

Toxic properties and allelopathic activity of *Melilotus officinalis* (L.) Pall.

Svetlana I. Mikhailova^{1,3}, Valeria Yu. Andreeva², Nadezhda S. Zinner^{1,2},
Elena S. Gulik¹, Svetlana A. Suchkova¹, Mikhail V. Belousov²

1 Tomsk State University, 36 Lenina prospect, Tomsk, 634050, Russia

2 Siberian State Medical University, 2 Moscovskiy Trakt, Tomsk, 634050, Russia

3 Tomsk Branch of All-Russian Plant Quarantine Center (“VNIIKR”), 109 A Frunze prospect, Tomsk, 634021, Russia

Corresponding author: Nadezhda S. Zinner (zinner@inbox.ru)

Academic editor: R. Yakovlev | Received 1 March 2022 | Accepted 24 March 2022 | Published 1 April 2022

<http://zoobank.org/F44E8456-C193-48C9-9236-F3D057A53D21>

Citation: Mikhailova SI, Andreeva VYu, Zinner NS, Gulik ES, Suchkova SA, Belousov MV (2022) Toxic properties and allelopathic activity of *Melilotus officinalis* (L.) Pall. Acta Biologica Sibirica 8: 89–99. <https://doi.org/10.14258/abs.v8.e03>

Abstract

Melilotus officinalis (L.) Pall., known as yellow sweetclover (Fabaceae), is widely used in medicine and agriculture. At the same time, yellow sweetclover is a weed and invasive plant in Siberia. In Russia, *M. officinalis* is cultivated as a valuable medicinal, fodder and honey plant. Its widespread use is due to its high ecological plasticity. In recent years, an interest in cultivation of *M. officinalis* as a low maintenance multipurpose crop has increased in biological agriculture. The herb *M. officinalis* contains a rich complex of biologically active compounds. However, along with positive properties, this species, though with a rich chemical composition and high physiological activity, is toxic towards different groups of living organisms. The toxic effect of *M. officinalis* extracts is primarily due to the presence of coumarin. A high allelopathic activity of *M. officinalis* was revealed. The phytotoxic effect of herb extracts on germination of crop and weed seeds was studied in detail. Data on the fungicidal and insecticidal activity of *M. officinalis* were obtained. Laboratory and in situ studies showed that the aboveground part of *M. officinalis* is a potential source of biopesticides with a broad-spectrum effect (bioherbicidal, insecticidal and fungicidal).

Keywords

Melilotus officinalis, coumarin, biological toxicity, allelopathy, biopesticides, repellent activity, biological agriculture

Introduction

Melilotus officinalis, known as yellow sweetclover, is widespread in Russia. In most regions of Siberia (Omsk, Novosibirsk, and Kemerovo regions, Altai Territory and the Republics of Altai and Khakassia), it is a native species. The plant grows in steppe meadows, sometimes saline, along roads, ditches, in fallow lands and in crops as a weed, on the plain in the forest, forest-steppe and steppe zones, and in the mountains – up to the middle belt of mountains. The species actively disperses within its natural and secondary ranges, including dispersion with crop seeds (Mikhailova et al. 2019). *M. officinalis* is often found as a weed plant (ruderal and segetal), and in some regions (Tomsk and Irkutsk regions, the Republics of Tyva and Buryatia, Krasnoyarsk Territory) of Siberia it is considered an invasive species (Khrustaleva 2016).

Melilotus officinalis is cultivated as a valuable medicinal, fodder and honey plant. The widespread use of sweetclover is due to its high ecological plasticity. *M. officinalis* is drought-resistant, winter-hardy, and undemanding to soil fertility; it can grow in a wide range of edaphic conditions (Luo et al. 2016). The plant exhibits high phytomeliorative properties and is recommended for improving soil fertility and phytoremediation of soils contaminated with heavy metals and hydrocarbons (Steliga and Kluk 2021).

