Biotechnology & Biotechnological Equipment

ISSN: 1310-2818 (Print) 1314-3530 (Online) Journal homepage: https://www.tandfonline.com/loi/tbeq20

Developmental and Feeding Alternations in *Leptinotarsa Decemlineata* Say. (Coleoptera: Hrysomelidae) Caused by *Salvia Officinalis* L. (Lamiaceae) Essential Oil

M. Kostić, S. Dražic, Z. Popović, S. Stanković, I. Sivčev & T. Živanović

DEVELOPMENTAL AND FEEDING ALTERNATIONS IN *LEPTINOTARSA DECEMLINEATA* SAY. (COLEOPTERA: HRYSOMELIDAE) CAUSED BY *SALVIA OFFICINALIS* L. (LAMIACEAE) ESSENTIAL OIL

M. Kostić[1], S. Dražić[1], Z. Popović[2], S. Stanković[3], I. Sivčev[4], T. Živanović[5]
Institute for Medicinal Plant Research "Dr Josif Pančić", Belgrade, Republic of Serbia[1],
University of Belgrade, Institute for Biological Research "Siniša Stanković", Department of Ecology, Belgrade, Republic of Serbia[2]
Institute for the Application of Science in Agriculture, Belgrade, Republic of Serbia[3]
Institute for Plant Protection and Enviroment, Belgrade, Republic of Serbia[4]
University of Belgrade, Faculty of Agricultural, Zemun, Republic of Serbia[5]
Correspondence to: Miroslav Kostić
Email: mkostic@iplb.co.yu

ABSTRACT
Secondary plant metabolites can express the regulatory effects on development and survival of other plant species, microorganisms or animals. In this study, we investigated the toxic and antifeedant effect of ethanol solutions of derivatives obtained from sage (the essential oil, five fractions of the same oil F1-F5, and the camphor) against the second instar larvae and adults of Colorado potato beetle (CPB). The bioassays were performed in laboratory conditions. Toxicity of tested solutions was negligible; with exception of S. officinalis essential oil which caused low mortality of insect (29.16% dead larvae and 20.83% dead adults, respectively). Alternations in development evaluated as number in both laid eggs and hatched larvae were insignificant. Antifeedant activity of tested solutions against the CPB larvae during the first 96h was significant; although their effectiveness decreased after 4 days, the LMD was still lesser in comparing with untreated control.

Keywords: *Salvia officinalis* L., *Leptionotarsa decemlineata* Say., essential oil, oil fractions, camphor, antifeedancy, toxicity

Introduction
Since the conventional pesticides have a long tradition and proved efficiency in the crop protection, the growers are mostly oriented to their application. Despite its ability for fast development of resistance (2, 3, 37), the Colorado potato beetle is one of the pests which are mostly controlled by these chemicals. Also, the most of pesticides which were intensively used in past (and some are in usage today) are directly responsible for ecosystem disturbances and injuries. Negative aspects of pesticide application force the efforts for development of alternative crop protection measures, which would meet the ecological and health requirements. However, new alternative measures could be incorporated into IPM strategies only if they are as efficient as conventional pesticides. Till now the best results against CPB have genetically modified Bt potatoes but they are cultivated only in experimental conditions in Europe (17). In finding the alternative agents for pest control, one of the possibilities is the application of essential oils and plant extracts with repellent, antifeedant or masking effect against the insect pests (28). For this purpose, the plant extracts and essential oils were widely investigated for their biological activities (7, 13, 14, 15, 16, 18, 38).

On the basis on promising results achieved with essential oil of *S. officinalis* against the various classes of pest organisms (4, 8, 20, 26, 30), in this study we investigated the effects of derivatives obtained from sage: the essential oil, five fractions of the same oil, and the camphor against the *L. decemlineata*.

Materials and Methods

Essential oil of *S. officinalis*
The essential oil of sage (*S. officinalis*) has been isolated by hydrodistilation using a Clavenger apparatus (29). The plants were collected from the plantings of Institute for Medicinal Plant Research (Pančevo), the origin of seeds was from seaside area. The overground plant parts were used as crude material for distillation. The essential oil and isolated fractions (F1-F5) were subjected to GC-MS analysis (20). Prior to bioassays, the essential oil, fractions and camphor (the component which was present in all isolated solutions) were diluted with ethanol 96% to obtain the solutions for testing: 0.5% concentrated solutions of essential oil, five fractions and camphor.

