# EFFECT OF WATER EXTRACT FROM MINT ON SELECTED CROP PESTS FEEDING AND SURVIVAL

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#### ABSTRACT

The aim of the study was to determine the effect of aqueous extracts of dried mint (Mentha piperita L.) in concentrations of 2%, 5% and 10%, and fresh parts of this plant, in concentrations of 10%, 20% and 30% on feeding of pea leaf weevil (Sitona lineatus L.) and survival of black bean aphid (Aphis fabae Scop.) on the leaves of broad bean (Vicia faba L.), variety "Bartek". In the studies of the effects of aqueous extracts of mint on the dynamics of Sitona lineatus feeding on the beans leaves, 9 laboratory's observations in 12-hour intervals was made. Leaves injuries on their edge surface caused by adult individuals were measured. Observations were carried out in 6 replicates divided into males and females. In these researches on impact of water extracts from mint on mortality of wingless females and larvae of black bean aphids, 14 laboratory's observations in the 8-hour intervals was made. Aqueous extracts of dry and fresh peppermint significantly limited the feeding of pea leaf weevil females, and the extracts from dried material were more effective. Extracts from peppermint caused mortality of black bean aphid larvae compared to wingless females to a greater extent, and the higher the concentration of the extract, the more beneficial effect was observed. An extract from dried material at a concentration of 2% was too weak to effectively combat the larvae of A. fabae, while in the case of aphid females, an effectiveness was only demonstrated for the extracts at the highest concentrations, i.e., 10% of dried and 30% of fresh peppermint.

Keywords: biological control, Mentha piperita L., plant extracts

# **INTRODUCTION**

The use of herbs as a base for the preparation of natural products protecting the crops is one of the safest (both for human and other living organisms) ways to fight the pests. Their production does not require high costs and specialized equipment [Isman 2000]. The effectiveness of protective measures using extracts of plants is determined by both the concentration of the substance, type and quality of plant material and the applied solvent (both aqueous and alcohol plant extracts are popular). Substances contained in herbaceous plants can act as a disincentive for further feeding of the pest in crops, cause disorders in development cycle and fertility, and even the death of the pest [Achremowicz and Cież 1992, Lamparski and Wawrzyniak 2004, Wawrzyniak and Dębek-Jankowska 2010, Wawrzyniak and Lamparski 2007].

Peppermint, or medical spearmint (*Mentha piperita* L.), is a plant species belonging to the mint family (*Lamiaceae* Lindl.), which is originally a blend of two other species – aquatic mint (*Mentha aquatica* L.) and spearmint (*Mentha spicata* L.), bred and introduced into cultivation in England [Szweykowska and Szweykowski 2003, Cavelius 2005]. The spread of this plant in the crops in most regions of the world was determined by its ease of cultivation, as well as vegetative propagation by rhizomes division, which causes an excessive expansiveness of peppermint, sometimes treated as a weed in other crops [Burnie 2005]. Peppermint is a valuable medicinal plant, since

it is characterized by analgesic, sedative, antibacterial, choleretic and diastolic activity, it also affects nerve receptors, causing a cooling sensation. Therefore, it is used in the food industry as an additive in beverages or food products. Intensely fragrant mint essential oil contained primarily in the leaves of this plants, can also be useful in the production of natural repellents in organic farming [Ciesielska et al. 2011]. The composition of peppermint oil mainly includes: menthol (monoterpene type) (above 50%), menthofuran, menthone (about 20%), menthol esters - menthol acetate and valerate (about 5%), phellandrene, pinene, cineole, menthofuran, piperitone, jasmone, tannins (6-12%), flavonoids (luteolin, apigenin, diosmetin) as well as mustard, phenolic acids and mineral salts [İşcan et al. 2002, Voirin et al. 1990, Karp et al. 1990]. The studies so far indicate the effectiveness of peppermint extracts in certain aphid species combating, e.g. peach-potato aphid (Myzus persicae Sulzer) [Ikeura et al. 2012]. In turn, the essential oils of peppermint were tested for toxicity against mosquitoes, as reported by Ansari et al. [2000] and Kumar et al. [2012].