In Russia, *M. officinalis* is typically grown as a medicinal raw material (*Meliloti herba*). In conventional medicine, the herb *M. officinalis* is included in the composition of sedative drugs (Vidal, electronic link). In traditional medicine, the above-ground part of *M. officinalis* is used to treat bronchitis and bronchial asthma, inflammatory processes in the ovaries, menstrual disorders, flatulence, furunculosis, etc. (Dubrovskikh, electronic link, Al-Snafi 2020). The experimentally proven pharmacological effects of the herb *M. officinalis* are as follows: antimicrobial, antioxidant, anti-inflammatory, hepato- and neuroprotective, antitumor, anxiolytic, rheological, immunostimulatory, etc. (Al-Snafi 2020, Aбышев et al. 2014, Venugopala, Patent 2006, Podkolzin et al. 1996). In Europe and China, the plant extract is used to treat inflammation, arthritis, rheumatism, phlebitis, venous insufficiency, hemorrhoids, brachygalgia, and bronchitis (Khare 2007).

The medicinal and poisonous properties of *M. officinalis* and its widespread cultivation and distribution in some countries as an invasive species triggered an intensive study of its allelopathic activity and biotoxicity.

The aim of the study was to analyze sources of information concerning the toxic properties of *M. officinalis* towards different groups of living organisms.

Material and methods

A literary search and a review of scientific articles available in the search databases was carried out using the keywords «*Melilotus officinalis*» and «biopesticides», «*Melilotus officinalis*» and «biological toxicity», «biological agriculture». The paper used

the resources of PubMed search engines, eLibrary, Scopus, Web of Science, for the review we used articles containing evidence-based experimental data on the toxic properties of *Melilotus officinalis* depending on their impact on different groups of organisms.

Results

Chemical composition

The herb *M. officinalis* contains a rich complex of biologically active compounds: phenolic compounds (coumarins, flavonoids, tannins), alkaloids, purines, essential oil (up to 0.02%), fatty oil (up to 4.3%), proteins (up to 17.6%), and mineral salts (Al-Snafi 2020). Phenolic compounds are represented mainly by coumarins, flavonoids, and derivatives of phenolcarboxylic acids. The predominant compounds include unsubstituted coumarin among coumarin compounds, rutin among flavonoid compounds, and ferulic acid among derivatives of phenolcarboxylic acids. The aboveground part of the plant contains flavonoids (luteolin, vitexin, hyperoside, herniarnin, hesperidin, robinin, quercetin, kaempferol, rutin, and others), coumarins (umbelliferon, scopoletin, 4-hydroxycoumarin, melilotin, melitoside), and derivatives of phenolcarboxylic acids (chlorogenic, coffee, cinnamon, and others) (Bubenchikova 2004).

About 60 components were found in the essential oil produced from the aboveground part of the plant by exhaustive hydrodistillation, the main of which was chamazulene, 4-epi-akoronene, benzyl alcohol, and some others (Efremov et al. 2012).

The content of polysaccharides and amino acids (glutamine, arginine, valine, threonine, etc.) (Fedoseeva, Kharlampovich 2013), fatty acids, and triterpenes was determined (Anwer et al. 2008).

The study of flowers assessed the content of the essential oil, coumarins (up to 0.87%), mucus, choline, resins, tannin, and flavone glycosides (kaempferol, quercetin, myricetin) (Nikolaev et al. 2019, Khare 2007).

The seeds contain the amino acid canavanine and alkaloid trigonelline (Khare 2007).

Toxic properties of sweetclover towards animals

Coumarins are considered to be the main group of pharmacologically active compounds of *M. officinalis*, which determine both therapeutic and toxic effects in humans and animals (Abyshv et al. 2014).

Basic data on the toxicity of *M. officinalis* refer to veterinary toxicology. In forage production (green feed, hay and haylage), it is necessary to consider the content

of coumarin in the grass, which can indirectly cause severe poisoning and death in different animal species. Sweetclover poisoning (“sweetclover disease”) was described by F.W. Schofield in 1921 (Schofield 1923) He unambiguously showed that spoiled sweetclover forage can cause fatal bleeding when fed to cattle or experimental animals. He also revealed the relationship between the presence of mold (typically located inside the stem) and the disease onset and concluded that the disease was caused either by the mold itself or by a decomposition product, yet he did not prove his conclusion.

