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*Research Article*

# EFFECT OF SAGE WATER EXTRACTS ON REDUCTION OF FEEDING OF PEA LEAF WEEVIL

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**ABSTRACT**
The aim of the experiment was to determine the effect of aqueous extracts of dried sage (*Salvia officinalis* L.) in concentrations of 2%, 5% and 10%, and the fresh parts of the plant in concentrations of 10%, 20% and 30% on the feeding of *Sitona lineatus* L. on the broad bean (*Vicia faba* L.) leaves. The olfactometry response of the pest (response to olfactory stimuli) was also determined in relation to the sage. Extracts of dry and fresh parts of sage reveal the inhibitory effect on feeding of both males and females of pea leaf weevil, the stronger, the higher their concentrations, however, no apparent differences were registered between the analysed sexes. Dry plant extracts in 10% concentrations and 30% extracts of fresh sage parts caused 2–3 time reduction of *Sitona lineatus* feeding after 4 days of the experiment. An apparent repellent response of aroma substances originating from sage towards weevil beetles (both males and females) was registered, which suggests potential applications of this plant as a neighbouring plant in mixed stands or, e.g., as a component of border belts.

**Keywords**: *Sitona lineatus* L., plant extracts, olfactometer, *Salvia officinalis* L.

## INTRODUCTION

The use of natural plant protection products, for example plant extracts is one of the safest way to pest control or control of plant diseases and can be used in organic farming.

The effective activity characterizes the extracts (aqueous or alcoholic) of herbal plants. In herbs there are substances, which can deter, discourage or affect the development and even cause the mortality of pests [Achremowicz and Cież 1992, Lamparski and Wawrzyniak 2004, Wawrzyniak and Dębek-Jankowska 2010, Wawrzyniak and Lamparski 2007]. The extracts are different in the manner of preparing, a composition, concentration or kind of solvent. Their applicability is very wide – from water extracts of dried or fresh parts of plant, applied as sprays or watering, to plant slurries.

Sage (*Salvia officinalis* L.) is the plant which has been used in medicine for ages. Currently, its properties have been even better known, which resulted in its application not only for pharmaceutic purposes, but also in cosmetic and food industries and in organic agriculture. In agriculture sage is used in a form of extracts, infusions or slurries. *S. officinalis* cultivated in the vicinity of other crops supports natural protection against pests or some diseases. For example when growing next to leeks it aided to reduce the harmfulness of tobacco thrips (*Thribs tabaci* Lind.) [Szafranek et al. 2012]. Sage extracts prepared with the use of methanol efficiently reduced the number of slugs (*Arion lusitanicus*) [Barone and Frank 1999] in rapeseed cultivation. Growth stimulation in beans, carrots or other herb plants (rosemary or fennel) [Pisulewska and Puchalska 2003, Szafranek et al. 2012), or even increase in yielding and improvement of coffee quality [Pacheto Bustos et al. 2008] are the benefits of sage vicinity in cultivations. The main active component, in this case – sage oil reveals a strong antibacterial and fungicidal effect [Akhondzadeh et al. 2003]. Active substances which it contains, such as:

thujone, cineole, camphor, borneol, pinene and thiamine, a number of vitamins, including B1, C, PP, as well as resins, diterpene lactone, tannins, flavonoids or fumaric acid allow a wide-scale application of sage [Perry et al. 1998].

Olfactory stimuli play a crucial role during food seeking by phytophages [Koschier et al. 2021, Koschier and Sendy 2003]. Properly maintained cultivation enriched by plant species with proven repellent or attractant effect on insects may bring measurable benefits. Phytophages may be effectively repelled whereas beneficial insects may be attracted by the plant aroma. Moreover, the presence of catechine tannins in sage oil influences lethality rise in most insects. It results from the reactions leading to the formation of reactive oxygen forms [Raymond et al. 2010].

The aim of the research was determining the effect of sage (*Salvia officinalis* L.) water extracts on feeding of pea leaf weevil (*Sitona lineatus* L.) males and females on broad bean (*Vicia faba* L.). The response of weevil beetles to the aroma of the above mentioned plant was also investigated.