Pea leaf weevil (Sitona lineatus L.) and black bean aphid (Aphis fabae Scop.) are two species of the pest of high economic importance, which have been effectively fought so far mainly by chemical methods [Księżak and Borowiecki 2003]. Sitona spp. beetles attack many species of Fabaceae plants, in turn, the larvae of these pests feed on the root nodules of these plants, causing them to rot and decay and reducing atmospheric nitrogen assimilation [Gospodarek et al. 2011, Borowiecki and Księżak 2001]. On the other hand, aphids occurrence in the crop always results in weakening the plants and contribute to yield reduction [Gospodarek 2012]. The measures aimed to find an effective, non-chemical methods of these pests combating are therefore particularly important.

The aim of the experiment was to investigate an effect of aqueous extracts of dry and fresh weight of peppermint (*Mentha piperita* L.) on the feeding of pea leaf weevil (*Sitona lineatus* L.) females and males, and the survival rate of larvae and wingless females of black bean aphid (*Aphis fabae* Scop.) on broad bean (*Vicia faba* L.).

### MATERIALS AND METHODS

The laboratory experiment involved fresh, young leaves of broad bean of Bartek cultivar, collected from the same level below the top of the plant, in order to eliminate an effect of leaf age on the intensity of pest feeding. The collected plant material was treated with aqueous extracts prepared from dry and fresh pieces of peppermint (leaves and young shoots). In order to prepare the extracts, 2 g, 5 g and 10 g of dry mass of the peppermint were weighed (referred to as the concentration of 2%, 5% and 10%), as well as 10 g, 20 g and 30 g of fresh pieces of the plant (referred to as the concentration of 10%, 20% and 30%) and then it was poured with 100 ml of cold double-distilled water. The extracts were allowed to stand for 24 hours in the dark at room temperature. Then, the resulting solutions were filtered through a filter paper, and immediately used for the experiment. The leaves of broad bean were immersed for 3 seconds in a suitable extract or double-distilled water (control), then dried at room temperature and placed in Petri dishes lined with moistened filter paper. Then, 2 individuals of pea leaf weevil were placed in each dish, the experiments were conducted separately for males and females. With regard to black bean aphid, the experiment was conducted separately for wingless females and 3-day larvae, placing 10 individuals of the pest in a dish. Both experiments were performed in 6 replicates. The filter paper in Petri dishes was wetted if necessary during the experiments to prevent drying the leaves. The area of losses caused by the feeding of pea leaf weevil beetles was measured nine times at 12 hour intervals, while 14 observations were performed in the case of black bean aphid, where mortality of the insects was investigated every 8 hours.

After conclusion of the conducted observations for pea leaf weevil, the following values were calculated:

- palatability index as the ratio of the percentage loss of the leaf blade in various combinations to the percentage loss of the leaf blade in the control,
- absolute deterrence index, which included the relationship between the area of leaf consumed by *Sitona* in the analyzed objects and the area of leaf consumed in the control [Kiełczewski et al. 1979]:

Adi = 
$$[(K-T): (K+T)] \cdot 100$$
 (1)

where:

K – the average area of leaf consumed by the pest in the control [mm<sup>2</sup>],

T – the average area of leaf consumed by the pest in the analyzed object [mm<sup>2</sup>].

The obtained results were then subjected to analysis by STATISTICA 12.5 PL software. The significance of differences between the means were tested by univariate analysis of variance, and the means were differentiated by Fisher's LSD test at  $\alpha = 0.05$ .

# RESULTS

In the case of almost all observation dates, the extracts of dry and fresh peppermint significantly limited the feeding of pea leaf weevil females, and slightly more potent activity was noted for the dried material extracts (Fig. 1, Tab. 1). Despite the significant difference with respect to the control, the activity of particular extracts did not differ from each other. Starting from 84-hour observation, the average area of losses on the broad bean leaves per one female was at least 2-fold

lower in the objects using aqueous peppermint extracts compared to the control.