The history of the discovery of 3,3'-methylenebis-(4-hydroxycoumarin), later named dicoumarol, its isolation from spoiled sweetclover hay (*Melilotus alba* and *M. officinalis*), crystallization, identification and synthesis is well known (Campbell and Link 1941; Campbell et al. 1940; Stahmann et al. 1941; Zobnin et al. 2013).

Cases of animal intoxication with dicoumarol have been reported regularly in the US and Canada (Alstad et al. 1985; Puschner et al. 1998; cited from Berny et al. 2005).

Dicoumarol is produced by the growth of fungi of the genera *Aspergillus* or *Penicillium* in *Melilotus* spp. The fungi secrete melilotic acid, mycotoxin responsible for dimerization of 4-O-coumarin in dicoumarol. The latter acts as an anticoagulant rodenticide: it reduces the level of prothrombin in the blood and affects blood coagulation. When an animal consumes toxic hay or silage for several weeks, dicoumarol changes the proenzymes required for prothrombin synthesis, which causes the hemorrhagic syndrome of hypoprothrombinemia (preventing active enzyme formation). It can also interfere with the synthesis of factor VII and other vitamin K-dependent coagulation factors. Dicoumarol concentrations of 20–30 mg/kg of hay ingested over several weeks are sufficient to cause poisoning in cattle. The signs of poisoning are most pronounced in cattle and to a limited extent in pigs and horses, while sheep are sufficiently resistant. The time between consumption of toxic sweetclover and the onset of clinical disease varies and depends on the dicoumarol content in the particular *M. officinalis* cultivar, the age of the animal, and the amount of feed consumed. If dietary dicoumarol is low or variable, animals may consume it for months before signs of disease appear. Hay containing a dicoumarol doze equal to 10–20 mg/kg can be fed for 100 days before poisoning develops. A 60–70 mg/kg doze of dicoumarol can cause poisoning after 21 days of its consumption. A 50 µg/g doze of dicoumarol causes severe clinical signs within 15 days (Puschner et al. 1998).

The first indication of dicoumarol poisoning in cattle is often death of one or more animals. Poisoned animals may be stiff and lame due to bleeding into muscles and joints. Cardiovascular signs (severe hemorrhage causes anemia, mucosal pallor, heart palpitations and death), respiratory signs (epistaxis), renal signs (hematuria), reproductive signs (excessive hemorrhage during calving), and ocular signs (bleeding into the anterior chamber of the eye). Death is generally caused by massive hemorrhage or bleeding. In horses, poisoning can lead to excessive bleeding, pallor of the mucous membranes, and weakness. A prothrombin time exceeding 40 seconds indicates faulty blood coagulation. Blood prothrombin, activated partial thrombo-

plastin time, and blood coagulation time become markedly increased (Baumann et al. 1942; Radostits et al. 1980; Puschner et al. 1998).

Low-coumarin varieties of *M. officinalis* are safe and do not cause a disease (Goplen 1980).

Dicoumarol became a new rodenticide and the first anticoagulant patented in 1941 as a pharmaceutical (Link 1959; Zobnin et al. 2013).

In the economic use of *M. officinalis* in feed formulations, its predisposition to the accumulation of fungal metabolites toxic to animals should be considered. A comparative study of mycotoxin contamination of legumes on natural fodder lands in the European part of Russia showed that more than 80% of *M. officinalis* samples were contaminated with the following mycotoxins: ergoalkaloids, alternariol, cyclopiazonic acid, and emodin; ochratoxin A and PR toxin were found in smaller amounts (Burkin et al. 2017).

At present, active breeding aimed at reducing the content of coumarin in sweet-clover grass is underway (Dzyubenko et al. 2018), and forage conservation technologies are being improved (Sagalbekov et al. 2016).

Toxicity of sweetclover towards humans

The herb *M. officinalis* is commonly used in herbal medicine as an angioprotective and antiplatelet agent to treat angina pectoris, chronic heart failure, and varicose veins. High dosages of *M. officinalis* can cause side effects such as dyspepsia, drowsiness, and headache (Lesiovskaia 2014). Moreover, improper harvesting of medicinal plant materials leads to dicoumarol formation. Hemorrhagic syndrome can develop in patients treated with preparations made on the basis of such raw materials (Zobnin et al. 2013).