Test insect
Bioassays were performed on Colorado potato beetle. Adults were collected from the locality Dobanovci (near Belgrade) from the potato plantation which hasn't been treated with pesticides. It was previously shown that this population of CPB is resistant to the organophosphate pesticides (39). The beetles were raised in laboratory under the conditions proposed for its optimal development (9). After the egg laying on the potato leaves, the adults were removed, and the biological cycle of new generation was continuously monitored. For the bioassays,

the individuals of define age were used: second instar larvae – 2 days old (L2), adult females and males – 5 days old (A), egg masses – 24h old (E1) and egg masses – 80h old (E2), prior to hatching.

Potato, *Solanum tuberosum* L.

We used potato Desire, 6-7 weeks old, prior to flowering, high 25-30 cm, which was grown in the glass house, without the any pesticide treatment.

Microclimate chamber

The experiments were performed in microclimate chamber equiped with comand table for the regulation the temperature, humidity and light (Danfoss, EKH 20, Netherland), at T= 27±1^{o}C, RH=60±5%, L/D=16/8 (neon diffuse light 30159.29 cd).

Leaf protective properties of tested solutions

The potato leaves were sprayed with tested solutions (40 ml per m^2) at the glass house (25^{0}C, shade) and air dried for 30 min. Thereafter, the plants were isolated with plastic cylinders, and the tested developmental stages of CPB were introduced. The experiments with L2 and A were performed separately, by introducing six individuals (6 larvae and 3 pairs of adults per replication, respectively). The experiment lasted 8 days, with evaluation on leaf mass damage at every 24h (0-100% scale). All experiments were settled in six repetitions.

Toxicity of tested solutions

1. Contact insecticidal effect

The contact effect of tested solutions was evaluated on glass medium: the aliquot of 0.3 ml of each solution was applied with sprayer on Petri dish (9 cm in diameter); totally 40 ml per m^2. The L2 and A were introduced in Petri dishes, covered by mosquito net (to avoid the negative effect of volatiles) and put into the microclimate chamber. The evaluation of insecticidal effect was done 48h after the introduction of insects. Experiments were done in six replications, with six individuals per replication. Results were compared with control (untreated variant) which was settled under the same conditions as treated Petri dishes.

2. Effect on embryogenesis

Prior to bioassay, the egg masses were obtained in microclimate chamber, where the five pairs of adults were put onto isolated potato plant, and removed after 24h. Obtained egg masses of known age (24h and 80h, respectively) were treated with test solutions using the sprayer. Evaluation on hatching the larvae from E1 was done 4 days after treatment, after the first instar larvae L1 were hatched. Evaluation on hatching the larvae from E2 was done 24h after the treatment (prior to hatching larvae from chorion). Also, two control egg masses were evaluated: untreated and treated with ethanol 96%. Experiments were six times replicated.

Statistical analysis

Both percentage mortality and the efficiency of effect of tested compounds were calculated using the corrected formula of Abbott (1). Leaf mass damage of treated leaves was presented as percentage based on 0-100% scale (25). The mean values of the experiments were separated using Duncan's multiple range test.

Results and Discussion

Chemical composition of tested solutions: The GC-MS analysis shown that the sage essential oil was composed of 14 constituents, with dominance of α- tujon (31.87% m/m) and camphor (24.65% m/m). Fractions obtained from this essential oil were significantly different regarding to their composition. F1 and F2 had 8 and 7 components, respectively, with the majority of α- thujon. Both F3 and F4 had 6 components, with the majority of camphor (46.99% m/m in F3, 44.42% m/m in F4, respectively), whereas F5 had 7 components of which the γ-selinen and α-humulen were present in the highest percent (19.57% m/m and 18.27% m/m, respectively). Camphor was the only constituent present in essential oil and all fractions (20).

