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ANTIFEEDANT EFFECTS OF SEVERAL NATURAL SUBSTANCES ON SOME PHYTOPHAGOUS INSECT SPECIES

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ABSTRACT

Nowadays increasing efforts are made to develop environmentally safer pest control methods. One such method can be the use of natural antifeedants. Laboratory experiments were conducted to determine the effects of 3 plant extracts (*Plantago major* L., *Brassica napus* var. *napus* L., *Tilia cordata* Mill.) and 1 enzyme inhibitor isolated from the desert locust (Schistocerca gregaria trypsin chymotrypsin inhibitor = SGTCI) on the food consumption of two major insect pests (*Leptinotarsa decemlineata* Say, *Sitona* spp.). The insect antifeedant activity of the four substances was investigated by circular leaf disc dual choice bioassay. After 24 hours the surfaces of the leaf disc remnants were measured with leaf area analyser. *Ajuga chamaepitys* L. extracts and copper were taken as standard. The tested 3 plant extracts were prepared from leaves diluted with water. SGTCI was tested with doses between 1-2 mg/ml. The most important results of the bioassay are the following (given in antifeedant activity: AF = (1-Treated/Control)x100: with *L. decemlineata* larvae: SGTCI (1,0 mg/ml): 59%, copper (as standard control): 45%, with *L. decemlineata* adults: SGTCI (1,0 mg/ml): 15%, copper (as standard control): 0%, Plantago extract: 35%, Tilia extract: 49%. With *Sitona* adults: SGTCI (1,0 mg/ml): 0%, Ajuga extract (as standard control): 100%, Brassica extract: 86%, Tilia extract: 94%. The results show that SGTCI showed appreciable antifeedant activity only against *L. decemlineata* larvae, while amongst the tested plant extracts Tilia was the most active as antifeedant against *Sitona* adults.

Key words: enzyme inhibitor, insect antifeedant, leaf disc bioassay, *Leptinotarsa decemlineata, Sitona* spp.

IZVLEČEK

ODVRAČALNI UČINEK NEKAJ NARAVNIH SNOVI NA NEKATERE FITOFAGNE VRSTE ŽUŽELK

Razvoj okolju prijaznih metod za varstvo rastlin postaja vse bolj pomemben. Ena od teh je uporaba naravnih odvračal. V laboratorijskih poskusih smo ugotavljali učinek 3 rastlinskih izvlečkov: velikega trpotca (*Plantago major* L.), ogrščice (*Brassica napus* var. *napus* L.), lipovca (*Tilia cordata* Mill.) in enega encimskega inhibitorja, izoliranega iz kobilice selke *Schistocerca gregaria,* tripsin himotripsin inhibitorja (SGTCI), na hranjenje dveh pomembnih škodljivcev: koloradskega hrošča (*Leptinotarsa decemlineata* Say) in obrobkarja (*Sitona* spp.). Odvračalni učinek 4 snovi je bil preučen z metodo "circular leaf disc dual choice bioassay". Po 24 urah smo izmerili ostanek listne površine s planimetrom. Kot standard smo uporabili izvleček rumenega skrečnika (*Ajuga chamaepitys* L.) in baker. Izvlečke testiranih rastlin smo pripravili z namakanjem listov v vodi. Testirani odmerki SGTCI so bili med 1 – 2 mg/ml. Najpomembnejši rezultati analize so naslednji (podani z odvračalnim učinkom): AF = (1 – tretirano/kontrola) x 100: pri ličinkah *L. decemlineata*: SGTCI (1,0 mg/ml): 59 %, baker (standard): 45 %, pri hroščih *L. decemlineata:* SGTCI (1,0 mg/ml): 15 %, baker (standard): 0 %, izvleček *P. major*: 35 %, izvleček *T. cordata*: 49 %. Pri odraslih osebkih vrst *Sitona*: SGTCI (1,0 mg/lm): 0 %, izvleček A. *chamaepitys* (standard): 100 %, izvleček *B. napus* var. *napus*: 86 %, izvleček *T. cordata*: 94 %. Rezultati kažejo, da ima

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SGTCI znaten odvračalni učinek le proti ličinkam *L. decemlineata*, med testiranimi rastlinskimi izvlečki pa je bil izvleček *T. cordata* najbolj učinkovit proti odraslim osebkom vrst *Sitona*.

Ključne besede: inhibitor encimov, odvračalni učinek, listni poskus, *Leptinotarsa decemlineata, Sitona* spp.