Traditionally, coumarins are used as a flavoring agent for food and alcoholic beverages. Coumarin was synthesized in 1868, and it was first put on the market as a flavoring substance (Abraham et al. 2010). However, coumarin was found to cause liver toxicity in human, and carcinogenicity and mutagenicity in animals, and it was withdrawn from usage in the USA in 1954. In 1964, coumarin was banned in the UK based on the experimental data on the ability of coumarin to cause benign and malignant tumors in the liver, kidneys and lungs of animals. In 1988, the European Union set a tolerable daily intake for coumarin from natural spices and herbs in food of 2 mg/kg (Lake 1999, cited in Shikh et al. 2015; Abraham et al. 2010), which was then reduced to 0.5 mg/kg, since hepatotoxicity in human could not be ruled out. In relation to safety, obvious differences in metabolism of coumarins present in foods and used as fragrances in cosmetics were revealed between susceptible species and other species, including humans. Conversion of this compound in the human body proceeds predominantly through the 7-hydroxylation pathway, which is safe for the body (Lake, 1999; Abraham et al. 2010).

Melilotus officinalis is included in the list of plants prohibited for use in food supplements (Nikolaev et al. 2019).

Allelopathic activity *in situ* and *in vitro*

There is a renewed interest, particularly among organic growers, in using yellow sweetclover (*M. officinalis*) as cover crop. Experiments were initiated in 1999–2002 in Canada to compare the effect of high- and low-coumarin cultivars of *M. officinalis* on weed suppression. Sweetclover termination at 70% bloom was often more effective in suppressing weeds than termination at the bud stage. In the summer and fall after termination, surface residues of Yukon, a high-coumarin and drought-tolerant cultivar, reduced *Chenopodium album* L. density by > 80% compared with the no sweetclover check and essentially eliminated *Descurainia Sophia* (L.) Webb. In the following spring, Yukon reduced *Kochia scoparia* (L.) Schrad. density by > 80% and *Avena fatua* L. biomass by > 30% compared with the no sweetclover check. It may be possible for organic growers to manage weeds with sweetclover in a reduced tillage system that leaves most of the plant residues on the soil surface (Moyer et al. 2007). It was suggested that *M. officinalis* suppresses weeds due to the allelopathic activity of the compounds released during its decomposition (Blackshaw et al. 2001).

Melilotus officinalis has the complex phytosanitary activity of spring wheat predecessor to agrocenoses of the southern forest steppe in Western Siberia (Novosibirsk Region). Yellow sweetclover improved the phytosanitary condition of soil by decreasing population densities of phytopathogens – agents of common root rots, weeds and phytophages (Toropova et al. 2014; Toropova et al. 2021).

Russian and Japanese scientists (Mardani et al. 2016) screened 178 species of Caucasian flora for the presence and degree of allelopathic activity. Laboratory experiments performed using the sandwich method showed that *M. officinalis* belongs to the group of highly toxic allelopathic plants. Aqueous extracts of the herb remarkably inhibited germination of lettuce (*Lactuca sativa*) seeds.

Aqueous extracts of *M. officinalis* with different concentrations (1, 3, and 5%) inhibited germination and growth of wheat and peas (Umer et al. 2010). Similar results were obtained for other crop test plants (Shinwari et al. 2013).

The allelopathic activity of aqueous extracts from leaves (15, 20, 25 and 30%) of *M. officinalis* was studied in relation to germination of seeds and growth of wheat seedlings. High concentrations of the extract (25–30%) significantly reduced seed germination, root length, and aerial part of wheat seedlings. Yet *M. officinalis* extracts were inferior in phytoinhibitory activity to leaf extracts of *Centaurea maculosa* (Siyar et al. 2017).

According to C.X. Wu et al. (2014), coumarin plays a predominant role in the inhibitory activity of *M. officinalis* extracts. The effect of coumarin, which inhibits plant seed germination and seedling growth, is consistent with the effect of aqueous extracts of *M. officinalis* on other plants (Wu et al. 2015).

