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ORIGINAL PAPER

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THE EFFECT OF THE PRODUCE OF PHENOLIC COMPOUNDS IN SOME BRASSICACEAE PLANTS ON *Dolycoris baccarum* AND *Carpocoris fuscispinus* FEEDING AND DEVELOPMENT (PENTATOMIDAE, HEMIPTERA)

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ABSTRACT

Background. The plants from the Brassicaceae family are the basic food product practically all over the world. In the growing season these plants can be attacked by insects. We studied the response of two phytophagous species from the family Pentatomidae to little-known but interesting plants from the family Brassicaceae.

Material and methods. In laboratory conditions, the effect of the total content of phenolic compounds (Folin-Ciocalteau colorimetric method) in a few plants from the family Brassicaceae (*Thlaspi arvense* L., *Descurainia sophia* (L.) Webb ex Prantl, *Berteroa incana* (L.) D.C. and *Diplotaxis tenuifolia* (L.) D.C.) on the feeding and development parameters of two herbivores from the family Pentatomidae (Hemiptera): *Dolycoris baccarum* L. and *Carpocoris fuscispinus* (Boh.) was studied.

Results. From among the above four plant species, *B. incana* contain the highest total amounts of phenolic compounds of 1.87% DM. *D. baccarum* much prefer feeding on *D. sophia* (53.3%) and the other one (*C. fuscispinus*) on *T. arvense*. The eggs need on average 11 days for embryonal phase of development. There is a strong correlation between the total content of phenolic compounds and the total pricks of plant tissues for *D. baccarum* (r = -0.7812) and for *C. fuscispinus* eggs hatching (r = 0.9299).

Conclusion. Among the two Pentatomidae insects, *C. fuscispinus* much prefer feeding and oviposition on all four plant species than *D. baccarum*.

Key words: Brassicaceae, phenolic compounds, shield bug, sloe bug

INTRODUCTION

Plants from the Brassicaceae family are known to contain high amounts of fat and proteins suitable for consumption by humans and animals. They are also a rich source of such nutrients as vitamins, minerals, carbohydrates, amino acids and a number of phytochemicals including indole phytoalexins, glucosinolates and phenolics (Jahangir *et al*., 2009; Cartea *et al*., 2011; Abu-Ghannam and Jaiswal, 2015). Yeshi *et al*. (2017) and Li *et al*. (2018) have found high levels of phenolics and flavonoids in plants of *T. arvense,* or field pennycress, which is a medicinal herb. Similarly, Khan and Wang (2012) wrote that *D. sophia* is a rich source of flavonoids and other phenolic compounds. This plant is an

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annual weed widespread in many countries and is known to contain very high concentrations of glucosinolates and flavonols which – with regular intake – have positive effect on health (Mohamed and Mahrous, 2009). The plant leaves of *D. tenuifolia*, in turn, contain flavonoids (quercetin, kaempferol and its glucosides, isorhamnetin glucosides and hydroxycinnamic acid derivatives). Its seeds and roots contain mainly 4-methylthiobutlyglucosinolate (Bennett *et al*., 2006; Cartea *et al*., 2011; Bell and Wagstaff, 2014; Modnicki, 2014). *B. incana* or hoary alyssum is an exotic annual to short-lived perennial plant, generally considered as a noxious weed (Özbek *et al*., 2015; Parkinson *et al*., 2017).

Plant phenolics have been a subject of intense studies for a long time because of their antiherbivore properties. It is known that the ecological relationships between plants and insects involve both physical and chemical interactions. These complex relationships depend on many factors, including hypersensitive reactions and plant resistance to insect-transmitted diseases. The plant components making them unattractive for feeding by insects are the secondary metabolites, but they must occur in concentrations high enough to exert the repelling effect. At present, it is generally assumed that plant phenolics are important plant protecting compounds. Plant phenolics induce serious disturbances in the functioning of trophic chains in a given environment. They disturb the process of digestion, which is often accompanied by irritation of the walls of herbivores alimentary canal, which in turn significantly deteriorates the absorbability of taken up food (Silva *et al*., 2005; Lattanzio, 2013).