1 INTRODUCTION

The widespread use of synthetic pesticides during the past several decades resulted many environmental problems throughout the world. Within the framework of sustainable agriculture, an increasing demand for new, more environmentally friendly pest control products is developed. Safer methods are needed and one such method can be the use of natural antifeedants against insect pests. One source of these kind of substances can be vegetable (isolated from plants). Many plants produce high amounts of secondary plant substances that inhibit the feeding activity of insects (for example: Neem-tree produces azadirachtin, Ajuga plants produce neoclerodane diterpenoids). The other source of feeding inhibitors can be animal. Several enzyme inhibitors (for example: cowpea trypsin inhibitor) are found to possess potential insect antifeedant activity. (However these enzyme inhibitors were also isolated from plants up to the present.)
Laboratory experiments were conducted to determine the effects of 3 plant extracts (*Plantago major* L., *Brassica napus* var. *napus* L., *Tilia cordata* Mill.) and 1 enzyme inhibitor isolated from the desert locust (Schistocerca gregaria trypsin chymotrypsin inhibitor = SGTCI) on the food consumption of two major insect pests (*Leptinotarsa decemlineata* Say, *Sitona* spp.). The results show that SGTCI showed appreciable antifeedant activity only against *L. decemlineata* larvae, while amongst the tested plant extracts Tilia was the most active as antifeedant against *Sitona* adults.

2 MATERIALS AND METHODS

Test insects belonged to the following species: *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) larvae (L3-instar) and adults, *Sitona* spp. (Coleoptera: Curculionidae) adults (3 species were identified: *Sitona humeralis* Stephens, *Sitona hispidula* Fabricius, *Sitona sulcifrons* Thunberg). Insects were collected from the fields near Keszthely two days before the experiment and were reared in plastic containers.
Food plants of the test insects were collected few ours before the experiment. Two species of plants were used: potato (*Solanum tuberosum*) for *L. decemlineata* and alfalfa (*Medicago sativa*) for *Sitona* spp.
Antifeedant activity of 4 substances were tested. 3 of the 4 substances were isolated from plant leaves (of 3 species: *Plantago major* L., *Brassica napus* var. *napus* L., *Tilia cordata* Mill.) with the simplest extraction method using water as solvent. Metzger and Grant (1932) found *Plantago major* extract made from the whole plant ineffective against *Popillia japonica* adults. We examined the effect of *P. major* extract made from the leaves on *L. decemlineata* adults. Ediz and Davis (1980) found the extract of *Brassica napus* seeds repellent to *Tribolium castaneum* and *T. confusum* adults. We wanted to know if the aqueous extract *of B. napus* leaves inhibits the feeding of *Sitona* adults or not. The antifeedant effect of *Tilia cordata* extract was tested on both insects. The 4th substance, Schistocerca gregaria trypsin chymotrypsin inhibitor = SGTCI, was originally isolated from the desert locust (Simonet *et al.,* 2001). It is a protease inhibitor, which is produced by transgenic bacteria and according to Patthy *et al.*, 2002 has the ability to inhibit both enzymes (trypsin and chymotrypsin). *Ajuga chamaepitys* L. extract (with methanol as solvent), which is proved to be a very effective feeding deterrent (Nádasy and Gál (1995)) and copper were taken as standard.
The insect antifeedant activity of the 4 substances was investigated by the circular leaf disc dual choice bioassay, which was set on the basis of the method used by Sáringer (1967) and Grieb (1976). It means that 8 leaf discs with equal diameter (leaf disc diameter was 27 mm in case of *L. decemlineata*, and 11 mm in case of *Sitona* spp.) were cut out from living leaves right before the

beginning of the experiment and the discs were placed in a hygrostat with the help of insect pins. A hygrostat is a round-shaped pot (diameter: 15 cm) made of glass in which a wet filter paper is placed to avoid desiccation of the leaf discs. Among the 8 alternately placed discs 4 were treated while the other 4 were untreated. 4 (or 8 in case of the *Sitona* spp.) hungry insects (each from the same species of course and from the same developmental stage) were placed in the hygrostat for 24 hours. The insects could choose between the treated and the untreated leaf discs. After 24 hours the surface of the leaf disc remnants were measured with leaf area analyser.

3 RESULTS AND DISCUSSION

The consumed quantity of the leaf discs can be determined by subtracting the area of the leaf disc remnants from the area of the intact leaf discs (the diameter was given). These values are shown in the tables in the following way: the treated and untreated (control) leaf disc surfaces were added up and the results of the 4 replicates were averaged. Standard deviation is shown in parentheses. Antifeedant activity (AF%) was calculated with the help of the following formula: (1-Treated consumption (cm^2)/Control consumption (cm^2))x100. The results of the bioassay done with *L. decemlineata* adults are shown in Table 1., while Table 2. shows the results of the bioassay with *L. decemlineata* larvae. Table 3. contains the results of the feeding bioassay which tested the food consumption of *Sitona* adults.