Of particular interest is the study of alleloherbicidal activity of *M. officinalis* in China (Wu et al., 2010; 2014; 2015; 2016; Wang and Qi 2014). C.X. Wu et al. (2016) reported the results of chemical studies of the herb *M. officinalis* and biotesting on different groups of plants. This study aimed to isolate, identify and quantify the pre-

dominant allelochemical composition of *M. officinalis* using organic solvent extraction, chromatography, thin layer chromatography (TLC), gas chromatography-mass (GC-MS) and nuclear magnetic resonance (NMR), as well as to assess its inhibitory activity on weeds using bioassays. The most active allelochemical components were extracted using petroleum ether. Coumarin, 2H-1-benzopyran-2-one, was isolated and recognized as the most predominant and active allelochemical. The coumarin content in the original extract of *M. officinalis* was 46.78 µg/ml or 1.152% of dry matter content. At a dose of 40 µg/ml, coumarin significantly inhibited seed germination and seedling growth in *Lolium multiflorum* Lam., *Polygonum aviculare* L., *Trifolium pratense* L., *Veronica persica*, *Poa pratensis* L., *Chenopodium album*, and *Plantago asiatica* ($P < 0.05$). Coumarin exhibited significant inhibitory activity on both seed germination and seedling growth of all plants studied ($P < 0.05$). It completely suppressed seed germination and seedling growth in *Lolium multiflorum*, *Polygonum aviculare*, and *Trifolium pratense*. Coumarin showed a strong inhibitory effect on seed germination and seedling growth in numerous weeds, which suggests that it can be used as a natural herbicide. According to A. Kaur and R. Kaur (2017), flowering leaves and flowers of *M. officinalis* can be used as a raw material for production of biopesticides.

There are few data on the effect of *M. officinalis* on insects, in particular on its repellent activity. Insecticidal activity of methanol extracts from *M. officinalis* was tested on 3rd instar larvae of the Egyptian cottonworm (*Spodoptera littoralis*). *M. officinalis* extracts were moderately toxic (LC₅₀ – 5.6 µg/ml). The relative growth rate, the rate of food intake and efficiency of conversion of the digested food were calculated. Clear correlations were found between weight increase, quantity of the ingested food, and quantity of excrements produced during the whole assay period. These results indicate an antifeedant activity of the tested extract of *M. officinalis* (Pavela and Chermenskaya 2004).

The repellent and insecticidal activity of *M. officinalis* on *Tenebrio molitor* L. were studied experimentally. The repellent activity of the dry crushed aboveground part of *M. officinalis*, added to oatmeal and spring wheat, was investigated against *T. molitor* larvae. The repellent efficacy of *M. officinalis* was observed in relation to *T. molitor* larvae when added to oatmeal. The insecticidal activity of an aqueous extract from the aboveground part of *M. officinalis* was studied on larvae and imago of *T. molitor*. *M. officinalis* was found to have a pronounced insecticidal effect on *T. molitor* imago, yet none of the effect was found for larvae (Gulik et al. 2021).

Conclusion

An increased interest in the cultivation of *M. officinalis* as a multi-purpose agricultural crop (medicinal, fodder, melliferous), particularly in biological agriculture, requires a comprehensive analysis of its chemical composition and physiological activity. A high coumarin content in *M. officinalis*, especially in wild plants, can have

a toxic effect on the biocenosis. Laboratory and in situ studies have shown that the aboveground part of *M. officinalis* is a potential source of broad-spectrum biopesticides (bioherbicidal, insecticidal and fungicidal). Genetic variation in germplasm of *Melilotus officinalis* is the foundation of successful plant breeding, and wild germplasm is a valuable source of new alleles associated with desirable characteristics, such as higher or lower coumarin content (Zhang et al. 2018).

Acknowledgements

The study was performed within the framework of a state assignment of the Ministry of Science and Higher Education of the Russian Federation (project No. FSWM-2020-0019).