## MATERIALS AND METHODS

Fresh leaves of broad bean (*Vicia faba* L.), Bartek c.v. were used for the analyses. In order to minimise the influence of the leaf age on feeding preferences of *Sitona* beetles, the leaves used for the experiment were collected only from the determined (second) level below the plant top. Respectively, 2 g, 5 g and 10 g of dry sage were weighted for the extract preparation (which provided the assumed concentrations of 2%, 5% and 10%) and 10 g, 20 g and 30 g of fresh fragments of the same plant (concentrations of 10%, 20% and 30%). Subsequently, 100 ml of cold redistilled water was poured over them [acc. to Wawrzyniak and Dębek-Jaworska 2010] and the extracts were left for 24 hours. They were kept in darkness at room temperature. After 24 hours ready extracts were filtered through filter paper and used immediately for laboratory experiments.

In order to determine the effect of sage extracts on pea leaf weevil beetles (*Sitona lineatus* L.) feeding, broad bean leaves were immersed for 3 seconds in the solutions of the respective extracts or in the redistilled water (control) and then dried at room temperature and finally placed on Petri dishes lined with wet filtration paper. Subsequently, 1 specimen of pea leaf weevil was placed on each dish (the experiment was conducted separately for males and females). The injured leaf area was measured nine times at 12 hour intervals. All observations were conducted in six replications.

The assessment of aroma preferences of adult specimens of pea leaf weevil was conducted by means of glass "Y-tube" olfactometer. The olfactometer is a device commonly used to test the insect response to olfactory stimuli [Giles et al. 1996, Desneux et al. 2004, Belda et al. 2010, Finlay-Doney et al. 2012, Cakmak et al. 2013, Sharma et al. 2013]. The olfactometer had one, so called supply arm and two test arms placed at 70$^{o}$ relative to each other. The air, previously purified by means of carbon filter was pressed by a pump (Power Cab, DC Power Supply 3050) and directed to each of the olfactometer test arms. The air flow was established at 900ml/min/arm. Before the air stream reached respective test arms it flew through the aroma source, i.e. a glass container containing respectively 30 g of fresh sage mass and a ring of filter paper moistened with distilled water (to ensure the proper air humidity) or only wet filter paper (control). The test insect was placed by the outlet of the tube constituting the supply arm and then its behaviour was watched for 10 minutes and the number of its entries in the respective olfactometer fields (arms) was registered. Each arm was washed with redistilled water and then by ethanol after testing each two specimens. The arrangement of the arms was changed to avoid visual effects. The experiment was conducted in 12 replications for each of the analysed sexes.

Statistical analysis of the obtained results was conducted using Statistica 10.0 PL programme. The significance of differences was tested using univariate analysis of variance and the means were differentiated by NIR Fisher test on significance level $\alpha = 0.05$. The t-Student test was applied for the results obtained using the olfactometer.

## RESULTS AND DISCUSSION

Observation of the rate of changes in broad bean leaf area losses due to female *Sitona lineatus* L. feeding from the beginning to the 60$^{th}$ hour of the experiment, for a majority of measurements demonstrated a significant effect of the individual sage extracts (Figure 1, Table 1). The greatest inhibition of *S. lineatus* females feeding was noted in the object where extracts from fresh fragments

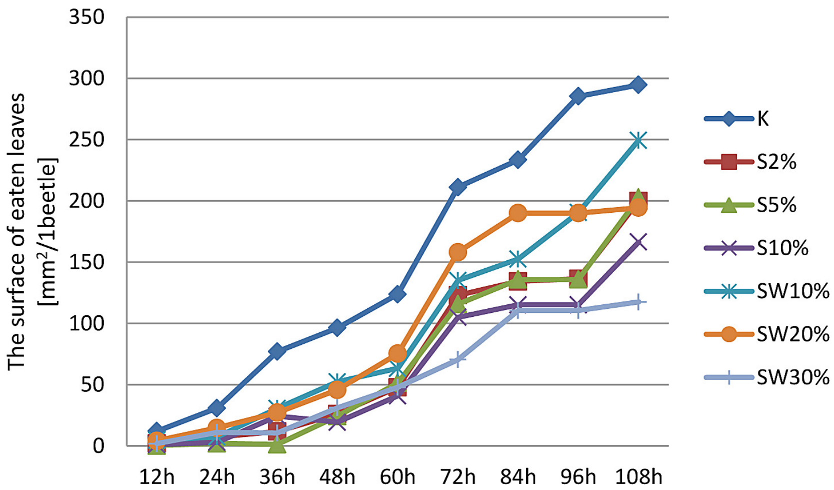


**Figure 1.** The dynamics of female *Sitona lineatus* L. beetles feeding on broad bean leaves, in cycle of 9 observations at 12-hour intervals, after application of the aqueous extract of dried sage in concentrations of 2% (S2), 5% (S5) and 10% (S10), and parts of fresh plants in concentrations of 10% (SW10), 20% (SW20) and 30% (SW30) compared to the control (K)