TABLE 1

Contact toxicity of tested solutions against both second instar larvae (L2) and adults (A) of CPB. C: concentration of tested solutions (%); M: mean mortality (%); E: efficiency in comparing with control/untreated (%)

Tested solution	C (%)	L2		A	
		M (%)	E (%)	M (%)	E (%)
S. officinalis ess. oil	0.5	29.16a	600.00	20.83a	400.00
F1	0.5	8.33b	100.00	4.16b	0.00
F2	0.5	8.33b	100.00	4.16b	0.00
F3	0.5	8.33b	100.00	4.16b	0.00
F4	0.5	4.16b	0.00	0.00b	0.00
F5	0.5	0.00b	0.00	0.00b	0.00
Camphor	0.5	4.16b	0.00	0.00b	0.00
Control	0.0	4.16b	0.00	4.16b	0.00
		LSD005=12.25 LSD001=16.67		LSD005=9.50 LSD001=12.93	

Toxicity of tested solutions on larvae and adults of CPB: Mortality caused by application of tested solutions on glass medium was low, i.e. insignificant in comparing with control. Only the essential oil of *S. officinalis* expressed moderate contact toxicity against the male L2 and A (**Table 1**). According to presented results, the initial toxicity and the efficiency of tested solutions completely missed.

Toxicity of tested solutions on embryogenesis: High percentage of larvae hatched from egg masses (both 24h and 80h) indices that tested solutions have not altered the embryogenesis in CPB (**Tables 2 and 3**).

Protective properties of tested solutions on potato leaves: The protection of leaf mass treated with tested solutions was different in experiments with larval stages and adults. Averagely, leaf mass damage caused by larvae (from L2 to nymph) was 35-60% lesser in treated than in untreated leaves (**Fig. 1**). The best protective function of tested solutions was expressed 4d after spraying, when larvae started to feed intensively and when the leaf mass damage below 10% (50% in control). After 4 days, the protective effect of sprayed solutions decrased. These compounds were not as much efficient in bioassay with adult insects, i.e. treated leaves were eaten approximately 20% lesser than untreated (**Fig. 2**). Also, the deterring effect of tested compounds decrased fastly, and after 5 days there were no differences between the damage of treated and untreated leaf mass.

TABLE 2

Effect of tested solutions on embryogenesis (eggs of 24h) of CPB. C: concentration of tested solutions (%); Egg: mean number of eggs in the trial; Larvae: number of hatched larvae (%); E: efficiency in comparing with control/untreated (%)

Tested solution	C (%)	Egg	Larvae	E (%)
S. officinalis ess. oil	0.5	19.00	96.03a	-2.82
F1	0.5	18.50	89.71a	3.95
F2	0.5	19.75	92.20a	1.28
F3	0.5	18.75	91.68a	1.84
F4	0.5	18.75	95.02a	-1.74
F5	0.5	19.00	94.65a	-1.35
Camphor	0.5	19.25	95.92a	-2.71
Control	0.0	19.25	93.65a	0.00
			LSD005=6.12 LSD001=8.32	

TABLE 3

Effect of tested solutions on embryogenesis (eggs of 80h) of CPB. C: concentration of tested solutions (%); Egg: mean number of eggs in the trial; Larvae: number of hatched larvae (%); E: efficiency in comparing with control/untreated (%)

Tested solution	C (%)	Egg	Larvae	E (%)
S. officinalis ess. oil	0.5	18.00	95.73a	0.46
F1	0.5	18.50	93.26a	3.03
F2	0.5	18.50	94.57a	1.66
F3	0.5	19.25	92.00a	4.34
F4	0.5	18.75	94.49a	1.74
F5	0.5	18.50	93.37a	2.91
Camphor	0.5	20.00	94.98a	1.24
Control	0.0	19.25	96.17a	0.00
			LSD005=4.55 LSD001=6.20	

Due to significant results achieved with the incorporation of non-conventional methods in both economic and ecological sense, the integrative crop protection became the most desirable way for pest control. Also, the effort given by researchers in finding new active compounds which wouldn't provoke the pest resistance and/or environmental disturbance had got a respectable extent.