References

- Abraham K, Wöhrlin F, Lindtner O, Heinemeyer G, Lampen A (2010) Toxicology and risk assessment of coumarin: Focus on human data. *Molecular Nutrition Food Research* 54 (2): 228–239. <https://doi.org/10.1002/mnfr.200900281>
- Abyshev AZ, Agaev EM, Abyshev RA (2014) Natural and synthetic coumarins and flavonoids (Current status and prospects for creation of new drugs based on coumarins and flavonoids). Edm and Takhsil Publishing House, Baku, 482 pp. [In Russian]
- Al-Snafi AE (2020) Chemical constituents and pharmacological effects of *Melilotus officinalis* – a Review. *IOSR Journal of Pharmacy (IOSRPHR)* 10(1): 26–36.
- Alstad AD, Casper HH, Johnson LJ (1985) Vitamin K treatment of sweetclover poisoning in calves. *Journal of the American Veterinary Medical Association* 187: 729–731.
- Anwer MS, Mohtasheem M, Azhar I, Ahmed SW, Bano H (2008) hemical constituents from *Melilotus officinalis*. *Journal of Basic and Applied Sciences* 4 (2): 89–94.
- Baumann CA, Field JB, Overman RS, Link KP (1942) Studies on the hemorrhagic sweetclover disease. *Journal of Biological Chemistry* 146: 7–14.
- Berny P, Alves L, Simon V, Rossi S (2005) Intoxication des ruminants par les raticides anticoagulants: quelle réalité? *Revue de Médecine Vétérinaire* 156 (8–9): 449–455.
- Blackshaw RE, Moyer JR, Doram RC, Boswell AL (2001) Yellow sweetclover, green manure, and its residues effectively suppress weeds during fallow. *Weed Science* 49 (3): 406–413. [http://dx.doi.org/10.1614/0043-1745\(2001\)049\[0406:YSGMAI\]2.0.CO;2](http://dx.doi.org/10.1614/0043-1745(2001)049[0406:YSGMAI]2.0.CO;2)
- Bubenchikova VN, Drozdova IL (2004) Study of the composition of phenolic compounds of sweetclover by HPLC. *Chemical Pharmaceutical Journal* 38 (4): 24–25. [In Russian]
- Burkin AA, Kononenko GP, Gavrilova OP, Gagkaeva TYu (2017) Mycotoxins in leguminous grasses of natural fodder lands in European Russia. *Agricultural biology* 52 (2): 409–417. [In Russian]
- Campbell HA, Link KP (1941) Studies on the hemorrhagic sweet clover disease. IV. The isolation and crystallization of the hemorrhagic agent. *Journal of Biological Chemistry* 138: 21–33.