In turn, the extracts prepared from dry and fresh weight of peppermint did not significantly limit the feeding of pea leaf weevil male on broad bean. The activity of the aqueous extracts of peppermint was also not varied in most cases with each other. 5% extract of dried peppermint even slightly stimulated the feeding of pea leaf weevil males.

Palatability index for pea leaf weevil females was on a level below 1 for each of the extracts used, indicating their inhibitory activity against the feeding. In the case of extracts from fresh peppermint pieces, the palatability index value decreased with an increasing concentration of the extracts, which was not noted in the case of extracts from dried material.

In the case of *S. lineatus* males, the values of palatability index were on average about



**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 mint 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 *S. 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 mint. See figure 1 for explanations.

Obiekt	12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h	108 h
K	C*	с	С	b	b	b	b	b	b
S 2%	ab	b	abc	а	а	а	а	а	а
S 5%	ab	ab	ab	а	а	а	а	а	а
S 10%	ab	а	а	а	а	а	а	а	а
SW 10%	а	а	а	а	а	а	а	а	а
SW 20%	ab	ab	bc	ab	а	ab	а	а	а
SW 30%	b	ab	abc	а	а	а	а	а	а

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



**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 mint 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 *S. 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 mint. See figure 1 for explanations.

Obiekt	12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h	108 h
K	ab*	ab	ab	ab	ab	а	ab	ab	ab
S 2%	а	а	а	а	ab	а	ab	ab	ab
S 5%	b	С	b	b	b	а	b	b	b
S 10%	ab	abc	ab	ab	ab	а	ab	ab	ab
SW 10%	ab	abc	ab	ab	ab	а	ab	ab	ab
SW 20%	ab	bc	ab	ab	ab	а	ab	ab	ab
SW 30%	а	ab	ab	ab	а	а	а	а	а

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





Obiekt	8h	16h	24h	32h	40h	48h	56h	64h	72h	80h	88h	96h	104h	112h
K	a*	а	а	а	а	ab	ab	ab	ab	ab	ab	abc	ab	ab
S2%	а	а	а	ab	а	а	а	а	а	а	а	а	а	а
S5%	а	а	ab	abc	ab	ab	ab	ab	ab	ab	а	ab	а	а
S10%	b	b	С	cd	b	с	bc	bc	bc	b	b	С	b	b
SW10%	а	а	ab	bc	ab	bc	abc	ab	ab	ab	ab	abc	ab	ab
SW20%	а	а	bc	bc	ab	abc	bc	bc	bc	b	b	bc	b	b
SW30%	b	b	d	d	С	d	с	С	с	С	с	d	С	с

**Table 3**. Results of the statistical analysis of wingless females of *Aphis fabae* Scop. mortality, in the cycle of 14 observations at 8-hour intervals, after application the aqueous extract from dried and fresh parts of mint. See figure 1 for explanations.

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

0.3-higher than for females (Fig. 4). This reflects weaker effect of aqueous peppermint extracts limiting feeding of this pest males. Higher index values were recorded for all extracts of dried peppermint.

The extracts of fresh pieces of peppermint effectively inhibited the feeding of *S. lineatus* males, as evidenced by significantly lower value of the palatability index.

All applied peppermint extracts were characterized by a clear inhibitory effect on pea leaf weevil females feeding, as evidenced by positive and quite high values of absolute deterrence index (Fig. 5). These values ranged from 31 to 70.

In the case of pea leaf weevil males, the value of absolute deterrence index in the case of an aqueous extract of dried plant at a concentration of 5% was negative. This demonstrates the stimulating, and therefore undesirable effect of this extract with respect to feeding of *S. lineatus* males (Fig. 6). Other peppermint extracts were characterized by positive, but lower than in the case of females, values of the absolute deterrence index.