A number of plant products which do not participate in metabolic processes are called secondary metabolites (i.e. alcaloids, glycosides, tanines, flavonoides, essential oils, saponins, 36). These compounds are oftenlly incorporated in plant defense against the insects and pathogens. Relationships between the insects and plants are mostly based on insect's sense of smell and/or taste. Therefore, the secondary plant metabolites primarly repell insects or inhibit their feeding, and in some cases disturbe digestion and absorption of ingested feed (22, 23, 40).

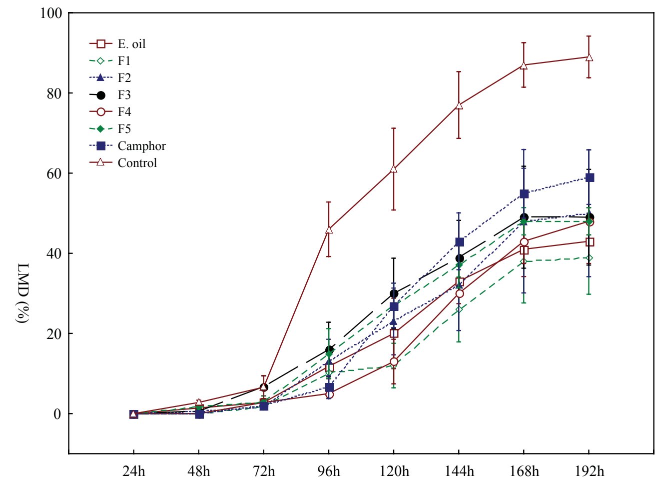

Fig. 1. Leaf mass damage (LMD) of treated potato leaves caused by different larval stages of CPB (24-48h: L2; 72-120h: L3; 144-168h: L4; 192h: nymph). Means ± S.E

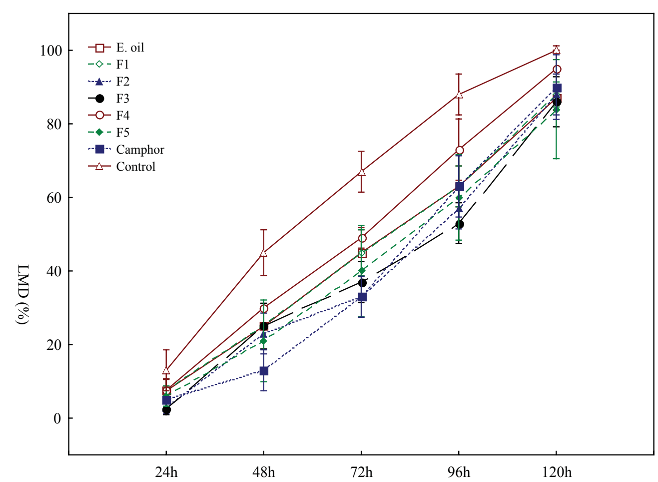

Fig. 2. Leaf mass damage (LMD) of potato leaves treated with tested solutions during the observation time caused adults of CPB. Means ± S.E

Plants which have compounds with antifeedant potential to insects (i.e. decrease or inhibit ingestion) are in intensively investigated (28, 32), as well as plant extracts and essential oils which could be applied at the same way as conventional insecticides (11) or acaricides (35). The first proposal on usage plant extracts as spray without insecticidal, but with confusing effect against pest insects was reported by Brinley (5). Generally, antifeedant properties of spraying these products don't have negative consequences against the predators or pollinators (6), which gives the great possibilities for pest control (33), making such antifeedants desirable as "environmentally friendly" products (21). It was reported that several plant extracts (*Arctium lappa, Bifora radians, Humulus lupulus, Verbascum songaricum* and *Xanthium strumarium*) applied in 200g kg^{-1} significantly decreased feeding of CPB larvae (10). Also, other plant extracts (*Hedera helix, Artemisia vulgaris, Xanthium strumarium, Humulus lupulus, Sambucus nigra, Chenopodium album, Salvia officinalis, Lolium temulentum* and *Verbascum sonyaricum*) were investigated for their toxicity in the sense of replacing the synthetic insecticides (11).

Masking and antifeedant properties of essential oils of both *Tanacetum parthenium* and *T. vulgare*, and their major components (camphor and thujon) against the CPB larvae were investigated (19). The results suggested that the most concentrated solutions of tested compounds (0.5%) could provide the masking effect of attractive host plant (potato), and the most respectable effect was achieved by application of camphor.