Table 1: Antifeedant activity of natural substances against the larvae of *Leptinotarsa decemlineata*

	Mean leaf area consumed (cm^2)[a]		
Treatments	Control	Treated	AF (%)[b]
SGTCI (1,5 mg/ml)	7,4 (3,90)	4,56 (1,55)	**39**
SGTCI (1,0 mg/ml)	10,75 (1,30)	4,41 (1,28)	**59**
Copper (3 mg/ml)	9,34 (2,50)	5,13 (3,52)	**45**

[a] Values are mean of the sum of 4 leaf discs (standard deviation) n = 4
[b] Antifeedant activity: AF=(1-T/C)x100

Among the examined substances the lower dose (1,0 mg/ml) of SGTCI had the highest (59%) antifeedant activity, which means that it inhibited the feeding of *L. decemlineata* larvae more than copper (used as standard control).

Table 2: Antifeedant activity of natural substances against the adults of *Leptinotarsa decemlineata*

	Mean leaf area consumed (cm^2)[a]		
Treatments	Control	Treated	AF (%)[b]
SGTCI (1,5 mg/ml)	4,53 (1,82)	3,60 (2,37)	**21**
SGTCI (1,0 mg/ml)	8,70 (2,17)	7,45 (4,77)	**15**
Copper (3 mg/ml)	8,28 (1,91)	8,35 (1,88)	**0**
Plantago major	8,97 (2,26)	5,9 (2,60)	**35**
Tilia cordata	13,34 (3,52)	6,33 (1,77)	**49**

[a] Values are mean of the sum of 4 leaf discs (standard deviation) n = 4
[b] Antifeedant activity: AF=(1-T/C)x100

The food consumption of *L. decemlineata* adults was not inhibited considerably by any of the tested substances. The ineffectiveness (0%) of the standard control (copper) was surprising. The effect of the *Tilia cordata* extract, which gained the best result (49%), is considered to be weak.

Table 3: Antifeedant activity of natural substances against the adults of *Sitona* spp.

Treatments	Mean leaf area consumed (cm^2)[a] Control	Treated	AF (%)[b]
SGTCI (1,0 mg/ml)	0,53 (0,43)	0,53 (0,14)	**0**
SGTCI (2,0 mg/ml)	0,57 (0,36)	0,38 (0,21)	**33**
Ajuga chamaepitys	1,06 (0,38)	0,00 (0,00)	**100**
Methanol	0,74 (0,29)	0,54 (0,33)	**27**
Brassica napus	1,08 (0,21)	0,16 (0,27)	**86**
Tilia cordata	0,86 (0,47)	0,05 (0,02)	**94**

[a] Values are mean of the sum of 4 leaf discs (standard deviation) n = 4

[b] Antifeedant activity: AF=(1-T/C)x100

SGTCI did not inhibit the food consumption of the *Sitona* adults (the higher dose reached 33% but it is not considerable). *Ajuga* used as standard control has a very powerful deterrent effect (100%). The effect of methanol, which was the solvent of *Ajuga* extract, modified the effect of the plant by 27%. Both plant extracts (*Brassica* and *Tilia*) decreased the food intake of the beetles. Especially, the effect of the *Tilia* extract (94%) is promising.

4 CONCLUSIONS

The results show that SGTCI showed appreciable antifeedant activity only against *L. decemlineata* larvae, while amongst the tested plant extracts Tilia was the most active as antifeedant against *Sitona* adults. However, the best result of SGTCI (59%) cannot be accepted without some reserve, because in that experiment only it was dissolved in an acid-containing solvent whose possible antifeedant effect cannot be eliminated. It should be taken into consideration that the examined enzyme inhibitor (SGTCI) has a different mode of action compared to the plant extracts. The insects have to consume a certain amount of SGTCI which can inhibit the enzymes within the body of the animals. That is why the SGTCI can never reach 100% antifeedant activity but plant extracts can. Another reason why the antifeedant activity of SGTCI was not considerable is that some insect species seem to be able to adapt their enzyme activity by switching to a proteinase not affected by the inhibitor. However the examined extracts may have long term or growth inhibiting effects too, but this method (24-hour leaf disc bioassay) is inappropriate to show possible postingestive effects.

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