The strongest activity causing mortality in wingless females of black bean aphid was demonstrated for 30% extract of fresh peppermint fragments (Fig. 3, Tab. 3). Other extracts from fresh parts of the plant (20% and 10%) also contributed to the increase in aphids mortality, but this effect in most cases was not statistically significant. In turn, in the case of dried peppermint extracts, only 10% extract appeared to be effective. An activity of this extract from the beginning of the observation up to 48 hour of the experiment significantly affected an increase in black bean aphid mortality on broad bean leaves. An average mortality in objects with fresh plant extracts ranged from 30-65%, while in the objects with dried material extracts it was 20-40%. Mortality of this pest at the end of the experiment in the control reached 25%.



**Figure 4**. Palatability index after applying extracts from fresh and dry matter of *Mentha piperita* L. at different concentrations (2 – 30%) for males of *Sitona lineatus* L. See Figure 3 for explanations.

Obiekt	8h	16h	24h	32h	40h	48h	56h	64h	72h	80h	88h	96h	104h	112h
К	a*	а	а	а	а	а	а	а	а	а	а	а	а	а
S2%	а	а	а	ab	ab	ab	а	ab	ab	ab	ab	ab	ab	ab
S5%	а	а	abc	bc	bc	bc	b	bc	bc	bc	bc	bc	bc	abc
S10%	а	b	С	cd	С	d	bc	cd	с	с	с	de	de	de
SW10%	а	а	ab	abc	abc	cd	b	с	с	bc	bc	bcd	cd	bcd
SW20%	а	а	bc	bc	bc	cd	bc	cd	cd	С	с	cd	cd	cd
SW30%	а	b	d	d	d	е	с	d	d	d	d	е	е	е

**Table 4**. Results of the statistical analysis of *Aphis fabae* Scop. larvae mortality, in the cycle of 14 observations at 8-hour intervals, after application the aqueous extract from dried and fresh parts of mint. See figure 1 for explanations.

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



Figure 5. Absolute deterrence index (Adi) of extracts from fresh and dry matter of *Mentha piperita* L. at different concentrations (2 – 30%) for females of *Sitona lineatus* L., calculated according to the formula:
Adi = [(K-T) : (K+T)] • 100, where: K – the average area of leaf consumed by the pest in the control [mm<sup>2</sup>], T – the average area of leaf consumed by the pest in the analyzed object [mm<sup>2</sup>]. S – dry matter, SW – fresh matter.



**Figure 6**. Absolute deterrence index (Bwd) of extracts from fresh and dry matter of *Mentha piperita* L. at different concentrations (2 – 30%) for males of *Sitona lineatus* L. See **figure 5** for explanations.



Figure 7. The effect of water extracts from dry matter of *Mentha piperita* L. 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) on mortality the wingless females of *Aphis fabae* Scop. [%]



Figure 8. The effect of water extracts from dry matter of *Mentha piperita* L. 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) on mortality of *Aphis fabae* Scop. larvae [%]

All the peppermint extracts contributed to an increase in the mortality of black bean aphid larvae feeding on broad bean leaves. The strongest effect, significant not only with respect to the control but also to other objects with peppermint extracts, was found in the case of 30% extract from fresh plant pieces, and was maintained for up to 16 hours to the end of the experiment (Figure 4, Table 4). A significant reduction of the survival of black bean aphid larvae was also noted on the objects with 5% and 10% dried peppermint extracts, as well as 10% and 20% fresh plant extracts. In the case of dried plant extracts, it was observed that the efficacy increased with an increasing concentration of the extract. Considerably higher average mortality was observed for larvae compared to wingless females. The experiment was terminated with an average mortality of larvae at about 60% (for dried material extract at a concentration of 30%) and 40% (for 20% fresh parts extract, and 10% dried peppermint extract), the other extracts increased the mortality rate of 10-20% relative to the control.

# DISCUSSION

The study showed that the efficacy of aqueous extracts of peppermint was dependent on the type and concentration of the extract and the stage of development, gender and insect species. High effectiveness with respect to larvae and wingless females of black bean aphid, as well as pea leaf weevil females, provides a basis for further research in this range.