Both species taken to the research, *D. baccarum* and *C. fuscispinus,* are representatives of the subfamily Pentatominae, family Pentatomidae, order Hemiptera. Pentatomidae belong to numerous families from among the heteropterans occurring in Poland; the number of identified species is 46 and they represent 27 genera. They are of relatively large size and have attractive coloration (Lis, 2000; Cieśliczka and Lis, 2015). In our country, *C. fuscispinus* have one generation in a year and feed on fruit and unripe seeds, they suck up the vascular bundles in stems and leaves. They can be found on Compositae plants and on *Allium* spp. seed, they also multiply on *Zea* spp. (Korcz, 1994; Lis, 2000; Durak and Kalender, 2012). From among the Brassicaceae, *Carpocoris* spp. attack: *Isatis tinctoria*, *Raphanus sativus* and *Cardaria draba* (Plant Host Records, 2018). In turn, in Poland *D. baccarum* is a pest of *Vaccinium* spp., *Rubus* spp., *Ribes* spp., some herbs: *Cirsium* spp., *Verbascum* spp. and *Zea* spp. (Korcz, 1994; Lis, 2000; Pytlik *et al*., 2017). From the family Brassicaceae, *Dolycoris* spp. attack *Hesperis* spp., *Brassica oleracea* and *Raphanus sativus* (Plant Host Records, 2018).

We have taken this problem because to the best of our knowledge, there has been no literature report yet on the impact of phenolic compounds in little-known but interesting plants (they have not been tested so far in this respect) from the family Brassicaceae on the two phytophagous species from the family Pentatomidae. We wanted to know how insects react to these less-popular plants. Does their defense system (secondary metabolites) affect insects?

The aim of the study was to characterize the impact of total content of phenolic compounds in a few plant species from the family Brassicaceae on the feeding and development parameters of two herbivores from the family of Pentatomidae (Hemiptera).

MATERIAL AND METHODS

All tests were performed in 2017 in the laboratory of the Department of Biology and Plant Protection, University of Science and Technology in Bydgoszcz, Poland and the Chair of Pharmacognosy at the Nicolaus Copernicus University, Collegium Medicum in Bydgoszcz, Poland. The study was conducted with the use of four plant species representing the family Brassicaceae: *T. arvense*, *D. sophia*, *B. incana* and *D. tenuifolia*.

The plants (which were before flowering) were taken from the collection of the Botanic Garden in Bydgoszcz. They were planted in pots of 15 cm in diameter and 15 cm in height. After one week, which was at about three weeks before the experiment, they were moved to the laboratory for acclimatization. All plants were supplied with mineral fertilizers in the amounts corresponding to the doses: 140 kg $N \cdot ha^{-1}$, 30 kg $P_2O_5 \cdot ha^{-1}$ and 60 kg $K_2O \cdot ha^{-1}$. They were watered once a week, with the amount of about 100 ml per pot. The plants were grown in an air-conditioned room, at 21 ± 1°C, L16:D8 photoperiod, and 70% RH (relative humidity).

The insects were collected and brought from fields (as last stage nymphs). The rearing procedure used in the study was obtained from the method described by Mohammed *et al*. (2017). They were placed on Brassicaceae plants in insulators (55 × 35 × 70 cm). The insulators with plants were kept under controlled conditions, at the laboratory of the Department of Biology and Plant Protection. Two species of Pentatomidae insects were studied: *D. baccarum* and *C. fuscispinus*. The species were identified based on the key for identification by Korcz (1994) and Lis (2000); male and female of Pentatomidae insects – sex differentiation – by Korcz (1994).

After introduction of the two insects, we counted the total number of pricks made by feeding insects actually sucking up the plant tissues (total pricks of plant tissues). Starting from the morning, the observations were made 5 times a day, every 2–3 hours for three subsequent days of feeding (Lamparski, 2016). The maximum number of the actively feeding pests was calculated as the product of the days of observation (3 days), the number of insects (2) and the number of observations per day (5), which gave 30. Results of the observations were presented as the ratio of the number of insects actually feeding to their maximum number, expressed in percentage. In order to evaluate the influence of Brassicaceae plants on egg laying (oviposition), one pair of insects from the family Pentatomidae was introduced into Petri dish for 3 days. The number of eggs laid was counted and the result was assumed as the number of eggs per a couple of insects. The duration of embryonal development (eggs duration) in days and the number of larvae in the stage L_1 (eggs hatching) in %. All insect tests were performed in 5 replicates.

