of the essential oils for poultry red mites

Table 4 shows the concentrations of the essential oil ( $\mu\text{L}/\text{cm}^2$ ) necessary for 50 and 90% mortality of poultry red mites.  $R^2$  coefficient (Nagelkerke) is in the range between 0 and 1 and shows how well the model fit data. Significant differences were observed in essential oils toxicity and used solvents. For the fitted model parameters, i.e.  $\text{LD}_{50}$  and  $\text{LD}_{90}$  are given fit 95% confidence interval in round brackets (Hubert et al. 2015).

Clove bud essential oil was the most effective with Tween 85 with water as solvent for the lethal doses  $\text{LD}_{50}$  and  $\text{LD}_{90}$  of poultry red mites, when there was necessary to be applied on the filter paper for  $\text{LD}_{50}$   $0.02 \mu\text{L}/\text{cm}^2$  and for  $\text{LD}_{90}$   $0.03 \mu\text{L}/\text{cm}^2$ . On the other hand clove bud was necessary to be applied on the filter paper in the biggest concentration with distilled water, for  $\text{LD}_{50}$   $0.04 \mu\text{L}/\text{cm}^2$  and for  $\text{LD}_{90}$   $0.08 \mu\text{L}/\text{cm}^2$ .

Lavender essential oil was the most effective with methanol as solvent for the lethal doses  $\text{LD}_{50}$  and  $\text{LD}_{90}$  of poultry red mites, when there was necessary to be applied on the filter paper for  $\text{LD}_{50}$   $0.02 \mu\text{L}/\text{cm}^2$  and for  $\text{LD}_{90}$   $0.19 \mu\text{L}/\text{cm}^2$ . On the other hand lavender was necessary to be applied on the filter paper in the biggest concentration with Tween 85 with water for  $\text{LD}_{50}$   $0.55 \mu\text{L}/\text{cm}^2$  and for  $\text{LD}_{90}$   $6.20 \mu\text{L}/\text{cm}^2$ .

Cinnamon essential oil was the most effective with Tween 85 with water as solvent for the lethal doses  $\text{LD}_{50}$  and  $\text{LD}_{90}$  of poultry red mites, when there was necessary to be applied on the filter paper for  $\text{LD}_{50}$   $0.004 \mu\text{L}/\text{cm}^2$  and for  $\text{LD}_{90}$   $0.02 \mu\text{L}/\text{cm}^2$ . On the other hand lavender was necessary to be applied on the filter paper in the biggest concentration with distilled water for  $\text{LD}_{50}$   $0.11 \mu\text{L}/\text{cm}^2$  and for  $\text{LD}_{90}$   $0.28 \mu\text{L}/\text{cm}^2$ .

Rosemary essential oil, essential oil derived from skin of Brazilian oranges and peppermint essential oils were tested only with distilled water as solvent. From their comparison ensue, that the most effective was skin of Brazilian oranges for the lethal doses  $\text{LD}_{50}$  and  $\text{LD}_{90}$  of poultry red mites, when there was necessary to be applied on the filter paper for  $\text{LD}_{50}$   $0.45 \mu\text{L}/\text{cm}^2$  and for  $\text{LD}_{90}$   $1.82 \mu\text{L}/\text{cm}^2$ . On the other hand rosemary was necessary to be applied on the filter paper in the biggest concentration, for  $\text{LD}_{50}$   $0.66 \mu\text{L}/\text{cm}^2$  and for  $\text{LD}_{90}$   $5.93 \mu\text{L}/\text{cm}^2$ .

Table 4 Dependence of the amount of the essential oil ( $\mu\text{L}/\text{cm}^2$ ) on the mortality of poultry red mites