Following the new tendencies, we reported a serial of studies regarding the antifeedant and toxic effect of plant derivatives against the various pests. The sage essential oil was effective against L2 of gypsy moth (especially F3 which expressed significant contact toxicity and antifeedancy, 20). In the same study, it was shown that sage essential oil was highly effective as repellent against *B. germanica*, but not against *A. aegypti*. Also, toxicity, repellency and anti-reproductive effect of sage essential oil against the rice weevil was proved (30).

The commercial preparation Neem (active compound azadiractin from *Azadirachta indica* , *Meliaceae*) is the best example that the natural compounds (preferably of botanical origin) are the near future in pest control. This preparation has the wide spectrum of activities against insects: antifeedant, inhibitor of chitin synthesis, inhibitor of development by blocking the ecdisone, inhibitor of oviposition, affecting the endocrine system by reducing/inhibiting the neurosecretory proteins (31).

Conclusions

In this study, we evaluated the antifeedant and toxic effect of sage essential oil, its fractions and camphor against the most serious potato pest, CPB. All tested compounds showed low contact toxicity against larvae and adults of CPB. Alternations in development evaluated as number in both laid eggs and hatched larvae were insignificant. Antifeedant effect of tested derivatives obtained from sage was significant against the feeding of larval stages, whereas the same solutions were not efficient against the adult feeding.

We suggest that the more investigations on masking, repellent and antifeedant properties of botanical compounds are needed, since these effects would not provoke the insect resistance. However, the possibilities of development of new adaptations in target insect after the repeated applications of proposed compounds have to be evaluated.

Acknowledgments

The research was supported by Serbian Ministry of Science, BTR # 6895.

REFERENCES

1. **Abbott S**. (1925) J. Econ. Entomol., **18**, 265-267.
2. **Alyokhin A., Dively G., Patterson M., Mahoney M., Rogers D., Wollam J.** (2006) American Journal of Potato Research, **83**(6), 485-494.
3. **Alyokhin A, Dively G., Patterson M., Castaldo C., Rogers D., Mahoney M., Wollam J.** (2007) Pest Management Science, **63**, 32-41.
4. **Bazzoni, E., Sanna-Passino, G., Moretti, M.D.L.** (2002) In: Recent progress in Medicinal Plants, (D.K. Majumdar, J.N. Govil, V.K. Singh, Eds.), 8 Phytochemistry and Pharmacology II, Houston, TX, Sci. Tech. Publishing LLC, 313-342.
5. **Brinley F.J.** (1926) J. Agric. Res., **33**, 177-182.
6. **Brum L., Emanuelli T., Souza D., Elisabetsky E.** (2001) Neurochemical Research, **26**(3), 191-194.
7. **Chiasson H., Belanger A., Bostanian N., Vincent C., Poliquin A.** (2001) J. Econ. Entomol., **94**, 167-171.
8. **Daferera, D.J., Zzogas, B.N., Polissiou, M.G.** (2000) Journal of Agricultural and Food Chemistry, **48**(6), 2576-2581.
9. **Ferro, D.N., Logan, J.A., Voss, R.H. and Elkinton, J.S**. (1985) Environmental Entomology, **14**, 343-348.
10. **Gökçe, A., Isaacs, R., Whalon, M.E.** (2006) Pest. Management Science, **62**(11), 1052-1057.
11. **Gökçe A., Whalon M.E., Çam M.E., Yanar Y., Demirtaş İ., Gören N.** (2007) Annals of Applied Biology, **1-6**, 32-41.
12. Handbook of insect rearing (eds. P.Singh and R.F. Moore), 1985, Vol II, 355- 368.
13. **Isman B.M.** (2000) Crop Protection, **19**, 603-608.
14. **Isman B.M.** (2002) Pesticide outlook, **13**(4), 152-157.
15. **Isman B.M.** (2005) Annu. Rev. Entomol., **51**, 45-66.
16. **Jovanović Z., Kostić M., Popović Z.** (2007) Ind. Crop Prod., **26**, 100-104.
17. **Kalushkov P., Batchvarova R.** (2005) Biotechnol and Biotechnol. Eq. **19**, 28-34.
18. **Kostić M., Sekulović D., Ristić M., Zabel A., Stanković S., Mihajlović Lj.** (1998) Book of Abstracts of the 9th International Congress on Pesticide Chemistry, August 2-7, 1998, London, United Kingdom, 3A - 051, topics 1-4.
19. **Kostić M., Dražić S., Zabel A., Manojlović B., Rajković S., Stanković S.** (2002) J. Sci. Agr. Res., **63** (221-222), 87-99.