- Campbell HA, Roberts WL, Smith WK, Link KP (1940) Studies of the hemorrhagic sweet clover disease: I. The preparation of hemorrhagic concentrates. *Journal of Biological Chemistry* 136: 47–55.
- Dubrovskikh T (2021) What is the use of *Melilotus officinalis*? [Electronic linck] URL: https://greenapteka.ru/donnik-lekarstvennyy/#Primenenie_donnika_lekarstvennogo (Accessed: 07/02/2022).
- Dzyubenko NI, Duk OV, Malyshev LL, Prosvirin YuA, Kosarev IA (2018) Screening of sweetclover (*Melilotus* Adans.) species diversity for resistance to chloride salinization. *Sel'skokhozyaistvennaya biologiya [Agricultural Biology]* 53 (6): 1294–1302. <https://doi.org/10.15389/agrobiology.2018.6.1294eng> [In Russian]
- Efremov AA, Zykova ID, Tselukovskaya MM (2012) Chemical composition of biological active sapsens *Melilotus officinalis* (L.). *Chemistry of Plant Raw Material* 3: 111–114. [In Russian]
- Goplen BP (1980) Sweetclover Production and Agronomy. *La revue veterinaire canadienne* 21(5): 149–51.
- Gulik ES, Mikhailova SI, Babenko AS, Qasim HA (2021) Repellent and insecticidal effect of the invasive plant *Melilotus officinalis* (L.) Pall. on *Tenebrio molitor* L. *Problems of Industrial Botany of Industrially Developed Regions. BIO Web of Conferences* 31, 00008. <https://doi.org/10.1051/bioconf/20213100008>
- Kaur A, Kaur P (2017) Green pesticides for clean environment. *International Journal of Engineering Development and Research* 5 (4): 1347–1349.
- Khare C (2007) *Melilotus officinalis* Linn. In: Khare C. (Eds) *Indian Medicinal Plants*. Springer, New York, NY. https://doi.org/10.1007/978-0-387-70638-2_993
- Khrustaleva IA (2016) *Melilotus officinalis* (L.) Pall. In: *The Black Book of flora of Siberia*. Vinogradova YuK and Kupriyanov AN (Eds) *Geo, Novosibirsk*, 250–254 pp. [In Russian]
- Lake B (1999) Coumarin metabolism, toxicity and carcinogenicity: relevance for human risk assesment. *Food and Chemical Toxicology* 37: 423–452.
- Lesiovskaya EE (2014) Evidence-based phytotherapy. Vol. 2. Moscow, 688 pp. [In Russian]
- Link KP (1959) Discovery of Dicumarol and Its Sequels. *Circulation* 19: 97–107.
- Luo K, Jahufer MZZ, Wu F, Di H, Zhang D, Meng X, Zhang J, Wang Y (2016) Genotypic variation in a breeding population of yellow sweet clover (*Melilotus officinalis*). *Frontiers in Plant Science* 7: 972. <https://doi.org/10.3389/fpls.2016.00972>
- Mardani H, Kazantseva E, Onipchenko V, Fujii Y (2016) Evaluation of allelopathic activity of 178 Caucasian plant species. *International Journal of Basic and Applied Sciences* 5 (1): 75–81. <http://dx.doi.org/10.14419/ijbas.v5i1.5631>
- Mikhailova SI, Ebel TV, Ebel AL (2019) Distribution of alien plants by speirochory in agrogenosis of Tomsk oblast. *Russian Journal of Biological Invasions* 10 (4): 358–364. <http://dx.doi.org/10.1134/S2075111719040064>
- Moyer JR, Blackshaw RE, Huang HC (2007) Effect of sweetclover cultivars and management practices on following weed infestations and wheat yield. *Canadian Journal of Plant Science* 87(4): 973–983. <https://doi.org/10.4141/CJPS06054>

- Nikolaev NA, Livazan MA, Skirdenko YuP, Martynov AI (2019) Biologically active plants and fungi of Siberia in clinical medicine. Vol. 1. Publishing House of the Academy of Natural Sciences, Moscow, 382 pp. [In Russian]
- Patent 2471495 RF, IPC A61K 36/48 (2006.01), A61P 7/00 (2006.01). Hemorrhological herbal preparation / Andreeva VYu, Aliev OI, Plotnikov MB, Kalinkina GI; applicant and patent holder Research Institute of Pharmacology SB RAMS, GBOU VPO Siberian State Medical University of the Ministry of Health and Social Development of Russia. 10.01.13, Bull. No. 1, 7 p. [In Russian]
- Pavela R, Chermenskaya T (2004) Potential insecticidal activity of extracts from 18 species of medicinal plants on larvae of *Spodoptera littoralis*. Plant Protection Science 40 (4): 145–150. <http://dx.doi.org/10.17221/464-PPS>
- Podkolzin AA, Dontsov VI, Sychev IA, Kobeleva GYU, Kharchenko ON (1996) Immunomodulating, antianemic, and adaptogenic effects of polysaccharides from plaster clover (*Melilotus officinalis*). Bulletin of Experimental Biology and Medicine 121: 597–599. <https://doi.org/10.1007/BF02447128>
- Puschner B, Galey FD, Holstege DM, Palazoglu M (1998) Sweetclover poisoning in dairy cattle in California. Journal of the American Veterinary Medical Association 212 (6): 857–859.
- Radostits QM, Searcy GP, Mitchall KG (1980) Moldy sweet clover poisoning in cattle. Canadian Veterinary Journal 21(5): 155–158.
- Sagalbekov UM, Sagalbekov E U, Syzdykov ET, Seytmaganbetova GT, Baydalın ME (2016) Innovational agricultural practices in melilot cultivation and harvesting in Northern Kazakhstan. Fodder Production 6: 27–30. [Russian]
- Schofield FW (1923) Damaged sweetclover: The cause of a new disease in cattle simulating hemorrhagic septicemia and blackleg. Journal of the American Veterinary Medical Association 64: 553–575.
- Shikh EV, Bulayev VM, Demidova OA (2015) The safety assessment of medicinal plants. Safety and Risk of Pharmacotherapy 2: 23–29. [In Russian]
- Shinwari MI, Shinwari MI, Fujii Y (2013) Allelopathic evaluation of shared invasive plants and weeds of Pakistan and Japan for environmental risk assessment. Pakistan Journal of Bototechnology 45: 467–474.
- Stahmann MA, Huebner CF, Link KP (1941) Studies on the hemorrhagic sweet clover disease. V. Identification and synthesis of the hemorrhagic agent. Journal of Biological Chemistry 138 (2): 513–527.
- Steliga T, Kluk D (2021) Assessment of the suitability of *Melilotus officinalis* for phytoremediation of soil contaminated with petroleum hydrocarbons (TPH and PAH), Zn, Pb and Cd Based on Toxicological Tests. Toxics 9: 148. <https://doi.org/10.3390/toxics9070148>
- Toropova E, Posazhennikov S, Marmuleva E (2014) Systemic phytosanitary role of spring wheat predecessors in the southern forest steppe of Novosibirsk region. Siberian Bulletin of Agricultural Science 4: 5–11. [In Russian]
- Toropova EY, Glinushkin AP, Insebaeva MK, Stetsov GY (2021) The conidia *Bipolaris sorokiniana* Sacc. Shoem. distribution in the soil of Altai and Kazakhstan arid regions.