The total phenolic content (TPC) in extracts of aerial parts of four Brassicaceae species plants was determined using the Folin-Ciocalteau colorimetric method, described in 10th Polish Pharmacopoeia (2014). All measurements were performed at least in triplicate and expressed as means ± standard deviation (S.D.). Finally, the amount of total phenolics was expressed as g of pyrogallol equivalents (PGE) per g of dry weight of plant material.

Statistical significance was estimated through Tukey's test for the data obtained from three independent samples. The correlations between the total phenolic content in the Brassicaceae plants (expressed as pyrogallol equivalents) and the feeding and development of Pentatomidae insects on Brassicaceae plants were checked using the analysis of linear correlation.

RESULTS AND DISCUSSION

To the best of our knowledge, there has been no literature report yet on the impact of the total content of phenolic compounds in the selected plants from the family Brassicaceae (*T. arvense*, *D. sophia*, *B. incana* and *D. tenuifolia*) on the parameters of feeding and development of the two phytophagous species from the family Pentatomidae (*C. fuscispinus* and *D. baccarum*).

At the first stage, the total content of phenolic compounds in the plants studied was evaluated (Fig. 1). Plants produced on average 1.71% DM of the total content of phenolic compounds. The highest TPC was found in *B. incana* (1.87%). The smallest content of these compounds was determined for three other plants. Generally, all studied extracts were found to be quite rich in phenols. Over the last two decades, the bioavailability and biological activity of phenolic compounds and flavonoids in plants have been of increasing interest. Phenolic compounds have many functions in plants, in particular they attract insects that would disperse their seeds and pollinate them. Moreover, phenolic compounds are engaged in the natural system protecting plants against insects, fungi, viruses and bacteria and can act as regulators of plant hormones. Also, recently phenolic compounds have been intensely studied from the aspect of their potential health promoting effects (Crozier *et al*., 2009; Jahangir *et al*., 2009; de Pascual-Teresa *et al*., 2010). It should be emphasized that the secondary metabolites occurring in *Brassica* crops are very sensitive to changes in the environmental conditions. The content of phenolic compounds depends on the biotic stress (attack of insects and pathogen infections) and abiotic stress (access to sunlight, temperature, nutrient supplies, access to water, conditions of growth and UV irradiation) as well as the storage conditions, post-harvest treatment and also the methods of their determination (de Pascale *et al*., 2007; Gawlik-Dziki, 2008). According to Young *et al*. (2005), the pak choi plants grown in ecological conditions showed a higher content of total phenols than the plants grown in conventional conditions. However, no differences in the phenolic content levels have been found between the organically and conventionally grown collards. According to these authors, the method of production was not responsible for an increase in the biosynthesis of phenolic compounds, but the organic conditions of growth provided higher opportunity for insect attack and in response to that, the organically grown pak choi synthesized more phenolic compounds.

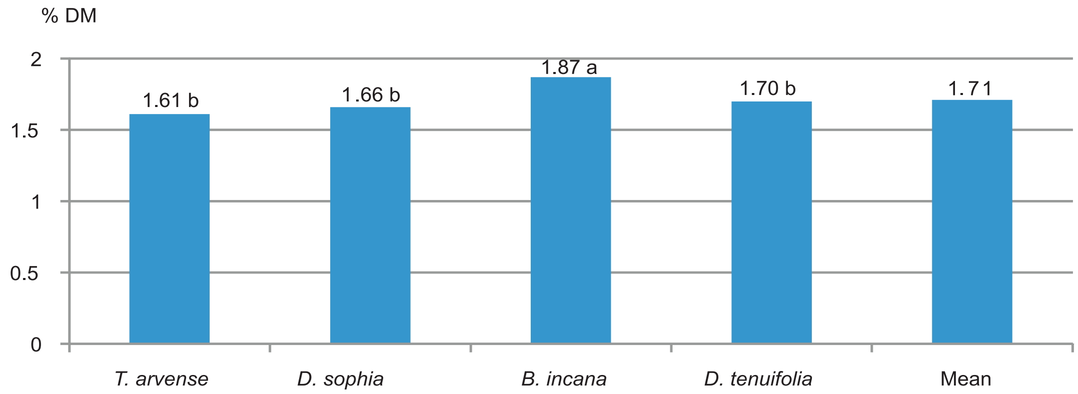

Fig. 1. Total contents of phenolic compounds in the Brassicaceae plants [% DM]

a, b… – values with the same letter are not significantly different at $P < 0.05$ according to Tukey's test