20. **Kostić M., Dražič S., Stanković S.** (2007) Uticaj etarskog ulja žalfije (*Salvia officinalis* L.) na insekte. Arhiv za poljoprivredne nauke, **68**, 242, 33-45.

21. **Langenheim J.** (1994) J. Chem. Ecol., **20**, 1223-1280.

22. **Marković, I., Norris D.M., Cekic M.** (1996a) J. Chem. Ecol., **22**, 2283-2298.

23. **Marković I., Norris D.M., Phillips J.K., Webster F.X.** (1996b) J. Agr. Food Chem., **44**, 929-935

24. **Marković I., Norris D.M., Nordheim E.V.** (1997) Entomologia Experimentalis et Applicata, **84**, 247-254.

25. **Mordue J., Zounos A., Wickramanada R., Allan J.** (1995) In: Integrated Crop Protection: Toward Sustainability? Ed. R.G. McKinley & D. Atkinson, BCPC Symposium Proceedings No. **63**, 187-194.

26. **Moretti, M.D.L., Peana, A.T., Franceshini, A., Carta, C.** (1998) Journal of Essential Oil Research, **10**, 157-160.

27. **Munakate K.** (1970) In: Control of Insect Behaviour by Natural Products, ed. by Wood DL, Silverstein RM and Nakajima M, Academic Press, London, UK, 179-187.

28. **Noris D. M.** (1986) In: Chemistry of Plant Protection 1, ed. by Hang G. and Hoffman H., Springer-Verlag, Berlin, 97-144.

29. Pharmacopoea Jugoslavica, editio quarta (Ph. Jug. IV) (1984) Federal Burean of Health Care, Belgrade.

30. **Popović Z., Kostić M., Popović S., Skorić S.** (2006) Biotechnol. & Biotechnol. Eq., **20**(1), 36-40.

31. **Puri, H.S.** (1999) Neem, The Divine Tree Azadirachta indica, Medicinal and Aromatic Plants - Industrial Profiles, (R. Hardman, Ed.) Harwood Academic Publishers, **6**, 121-129.

32. **Regnault-Roger C.** (1997) Integrated Pest. Management Reviews, **2**(1), 25-34.

33. **Renwick J.A.A.** (1983) In: Plant Resistance to Insects, (P.A. Hedin, Ed.) ACS Symp., Ser. 208, ACS, Washington DC, 199-213.

34. **Rosa M.D., Mendonca-Filho R.R., Bizzo H.R., Rodrigues I.D., Soares R.M., Souto-Padron T., Alviano C.S., Lopes A.H.** (2003) Agents Chemother., **47**(6), 1895-1901.

35. **Sekulić D., Jovanović Z., Kostić M., Sekulović D.** (1995) Pharmazie, **50**(12), 835.

36. **Southwood T.R.E.** (1973) In: Insect/Plant Relationships, (Van Emden H.F., Ed), The Royal Entomological Society, London, U.K., **6**, 3-30.

37. **Stanković S., Zabel A., Kostić M., Šestović M**. (2004) Pesticidi, **1**, 59-175.

38. **Szczepanik M., Dams I., Wawreńczyk C.** (2005) Environ. Entomol., **34**(6), 1433-1440.

39. **Zabel A., Manojlović B., Rajković S., Stanković S., Kostić M**. (2002) J. Pest. Sci., **75**(1), 19-25.

40. **Zabel A.** (1991) Medjuzavisnost otpornosti krompirove zlatice (*L. decemlineata* Say.) na organofosforne insekticide i delovanje esteraza. PhD Thesis (in Serbian), Faculty of Agronomy, Novi Sad.