ETHANOLIC EXTRACT AND ESSENTIAL OILS FROM *MONARDA FISTULOSA* L. WITH OVICIDAL, INSECTICIDAL AND ANTIFEEDANT ACTIVITY AGAINST COLORADO POTATO BEETLE

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Abstract

Species *Monarda fistulosa* L. (Lamiaceae), which is currently successfully cultivated in the conditions of the Republic of Moldova, is of great interest due to the content of various components that possess biological activity. Studying the effect of ethanolic extract and essential oils from *M fistulosa* in the laboratory conditions showed the presence of ovicidal, insecticidal, and antifeedant properties against the Colorado potato beetle. Hatching of larvae of *L. decemlineata* in all experimental variants was completely suppressed (100 %) in comparison with the control. Essential oils showed higher insecticidal activity than ethanol extract. The death of the larvae of *L. decemlineata* in the variant with 5 % ethanol extract is 13.3 %, and in the variants with essential oils (with and without timoquinone) – 93.3 and 60.0%, respectively. It was established that the essential oil with thymoquinone has a higher insecticidal properties (by 33.3%) than the oil without thymoquinone.Mathematical analyses revealed some significant differences of insecticidal activity between variants: ethanolic extract, essential oils and control ($p \leq 0,05$, $LSD_{0.05}= 6.7$). The larvae in the variants with essential oils did not feed on the treated leaves of potatoes. In the variant with ethanolic extract treatment (EE), the larvae tried to feed, but the damage to the leaf plate did not exceed 10 %. In the control the leaves damage was 100 %.

Key words: *Monarda fistulosa*, ethanolic extract, essential oils, ovicidal, insecticidal, antifeedant activity

Species *Monarda fistulosa* L. (Lamiaceae) – wild bergamot or bee balm, native to North America (Canada and 48 lower states of the USA). Now it distributed in subtropical regions of the planet. *M. fistulosa* cultivated as an essential oil plant since 1637. At the moment it occurs in Canada, the United States, European countries, the Crimea and the Caucasus, in the Russian Federation. There are no links that this species can be invasive. At home in North America – in the United States and Mexico is found quite widely, sometimes turning into a weed plant.

M. fistulosa is a perennial herbaceous polycarpic plant with numerous upright, branched stems, 65–120 cm in height (*figure 1*). Stems with a light or partially anthocyanin coloration. The leaves are simple, opposite, broadly lanceolate forms, jagged along the edge, gray-green due to strong pubescence with fine hairs. The plant forms many shoots, but not all of them bloom. Flowers numerous, small, connected in axillary round pseudoglobular verticils, surrounded by reddish bracts along the nerves, gathered in compact spherical anthodiums, located at the ends of the main and lateral axillary brunches (*figure 2, 3*). On each flower-bearing shoot there are from five to nine inflorescences with a diameter of 5–7 cm. There are 190–260 flowers in each inflorescence. Corolla with a long tube, deep-eared. The flowers are predominantly lilac in contrast to purple, violescent, purple-pink, and occasionally white in natural habitats (or in hybrids). The fruit is aggregate nut (*figure 4*), consist of four small dark brown nuts (Gorlacheva, 2009; Feskov, Shevchuk, 2017).

The aerial parts of the *M. fistulosa* contain essential oil, terpenoids, polyphenols (hydroxycinnamic acids, flavones, flavonoids, anthocyanins) monardein, amino acids, bitterness, tannins, cellulose, pectins, vitamin C – 29.3 mg % (Oparin et al., 2000). The leaves contain 1.73 % thymol and carvacrol; 3.30 % hydroxycinnamic acids and 1.78 % flavone glycosides (Casian et al., 2017). The content of flavonodes in the dry above-ground mass was 4702.1–6156.2 mg %,

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hydroxycinnamic acids – 3969 mg % (Bobrovich et al., 2012), timoquinone – 0.23 %; tannins – 3.74 %, coumarins, phenol carboxylic acids and flavanoids – 11.61 %. The maximum content of essential oil – in the inflorescences and leaves, the smallest – in the stems (Casian et al., 2017). The main component of the essential oil are thymol and carvacrol (60–61 %).