**Table 1.** Results of the statistical analysis of female *Sitona lineatus* beetles feeding on broad bean leaves, in the cycle of 9 observations at 12-hour intervals, after application the aqueous extract from dried and fresh parts of sage. See figure 1 for explanations.

| Object | 12h | 24h | 36h | 48h | 60h | 72h | 84h | 96h | 108h |
|---|---|---|---|---|---|---|---|---|---|
| K | *b | c | b | b | b | b | b | b | b |
| S2% | a | ab | a | a | a | ab | ab | a | ab |
| S5% | a | a | a | a | a | ab | ab | a | ab |
| S10% | a | a | a | a | a | a | a | a | ab |
| SW10% | a | ab | a | a | a | ab | ab | ab | ab |
| SW20% | a | b | a | a | a | ab | ab | ab | ab |
| SW30% | a | ab | a | a | a | a | a | a | a |

* Values marked with different letters differ significantly at $\alpha = 0.05$.

of *S. officinalis* were used in a concentration of 30%. 10% dry sage extract revealed similar effect discouraging *S. lineatus* females from feeding. On the other objects a lesser interest of females in food was also observed, however, these differences were not always statistically proved. Since the 72nd hour of observation, the effect of all extracts was slightly weakened. No significant differences in the intensity of respective kinds of extracts and their concentrations effect among one another were registered.

The statistical analysis, conducted for the experiment involving the observation of the rate of changes in the broad bean leaf blade area loss due to male *Sitona lineatus* L. beetles feeding, revealed significant differences in most measurements (Figure 2, Table 2). Slowing down of *Sitona lineatus* males feeding in result of applied extracts was observed already during the first measurement (the 12th hour of the experiment). The subsequent measurement (after 24 hours) revealed that 2% and 10% extracts of dry sage and 30% extract of fresh sage fragments apparently limited consumption of broad bean lead blade. According to expectations, during the subsequent observations, the extracts with higher concentrations, i.e. 10% dry sage extract and 30% extract of fresh fragments had the strongest limiting effect.

At the same time, the statistical analysis revealed the differences significant only for the control, whereas the individual objects with extracts did not differ from one another.

The analysis of the number choices of olfactometer fields made by the insects revealed a significant, repellent effect of sage olfactory stimuli, both for males ($t = 2.67$, $P = 0.015$) and females ($t = 2.77$, $P = 0.014$) (Table 3). The insects were choosing the control field over twice more often than the field where aroma substances from sage were supplied.

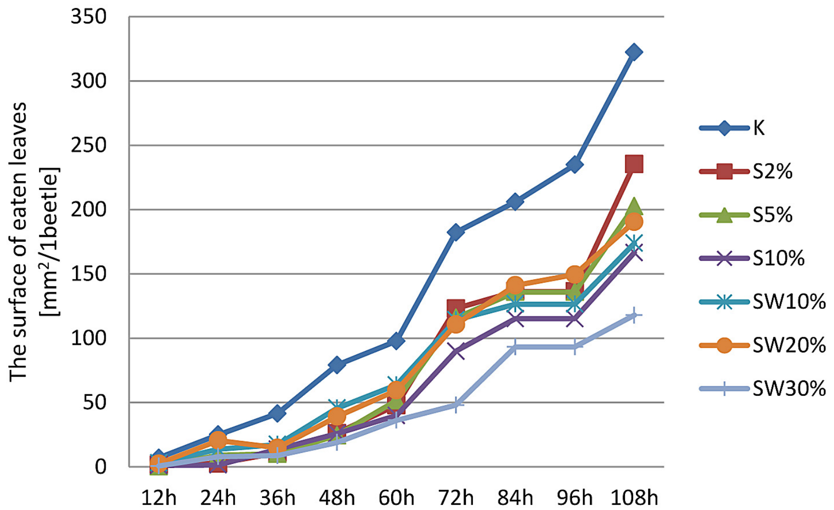


**Figure 2.** The dynamic of male *Sitona lineatus* L. beetles feeding on broad bean leaves, in cycle of 9 observations at 12-hour intervals, after application of the aqueous extract of dried sage in concentrations of 2% (S2), 5% (S5) and 10% (S10), and parts of fresh plants in concentrations of 10% (SW10), 20% (SW20) and 30% (SW30) compared to the control (K)

**Table 2.** Results of the statistical analysis of male *Sitona lineatus* beetles course feeding on broad bean leaves, in the cycle of 9 observations at 12-hour intervals, after application the aqueous extract from dried and fresh parts of sage. See figure 1 for explanations.