The efficacy of aqueous extracts of plants is much lower than the essential oils of these plants, however, extracts preparation is much simpler and requires no specialized equipment [Isman 2000]. Most of the information in the available literature on peppermint use to protect the plants against pests is related to the use of essential oils. It was demonstrated that peppermint oil applied at a dose of 3 mL per 1 m<sup>2</sup> of aqueous surface resulted in 100% mortality of Culex quinquefasciatus mosquito larvae, and 90% mortality of Aedes aegypti and 85% of Anopheles stephensi mosquitoes after 24 hours of the experiment [Ansari et al. 2000]. 100% mortality of the last two species was also observed after 48 hours, and 24 hours after application of 4 ml/m<sup>2</sup> of aqueous experimental surface. Peppermint oil also resulted in deterring adult mosquitoes, hence - an inhibition of egg laying. Kumar et al. [2012] also studied the effect of peppermint oil on the survival of Aedes aegypti mosquitoes and determined the value of  $LC_{50}$  at  $0.54 \,\mu$ l/cm<sup>2</sup> of the dish with water and the larvae of this mosquito, and 48.4 µl/l of peppermint oil in the fumigation treatment of adults after 24 hours. However, there is very little information on the effectiveness of peppermint extracts against any pests, and in particular there is the lack of data on the use of aqueous extracts. Ikeura et al. [2012] examined the effectiveness of peppermint extract prepared by pouring and homogenization of 10 g fresh leaves with 40 ml of 50% EtOH with respect to peach-potato aphid (Myzus persicae Sulzer). In the laboratory conditions, they applied the dose of 0.5 ml of peppermint extract for 24 hours at radish leaf, and after that they put 20 aphids on the treated leaf on a dish lined with paper. The repellent activity of peppermint extract determined as the number of aphids, which was permanently repelled and discouraged from staying on the leaf treated with plant extract was 55%, and the peach-potato aphid mortality reached 50%. In our study, at the end of the observation (i.e., after 112 hours) after the application of 30% concentration of the extract from fresh peppermint fragments, the mortality of females was 65%, while in the case of larvae less than 60%.

It can be concluded regarding our findings to the use of aqueous extracts from other herbal plants to black bean aphid that peppermint exhibits slightly lower effectiveness than other species, e.g. winter savory (*Satureja montana* L.) [Rusin et al. 2016]. It was demonstrated that aqueous extract from winter savory at a concentration of 10% prepared from the dried material, and 30% and 20% fresh plant extracts, contributed to the 100% mortality of wingless *A. fabae* females, after 84, 96 and 108 hours, respectively. In comparison to the effectiveness of aqueous extracts of common nettle (Urtica dioica L.), peppermint extracts seem to have a similar effect [Biniaś et al. 2015]. The highest mortality rate of 60% with respect to black bean aphid larvae after 120 hour of the experiment was noted for 30% extract of fresh nettle pieces. In turn, with respect to wingless female, 55% mortality rate after 120 hour of the experiment was demonstrated for 30% extract from fresh nettle pieces, and 52% mortality was found for dried material extracts at a concentration of 10%, and fresh plant extracts at a concentration of 20%. Also aqueous and alcohol extracts of wormwood and sage, as well as the combinations of these herbs with garlic, were tested in combating the black bean aphid on broad bean [Achremowicz and Cież 1992]. However, very high efficiency reaching 70-100% on the second day of the observation in the objects with sage and wormwood was only noted in the case of alcohol extracts, while aqueous extracts caused mortality at a level of 20-40%.