This species was successfully introduced on the territory of the Republic of Moldova, where it allows to obtain yields of 15.2 ± 4.4 t / ha of fresh biomass or 68.0 ± 4.3 kg / ha of essential oil (Bodrug, 1993). Due to reach chemical compounds *M. fistulosa* represents valuable raw materials.

The purpose of this work was to study the ovicidal, insecticidal and antifeedant activity of essential oils and ethanolic extract from the *M. fistulosa* against the Colorado Potato Beetle.

MATERIAL AND METHOD

As raw materials, we used leaves and flowers of wild bergamot collected at the beginning of the flowering period (*figure 1, 2, 3*).

Ethanolic extract and essential oils was prepared and quantitative analyzed in the Scientific Center for Drug Research of "Nicolae Testemitanu" State University of Medicine and Pharmacy (Casian et al., 2017).

Testing of the ovicidal, insecticidal and antifeedant activity of extracts and oils were carried out in the laboratory conditions during 2018 year in the Institute of Genetics, Physiology and Plant Protection, Chisinau, Republic of Moldova and in the Institute of Biodiversity Conservation and Biosafety, Department of Genetics and Plant Breeding, Slovak University of Agriculture in Nitra.

Figure 1 **The plants of the *Monarda fistulosa* L. (Lamiaceae), May 2018**

The ethanolic extract (EE) (70% alcohol, the ratio of raw materials: solvent 1: 5), in a concentration of 5.0 % and essential oils of *M. fistulosa* with (EO_1) and without (EO_2) thymoquinone, in a concentration of 0.01 % were tested. As a standard was used the water.

Figure 2 **Plants of *Monarda fistulosa* during flowering, 2018**

Figure 3 **The flower of the *Monarda fistulosa* L. (Lamiaceae), 2018**

Figure 4 **Seeds of *Monarda fistulosa***

Determination of ovicidal, insecticidal and antifeedant activities of ethanolic extract and essential oils were carry out on the ovipositions and

2-3 instars larvae *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) according to standard methods (Indrumări metodice..., 2002).

Ovicidal activity. Nine fresh-laid ovipositions of Colorado potato beetles (on potato leaves) were treated with the prepared solutions by immersion for 30 seconds. After that, they were laid out on filter paper and kept in the open air until the solutions evaporated from the leaves. Then three leaves with egg-laying were placed in Petri dishes, each variant had three replications. During 8 days, we recorded the hatching and death of hatched larvae. The percent of ovicidal activity was assessed at 120 hours' post-treatment using the following formula (1):

$$O = \frac{N_1}{N_0 - N_c} \times 100, \quad (1)$$

where O – ovicidal activity, %;
N_1 – number of unhatched eggs;
N_0 – number of total eggs in the variant;
N_c – number of unhatched eggs in the control.

The death of the larvae after hatching was taken into account as the insecticidal effect of preparations on insects.

Insecticidal activity. Leaves of potato were treated by immersion in solutions of the ethanolic extract and essential oils, and then were stored in an exhaust box for 1 hour to complete evaporation of solvent. The leaves, treated using water, were served as the control. After that, leaves were placed into double (Petri) dishes with insects. Insecticidal activity was evaluated by counting of dead insect number for three days in comparison with the control according formula (2).

$$I = \frac{N_1}{N_0 - N_c} \times 100, \quad (2)$$

where I – insecticidal activity, %;
N_1 – number of dead larvae;
N_0 – number of total larvae in the variant;
N_c – number of dead larvae in the control.

Antifeedant activity of extracts was estimated three days after beginning of each experiment according to the point scale (tab. 1).

Table 1

Scale of antifeedant activity

Browsing of leaf surface, %	Level of antifeedant activity	Points
0 to 5 %	very high	1
6 to 25 %	high	2
26 to 50 %	moderate	3
51 to 75 %	low	4
76 to 100 %	very low (zero)	5

Statistical processing of obtained data was carried out according to the one-way ANOVA test and Microsoft Excel software.

RESULTS AND DISCUSSIONS

All preparations - the ethanolic extract and essential oils from *Monarda fistulosa* shoved high ovicidal activity (*table 2*).