| Obiekt | 12h | 24h | 36h | 48h | 60h | 72h | 84h | 96h | 108h |
|---|---|---|---|---|---|---|---|---|---|
| K | *b | c | b | b | a | b | a | b | b |
| S2% | a | a | a | a | a | a | a | a | a |
| S5% | a | abc | a | a | a | a | a | a | a |
| S10% | a | a | a | a | a | a | a | a | a |
| SW10% | a | abc | a | ab | a | a | a | a | a |
| SW20% | a | bc | a | ab | a | a | a | a | a |
| SW30% | a | ab | a | a | a | a | a | a | a |

* Values marked with different letters differ significantly at α = 0.05.

**Table 3.** Responses of pests to odors derived from *Salvia officinalis* L. fresh matter expressed as a number of choices of selected areas of Y-tube olfactometer per one insect

| Szkodnik/Pest | Kontrola/Control | *Salvia officinalis* L. |
|---|---|---|
| Females* | 0.88 | 0.33 |
| Males* | 0.82 | 0.30 |

* Differences significant at α = 0.05.

The presented research demonstrated that the effect of sage used in a form of water extracts was diversified depending on the kind of plant material used and the extract concentration. The higher the concentration, generally the more limited the insect feeding. The extract of fresh parts acted more strongly than dry sage extract.

Despite numerous experiments using herb plant extracts for crop pest control, the available literature lacks information about the effect of sage extracts on pea leaf weevils. Moreover, a majority of papers in this area focuses on the effect of sage oil, not sage extracts. Application of sage oil for biological plant protection caused over 80% of lethality among adult spider mites (*Tetranychus urticae* Koch) [Won-Il et al.]. Similar results for the above mentioned pest (95–100% lethality when 0.10% and 0.25% sage oil was applied) were obtained in the research of Laborda et al. [2013]. The experiments conducted by Koschier and Sendy [2003] revealed between 45 and 60% lower oviposition by *Thribs tabacci* Lind. females under the influence of 0.1% and 1.0% sage oil. Sage oil (2%) revealed over 27% lethality, repellent effect in almost 64% of specimens and anti-reproductive effect in adult *Sitophus oryzae* L. specimens after 48 hours of the experiment [Popović et al.2005]. Regnault-Roger et al. [1993] determined $LC_{50}$ for *Acantoscelides obtectus* Say at sage oil concentration lower than 10 mg/dm$^3$. Sage oil dose lethal for housefly (*Musca domestica* L.) is 5–10 microgram/cm$^2$ of a paper ring [Pavela 2008].

The obtained results demonstrated slight differences in the amount of consumed food between male and female specimens. Females consumed slightly more food, but it was visible only at the initial stage of the experiment. The moment when the observations and test of weevil beetles were conducted overlapped with the reproductive season of these insects, which may explain higher demand for food in females. In the research of other authors [Landon et al.1997] the differences for various insect sexes were also noted – weevil females showed greater activity in feeding than males.

As demonstrated by the experiments using olfactometer and electroantennography (EAG), the response of weevil beetles to chemical substances in pea (*cis-3*-h*exen-1*-yl acetate) combined with the plant aroma became a key stimulus helping insects to seek host plants among the other pulses [Landon et al.1997], which evidences a crucial role of olfactory stimuli for this species. The analysis of olfactometric response conducted in the presented research clearly showed repellent properties of sage aroma towards *S. lineatus* males and females. A similar effect of other herb plants (fennel and coriander) visible as inhibition of broad bean leaves injuring by *Sitona lineatus* L. beetles under the influence of the above mentioned plants presence in the parallel broad bean cultivation were observed in previous investigations [Gospodarek et al. 2011]. No visible differences in olfactometric response were noted between both sexes in the presented experiment, although acc. to Landon et al. [1997] pea leaf weevil females show much greater sensitivity to olfactory stimuli.

## CONCLUSIONS

1. Extracts of dry and fresh parts of sage reveal the inhibitory effect on feeding of both males and females of pea leaf weevil, the stronger, the higher their concentrations, however, no apparent differences were registered between the sexes.
2. Dry plant extracts in 10% concentrations and 30% extracts of fresh sage parts caused 2-3 time reduction of *Sitona lineatus* feeding after 4 days of the experiment.
3. An apparent repellent response of aroma substances originating from sage towards weevil beetles (both males and females) was registered, which suggests potential applications of this plant as a neighbouring plant in mixed stands or, e.g., as a component of border belts.

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