As in the case of black bean aphid, also in relation to pea leaf weevil there is no data in the literature on the effectiveness of aqueous extracts from peppermint to limit their feeding. Our studies demonstrated a clear effect with respect to pea leaf weevil females in the case of all extracts used, however, the effect of these made of dried material exhibited slightly stronger activity, while the effect on males feeding was not statistically proven. For a comparison, extracts of dry and fresh parts of sage (Salvia officinalis L.) limited the feeding of both females and males of this pest, and 30% extract of fresh fragments of the plant to the highest degree limited the feeding [Biniaś et al. 2016]. The activity of sage extracts were stronger with their increasing concentration. In turn, Rusin et al. [2016] examined the effect of aqueous extracts of the wormwood (Artemisia absinthium L.) on this pest feeding. It was demonstrated in the study that 10% of extract of dried plants to the highest degree limited the feeding of both females and males. The area of losses caused by females on the object with this extract was on average about 107 mm<sup>2</sup> lower than in the control after 120 hours of the experiment. In turn, this value was significantly lower in the case of males. The reduction in areas of the losses on broad bean leaves in relation to the control was only 34 mm<sup>2</sup> for dried material extracts at a concentration of 10%, and 28 mm<sup>2</sup> for fresh fragments at a concentration of 20% and 30%. Other concentrations and types of the extracts exhibited even lower activity. The above study using peppermint extracts demonstrated significantly more potent activity limiting leaf lamina consumption by pea leaf weevil females in case of 5% of dried plant extract, amounting up to 335 mm<sup>2</sup> of the difference between the control and examined extract after 108 hours of the experiment. With respect to males, the same extract demonstrated slightly stimulating activity. A comparison of the values of absolute deterrence index for particular extracts of wormwood suggest that the effect of peppermint extracts was much stronger. In the case of pea leaf weevil females, the highest value of the index was 16 when using the wormwood extracts, other values ranged from 5 to 8, with the exception of an object with 20% extract from fresh pieces of wormwood, where the value of absolute deterrence index was negative and amounted to -4.5. In the case of pea leaf weevil males, the maximum value of absolute deterrence index was 8 for aqueous extracts from dried material at a concentration of 10%, and fresh parts of the plants at a concentration of 20%. The lowest value, i.e., -12.5, proving the lack of limiting activity, and even stimulation of the feeding, was noted for 5% extract of dried wormwood. In our study with the use of aqueous extracts of peppermint against pea leaf weevil females, the highest value of absolute deterrence index of up to 70 was observed for 5% extract of dried peppermint, and the lowest value, but still high compared to studies with extracts of wormwood, at the level of about 32% was demonstrated for 10% extract of dried peppermint, and 10% and 20% for fresh fragments extracts. In the case of pea leaf weevil males, the highest value of an index to 52 was noted for the strongest, 30% extract of fresh fragments of the plant, and the lowest value of -11 stimulating the feeding was found for 5% extract of dried peppermint. The value of palatability index in the case of wormwood for females was the lowest in the object with 10% extract of dried material, where it was 0.6, and the highest in the object with 20% extract from fresh plant fragments and amounted to less than 1. In turn, the lowest value for males, equal to 0.7, was observed for 20% extract from fresh fragments of the plant, and the highest for 10% extract of dried plants reaching the value of 1.3. The values of palatability index obtained using peppermint extracts, especially in females, were slightly lower, what

suggests higher efficiency of peppermint extracts compared to wormwood.

# CONCLUSIONS

- 1. Aqueous extracts of dry and fresh peppermint significantly limited the feeding of pea leaf weevil females, and the extracts from dried material were more effective. However, no significant reduction in males feeding was proved using these extracts.
- 2. Extracts from peppermint to a greater extent caused mortality of black bean aphid larvae, compared to wingless females, and the higher the concentration of the extract the more beneficial effect was observed, and extracts from fresh plant proved to be more effective than those prepared from dried material. An extract from dried material at a concentration of 2% was too weak to effectively combat the larvae of *A. fabae*, while in the case of aphid females, the effectiveness was only demonstrated for the extracts at the highest concentrations, i.e., 10% of dried and 30% of fresh peppermint.
- 3. Comparing the effectiveness of aqueous peppermint extracts in the reduction of black bean aphid and pea leaf weevil with the extracts prepared on the basis of other herbaceous plants, it can be considered that it is at a medium level.

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