Therefore, the attack of insects can be a biotic stress factor stimulating a higher biosynthesis of total phenolic compounds in some fruits and vegetables grown in ecological conditions (Chassy *et al*., 2006; Singh *et al*., 2009). In organic farming conditions, when plants may be more susceptible to infection, the presence of pests and weeds, increasing the production of these phenolic compounds, seems justified (Dixon and Paiva, 1995; Asami *et al*., 2003; Winter and Davis, 2006).

According to Scutareanu *et al*. (1996), the polyphenolic compounds found in old infested leaves, collected in August, were characterized by the same retention times (so were similar) and the same amounts as in the leaves uninfected by the insects and collected in May. These observations show that the damage caused by *Psylla pyricola* (Heteroptera) stimulates in response the synthesis of polyphenolic compounds mainly in young leaves. As reported by Silva *et al*. (2005), sugarcane attacked by froghopper *Mahanarva fimbriolata* had a higher total content of phenols in the leaves and roots. In response to the attack of *M. fimbriolata* the variety SP80-1816 accumulated higher content of phenolic compounds in roots. However, in the leaves and roots of the control plant SP86-42, higher levels of phenolic compounds were found in the leaves and roots, which can indicate the lack of preferences. The increase in the total content of phenolic compounds in sugarcane infested with root-sucking froghopper nymphs cannot be used to evaluate the resistance to this pest. According to Onyilagha *et al*. (2004) the flavonoids biosynthesized by *B. napus*: 3-sophoroside-7-glucoside and kaempferol 3-diglucoside are effective deterrents of armyworm.

In our study, the correlations between the feeding and development parameters of the two herbivores from the family Pentatomidae and the total content of phenolic compounds biosynthesized by the Brassicaceae plants studied were usually insignificant (Table 2). In contrast to our results, in 16 out of 20 cabbage genotypes a negative correlation was found between the antioxidative potential in the outer leaves and the extent of damage made by cabbage stink bugs sucking on the outer leaves and cabbage heads. As shown by Marković *et al*. (2014), a higher antioxidative potential is related to a lower extent of damage caused by *Eurydema* spp. The negative correlation between the concentration of secondary metabolites and the rate of plant growth is a compromise between the energy used for the plant growth and that used for biosynthesis of defensive compounds. Plants have limited energy resources to support their physiological processes and all their needs cannot be met simultaneously. Thus, if the plant growth is restricted by a physiological or ecological factor, more carbon is directed towards production of secondary metabolites than to growth (Lattanzio, 2013).

The Pentatomidae species in our study readily and frequently sucked up the plant juices (Table 1). The total number of plant tissue pricks was on average of 38%. *C. fuscispinus* was observed to intake plant juices significantly more often than *D. baccarum* (42.3% and 34.7%, respectively). Both species distinctly preferred feeding on *D. sophia* (53.3% and 43.3%, respectively).

Table 1. The feeding and development of Pentatomidae insects (I) on Brassicaceae plants (II)