| Essential oil             | Solvent            | R <sup>2</sup> | LD <sub>50</sub>     | LD <sub>90</sub>  |
|---------------------------|--------------------|----------------|----------------------|-------------------|
| Clove bud                 | ddH <sub>2</sub> O | 0.57           | 0.04 (0.04/0.04)     | 0.08 (0.07/0.09)  |
|                           | CH <sub>3</sub> OH | 0.64           | 0.03 (0.02/0.03)     | 0.05 (0.05/0.06)  |
|                           | Tween 85           | 0.8            | 0.02 (0.02/0.02)     | 0.03 (0.03/0.04)  |
| Lavender                  | ddH <sub>2</sub> O | 0.71           | 0.13 (0.12/0.17)     | 0.22 (0.17/0.38)  |
|                           | CH <sub>3</sub> OH | 0.23           | 0.02 (0.01/0.02)     | 0.19 (0.15/0.24)  |
|                           | Tween 85           | 0.17           | 0.55 (0.39/0.89)     | 6.20 (3.03/17.91) |
| Cinnamon                  | ddH <sub>2</sub> O | 0.57           | 0.11 (0.10/0.12)     | 0.28 (0.25/0.32)  |
|                           | CH <sub>3</sub> OH | 0.45           | 0.03 (0.03/0.03)     | 0.07 (0.07/0.09)  |
|                           | Tween 85           | 0.19           | 0.004 (0.00001/0.01) | 0.02 (0.002/0.03) |
| Rosemary                  | ddH <sub>2</sub> O | 0.15           | 0.66 (0.50/0.98)     | 5.93 (3.02/18.23) |
| Skin of Brazilian oranges | ddH <sub>2</sub> O | 0.32           | 0.45 (0.39/0.53)     | 1.82 (1.35/2.72)  |
| Peppermint                | ddH <sub>2</sub> O | 0.17           | 0.49 (0.40/0.66)     | 4.97 (2.89/10.90) |

For the fitted model parameters, i.e. LD<sub>50</sub> and LD<sub>90</sub> are given fit 95% confidence interval in round brackets.

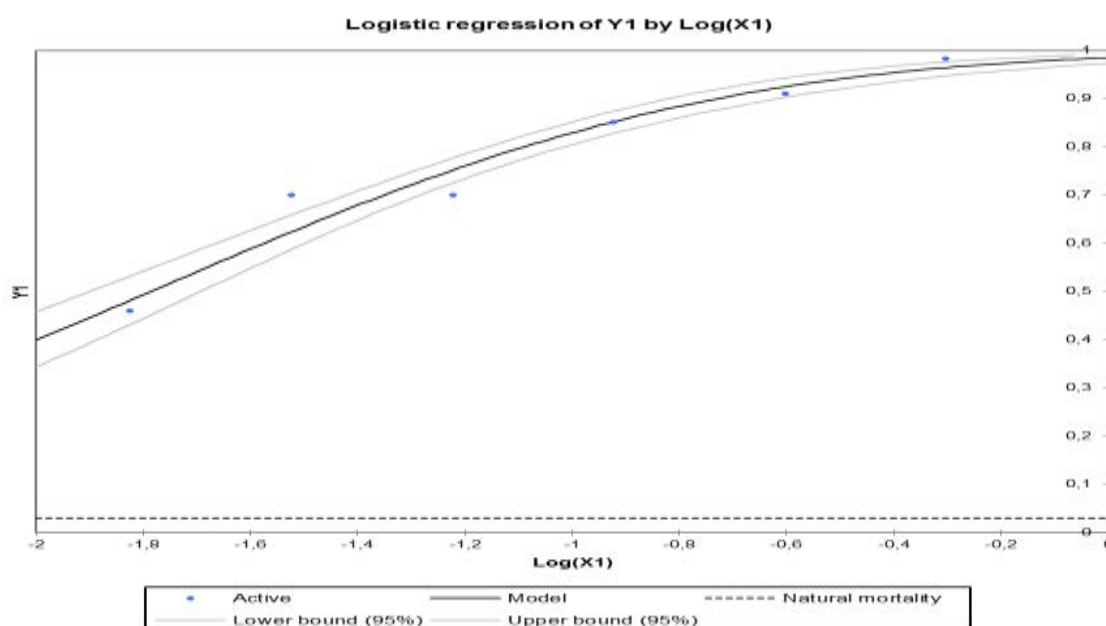
Figure 1 Dependence of the concentration of the lavender essential oil with methanol as solvent for the lethal doses of poultry red mites on  $\text{cm}^2$ 

Figure 1 shows the correlation between the amount of lavender essential oil with methanol as solvent ( $\mu\text{L}/\text{cm}^2$ ) and the mortality of the poultry red mites population, which is represented by a black curve in the graph. Gray curves show confidence intervals and blue points measurements. It was necessary to be applied on the filter paper for LD<sub>50</sub> 0.02  $\mu\text{L}/\text{cm}^2$  and for LD<sub>90</sub> 0.19  $\mu\text{L}/\text{cm}^2$ .

## CONCLUSION

The results of these experiments suggest that certain essential oils may make effective natural botanical pesticides against poultry red mites. The highest mortality was observed with clove buds and cinnamon essential oils. For practical usage may enough exerting more than 50% mortality. Plant essential oils have little or no harmful effects on non-target organisms. Perhaps the greatest constraint to the use of essential oils like effective natural botanical pesticides in poultry red mites control is their relative lack of standardization and consequent inconsistent efficacy.

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