- Journal of Physics: Conference Series 1942 (1): 012078. <https://doi.org/10.1088/1742-6596/1942/1/012078>
- Umer A, Yousaf Z, Khan F, Hussain U, Anjum A, Nayyab Q, Younas A (2010) Evaluation of allelopathic potential of some selected medicinal species. African Journal of Biotechnology 9 (37): 6194–6206. <https://doi.org/10.5897/AJB09.1288>
- Venugopala KN, Rashmi V, Odhav B (2013) Review on natural coumarin lead compounds for their pharmacological activity. BioMed Research International. Article ID: 963248. <https://doi.org/10.1155/2013/963248>
- VIDAL. Drug guide [Electronic version] URL: <https://www.vidal.ru/drugs/molecule-in/651> (Date of access: 18.08.2021).
- Wang ZB, Qi CH (2014) Allelopathy effect of water extracts of *Melilotus officinalis* on three kinds of weeds. Seed 33 (12): 40–43.
- Wu C, Guo X, Li Z, Shen Y (2010) Feasibility of using the Allelopathic potential of yellow sweetclover for weed control. Allelopathy Journal 25: 173–183.
- Wu CX, Liu SJ, Zhao GQ (2014) Isolation and identification of the potential allelochemicals in the aqueous extract of yellow sweet clover (*Melilotus officinalis*). Acta Prataculturae Sinica 23: 184–192. [In Chinese] <https://doi.org/10.11686/cyxb20140521>
- Wu CX, Liu SJ, Zhao GQ, Xu J (2015) The allelopathy of yellow sweetclover on weeds. Acta Agrestia Sinica 23: 137–143. [In Chinese]
- Wu CX, Zhao GQ, Liu DL., Liu SJ, Gun XX, Tang Q (2016) Discovery and weed inhibition effects of coumarin as the predominant allelochemical of yellow sweetclover (*Melilotus officinalis*). International Journal of Agriculture and Biology 18: 168–175. <https://doi.org/10.17957/IJAB/15.0082>
- Zhang J, Di H, Luo K, Jahufer Z, Wu F, Duan Z, Stewart A, Yan Z, Wang Y (2018) Coumarin content, morphological variation, and molecular phylogenetics of *Melilotus*. Molecules 23: 810. <https://doi.org/10.3390/molecules23040810>
- Zobnin YuV, Kutateladze RG, Malykh AF, Pazyukov EA, Provado IP, Teterina IP (2013) Acute poisonings with anticoagulants according to the data of the Irkutsk toxicological center. Siberian Medical Journal 5: 131–134. [In Russian]