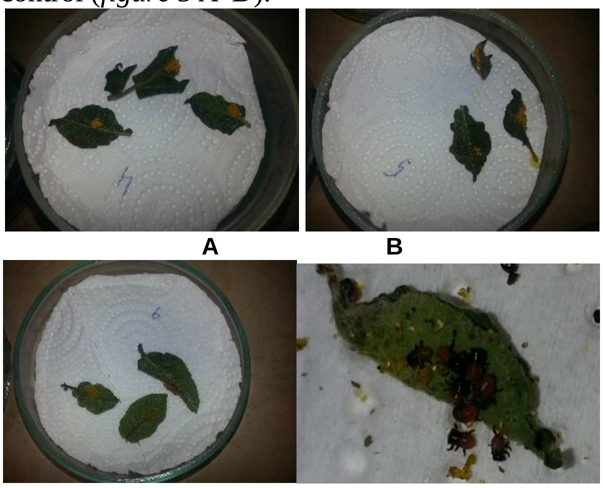
In the control variant all the larvae have hatched: in the first day – more than 30% of hatching, on the second day – another 40%, and the rest have hatched on the sixth day. The treatment of egg-laying with extract and oils from wild bergamot caused the larvae of *L. decemlineata* not to hatch.

Table 2

Ovicidal, insecticidal and antifeedant activity of the ethanolic extract and essential oils from *Monarda fistulosa* against *Leptinotarsa decemlineata* in the laboratory conditions, 2018

Variants	Ovicidal activity, %	Insecticidal activity, %	Antifeedant activity, points
Control	0	0	5
EE 5.0 %	100	13.3	2
EO_1, 0.01 %	100	93.3	1
EO_2, 0.01 %	100	60.0	1
		$LSD_{0.05}$= 6.7, p≤0,05	

The ovicidal effect of preparations from *M. fistulosa* on the egg-laying is shown in figure 5 in comparison with the hatching of larvae in the control (*figure 5 A–D*).



C D

Figure 5. **Non-hatching of the larvae of the Colorado beetle from oviposition under the action of extract and essential oils from *Monarda fistulosa* in the laboratory conditions, 2018 (A –*ethanolic extract*; B, C – *essential oil EO_1 and EO_2*; D - hatching of the larvae of the Colorado beetle from oviposition in the control)**

Essential oils of wild bergamot showed high insecticidal activity (60.0-93.3%), but ethanolic extract caused death only 13.3% of the larvae *L. decemlineata* (*table 2*). At the same time, a negative (phytotoxic) impact of essential oil on potato foliage was observed (*figure 6 A*). There was no phytotoxicity in control (*figure 6 B*). Hereafter, should be explored whether the phytotoxicity of essential oils is manifested when the preparation is sprayed on the potato bushes.

Mathematical analyses revealed some significant differences of insecticidal activity between variants: ethanolic extract, essential oils and control ($p \leq 0,05$, $LSD_{0.05} = 6.7$) (*table 2*).

It was also found that the essential oil had the antifeedant effect (*table 2*). The larvae in the variants with essential oils did not feed on the treated leaves of potatoes. In the variant with ethanolic extract treatment (EE), the larvae tried to feed, but the damage to the leaf plate did not exceed 10 %. In the control the damage reached to 100 %.

A

B

Figure 6. **The action of essential oil from *Monarda fistulosa* on the larvae *L. decemlineata* and on potato leaves in the laboratory conditions, 2018 (A – *essential oil* EO_2; B – control).**

Earlier, the authors Tabanca *et al* (2013) found that thymol, isolated from an essential oil of wild bergamot, showed the best larvicidal activity (compared with the other components - carvacrol, eugenol and carvacrol methyl ether) against one-day larvae of the yellow-fever mosquito *Aedes aegypti* L. (Diptera: Culicidae) – LD_{50} 9 mg / m^3. In ours experiments established that the essential oil with thymoquinone has a higher insecticidal properties (by 33.3%) than the oil without thymoquinone. Thus, thymoquinone possesses insecticidal properties, and probably its addition to other herbal preparations could to enhance their biological effects. Therefore, we believe that the cumulative (total) effect of the essential oil will be higher than when separated into individual components. However, it is also possible to use essential oil without timoquinone against pests, as it has several advantages, one of which is the transparent color of the oil.

CONCLUSIONS

The pronounced ovicidal, insecticidal and antifeedant properties of the ethanolic extract and essential oils from medicinal and aromatic plant *Monarda fistulosa* were established. Essential oil with thymoquinone had the highest activity (100% effect) against Colorado potato beetle.

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