I	II				
	T. arvense	*D. sophia*	*B. incana*	*D. tenuifolia*	Mean
Total pricks of plant tissues [%]					
D. baccarum	23.3 ± 3.33	53.3 ± 2.36	36.7 ± 2.36	25.3 ± 1.83	34.7 ± 0.75
C. fuscispinus	50.7 ± 3.65	43.3 ± 3.33	37.3 ± 2.79	38.0 ± 2.98	42.3 ± 1.09
Mean	37.0 ± 2.17	48.3 ± 1.67	37.0 ± 2.47	31.7 ± 1.18	38.5 ± 0.63
$HSD_{0.05}$: I = 1.85; II = 3.47; II/I = 4.91; I/II = 3.67					
Oviposition [individuals]					
D. baccarum	6.8 ± 1.30	9.8 ± 1.30	9.6 ± 1.52	10.2 ± 1.10	9.1 ± 1.67
C. fuscispinus	13.8 ± 1.30	12.0 ± 2.12	10.8 ± 0.84	16.2 ± 1.48	13.2 ± 1.87
Mean	10.3 ± 0.76	10.9 ± 1.39	10.2 ± 0.98	13.2 ± 1.04	11.2 ± 1.61
$HSD_{0.05}$: I = 0.90; II = 1.70; II/I = 2.40; I/II = 1.79					
Eggs duration [days]					
D. baccarum	10.2 ± 0.45	11.0 ± 0.71	10.8 ± 1.10	10.4 ± 0.55	10.6 ± 1.30
C. fuscispinus	11.4 ± 0.89	11.8 ± 0.84	12.4 ± 0.55	10.8 ± 0.84	11.6 ± 0.45
Mean	10.8 ± 0.45	11.4 ± 0.42	11.6 ± 0.65	10.6 ± 0.55	11.1 ± 0.79
$HSD_{0.05}$: I = 0.49; II = 0.92; II/I = 1.30; I/II = ns					
Eggs hatching [%]					
D. baccarum	60.3 ± 9.41	63.1 ± 8.23	68.1 ± 9.79	62.4 ± 5.79	63.5 ± 9.40
C. fuscispinus	58.6 ± 9.11	64.1 ± 7.04	61.3 ± 8.17	63.4 ± 6.64	61.9 ± 8.38
Mean	59.5 ± 9.55	63.6 ± 6.40	64.7 ± 7.22	62.9 ± 2.51	62.7 ± 6.19
$HSD_{0.05}$: I = ns; II = ns; II/I = ns; I/II = ns					

Data are presented as mean ± SD, ns – not significant differences

Compared to these results, the bird cherry-oat aphids also readily and frequently sucked up the plant juices from spring barley. The total number of pricks of plant tissues was on average 61.1% and they could be detected only with the use of appropriate preparations (EM and Asahi) (Lamparski, 2016). However, according to Gabryś and Pawluk (1999), the aphid activities have not been suppressed on *T. arvense* and cabbage aphid, *Brevicoryne brassicae* (Homoptera, Aphididae), spent on average 3 h in the phloem during the 8-h experiment. According to Sempruch *et al*. (2009), the average time and number of sap ingestion affects the plant condition and may be related to the different course of infection by plant viruses. Goggin (2007) found that during total activity within plant tissues aphids get into wounding, which leads to the production of secondary plant metabolites, then reduction of feeding by pests (herbivores) which in consequence may limited the insects number on plants.

Another subject of our interest was the development parameters of the two species studied. In our study, we found that in the three-day period of observations, each couple of insects of both species, *D. baccarum* and *C. fuscispinus*, laid on average 11.2 eggs (Table 1). On the four plants studied, *C. fuscispinus* always laid more eggs (13.2) than *D. baccarum* (9.1), so the former laid almost half more eggs than the latter. Both species preferred laying eggs on *D. tenuifolia*. The eggs needed on average 11 days for the embryonal development, however, *C. fuscispinus* needed significantly more time than *D. baccarum*, and the difference was 1 day (Table 1). The significant differences in the duration of embryonal development of the insect eggs were noted on the four plants studied. However, the shortest period of embryonal development, thus the most beneficial for the plant, was noted for *D. tenuifolia* (10.6 days), while the longest for *B. incana* (11.6 days). The range of eggs hatching was from 68.1 for *D. baccarum* on *B. incana* to 58.6 for *C. fuscispinus* on *T. arvense* (Table 1). No statistically significant differences were found between the four plants as well as between the two species. Compared to these results, Mohammed *et al*. (2017) have reported that the lifetime fecundity of sloe bugs ranged from 84.6 to 115.5 eggs per female, while the median fecundity was 102.6 eggs per female. The females laid eggs on average for 33 days (oviposition period), so on average, one female laid 3 eggs per day. According to Matesco *et al*. (2009), each female of *Chinavia longicorialis* laid an average of 5.8 ± 3.86 egg mass and 103.8 ± 91.78 eggs and with a marked peak at 17.7 ± 7.38 eggs per egg mass. Slightly shorter embryonal development periods have been reported by Mohammed *et al*. (2017), who claim that the mean of incubation period (± SE) was 7.5 ± 1.55 (range 6–9) days. Shafi *et al*. (2013) have observed that the incubation period of *D. indicus* ranged from 3 to 6 days, while the development time of *Chinavia impicticornis* eggs (days) was 5.2 ± 1.5 and that of *Chinavia ubica* eggs (days) was 5.7 ± 0.9 (Silva *et al*., 2015). We also evaluated the percentage of egg hatching, which was on average over 62%. Similar results have been reported by Mohammed *et al*. (2017). In their study, the percentage of egg hatching reached on average 68.4 ± 4.10 eggs (range 60.6-80.9). In the study described by Abdulla (2013) on *C. coreanus*, the percentage of hatching ranged from 66.6% to 88.8% with the average of 79.4%.

Table 2. Correlation between total contents of phenolic compounds in the Brassicaceae plants and feeding and development of Pentatomidae insects

Specification	Total pricks of plant tissues	Oviposition	Eggs duration	Eggs hatching
D. baccarum	-0.7812***	-0.4443	0.6347**	0.0988
C. fuscispinus	0.1275	0.4829	0.3766	0.9299***
Mean	-0.2184	-0.1516	0.6164**	0.6800**

* $r_{crit.}^{0.1} = 0.497$, ** $r_{crit.}^{0.05} = 0.576$, *** $r_{crit.}^{0.01} = 0.708$

CONCLUSIONS

Among the investigated species, *D. sophia* has a quite high total phenolic content. *D. baccarum* much prefer feeding on this plant. Among the two Pentatomidae insects, *C. fuscispinus* much prefer feeding and oviposition on all four plant species than *D. baccarum*. Strong correlation between the total content of phenolic compounds and the total pricks of plant tissues for *D. baccarum,* for eggs duration for *D. baccarum* and for eggs hatching for *C. fuscispinus* was observed.

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WPŁYW OGÓLNEJ ZAWARTOŚCI ZWIĄZKÓW FENOLOWYCH W WYBRANYCH ROŚLINACH KAPUSTOWATYCH NA ŻEROWANIE I ROZWÓJ DOLYCORIS BACCARUM I CARPOCORIS FUSCISPINUS (PENTATOMIDAE, HEMIPTERA)Streszczenie

Rośliny kapustowate są podstawowym produktem żywnościowym praktycznie na całym świecie. Podczas wzrostu w sezonie wegetacyjnym mogą być atakowane przez owady. W warunkach laboratoryjnych badano wpływ zawartości sumy związków fenolowych (metoda kolorymetryczna Folin-Ciocalteau) w wybranych roślinach z rodziny Brassicaceae (*T. arvense*, *D. sophia*, *B. incana* i *D. tenuifolia*) na żerowanie i rozwój dwóch owadów z rodziny Pentatomidae (Hemiptera): *D. baccarum* i *C. fuscispinus*. Stwierdzono, że największą ilość polifenoli zawierał ekstrakt z części nadziemnych *B. incana* (1,87% SM). *D. baccarum* zdecydowanie preferowały żerowanie na *D. sophia* (53,3%), zaś *C. fuscispinus* na *T. arvense*. Jaja owadów potrzebowały średnio ok. 11 dni na przejście okresu rozwoju embrionalnego. Stwierdzono silną ujemną korelację pomiędzy zawartością sumy związków fenolowych a sumą nakłuć tkanek roślinnych przez *D. baccarum* (r = -0,7812) oraz z wylęgiem larw L_1 dla *C. fuscispinus* (r = 0,9299). Wykazano, że spośród dwóch pluskwiaków z rodziny tarczówkowatych, *C. fuscispinus* chętniej żeruje i składa jaja na badanych gatunkach roślin niż *D. baccarum*.

Słowa kluczowe: *Carpocoris fuscispinus*, *Dolycoris baccarum*, kapustowate, suma polifenoli