Bioactivity evaluation of prenylated isoflavones derived from *Derris scandens* Benth against two stored pest larvae

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ABSTRACT

The development of environment friendly bio-pesticides is now an area of intense research in the stored commodities. In the present research, we studied the feeding deterrent and contact toxicant properties of prenylated isoflavones derived from *Derris scandens* Benth against test larvae of red flour beetle, *Tribolium castaneum* Herbst and rice moth, *Corcyra cephalonica* S. Among all the compounds, Osajin (2), Lupalbigenin (4), Scandinone (5), Sphaerobioside (8) and Genistein (9) produced 100% contact toxicity to *T. castaneum* after 10th day and C. *cephalonica* after 15th day of treatment in food treatment assays. In the flour disc bioassay, compounds 2, 4, 5, 8 and 9 produced feeding deterrent activity against both the test larvae at the higher concentration tested. In the same assay, the relative growth rate (RGR), relative consumption rate (RCR) and food utilization (ECI) of test insects was significantly reduced with above test compounds even at lower concentrations. Compounds 2, 4, 5, 8 and 9 showed higher toxicity against both the larvae than other test compounds at 10 µg /larva after 14th day of treatment in topical application method. Isolation of prenylated isoflavones from *D. scandens* may be important as a source of this material for stored pest control on wheat and jowar commodities.

Keywords: *Corcyra cephalonica, Derris scandens,* feeding deterrent, insecticidal activity, *Tribolium castaneum*

INTRODUCTION

The post-harvest damage caused by insect pests in stored grain may amount to 10-40% in developing countries, where modern storage technologies have not been introduced (Raja *et al*., 2001). Over 60 species of insects infest on the stored grains. Care must be taken to protect the stored products against deterioration, especially loss of quality and weight during storage. *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is one of the most destructive insect pests of stored grains (Athanassiou *et al.*, 2008, Usha Rani *et al.,* 2011). These insects cause severe loss in storage due to its high reproductive potential and their breeding capacity throughout the year (Prakash *et al.,* 1987). *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) has been reported to infest many types of stored products from different parts of the world (Hodges, 1979) and their larvae besides voraciously feeding the grain ,also forms webbing by forming the silken threads noticeably dense and tough whereever they move, adding to the damage caused (Ayyar, 1934; Carmona, 1958).

Conventional methods mainly focus on the repeated applications of synthetic insecticides in stored grain pest management (Hasan and Reichmuth, 2004). The continuous use of synthetic insecticides and insect growth regulators for the eradication of insects has already led to the development of pest strains resistant to many pesticides (Mohan and Fields, 2002). During previous centuries, botanicals were used in traditional grain storage in tropical countries for effective insect control in stored grains. Efficiency and optimal usage of these extracts are still needed to be assessed in order to make them proper means of insect control. They are cheaper compared to their synthetic counterparts, simpler to use and are reported to constitute a rich source of bioactive chemicals (Arnason *et al.*, 1989). Berenbaum *et al.* (1989) stated that the plant based insecticides provide novel modes of action that can eliminate the risk of resistance of pests.

Derris scandens Benth (Fabaceae), known by its common name Gonj (Hindi), is widely distributed throughout India (Kirtikar and Basu, 1987). Many varieties of the Derris species all over the world have been reported for their potential use in control of the various pests. Alcoholic extracts of the stem were reported to have both antimicrobial (Dhawan *et al*., 1977) and immunostimulating activities (Chuthaputti and Chavalittumrong, 1998). Chavalittumrong *et al.* (1999) reported that *D. scandens* root is an excellent insecticide being harmful to the chewing and sucking insects, but not to human beings. There are a few reports regarding, isolation of insecticidal constituents, rotenone and lonchocarpic acids (Seshadri,

1959) from the roots of *D. scandens*, which showed high insect feeding deterrent activity against the polyphagus lepidopteran pest, *Spodoptera litura* F. The insecticidal and feeding deterrent activities of the isolated derivative compounds from *D. scandens* against castor semilooper, *Achaea janata* L were examined (Sreelatha *et al*., 2010). The fumigation toxicity of these chemicals against the adult beetles of the four major stored product pests was reported by Hymavathi *et al*. (2011). However, the contact susceptibility of the larval population of the adult *T. castaneum* and *C. cephalonica* to the isolated prenylated isoflavone derivatives from *D. scandens* has not been tested. The present work is therefore designed to evaluate the contact toxicity, nutritional and feeding deterrent properties of *D. scandens* derived prenylated isoflavones (1-9) on the larvae of red flour beetle, *T. castaneum* and rice moth, *C. cephalonica.*

MATERIALS AND METHODS

Plant material

Derris scandens plants were collected from Tirumala forest, Tirupathi, Andhra Pradesh, India in August 2012. It was authenticated by K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati, India. A voucher specimen was deposited in the herbarium of the Botany department, Sri Venkateswara University, Tirupati.

Extraction and isolation of the compounds

The roots of Derris *scandens* (5 kg) were shade dried, powdered, and extracted with chloroform in a soxhlet apparatus for 72 hrs. The resulting chloroform extract (21 g) was subjected to column chromatography on a silica gel column (60-120 mesh) to give prenylated isoflavone derivatives, 1-11 among which compounds identified by nuclear magnetic resonance (NMR), gas chromatography mass spectrum (GCMS) and high performance liquid chromatography (HPLC) are scandenone (1) (Mizuno *et al.*, 1990), osajin (2) (Mizuno *et al.*, 1990), laxifolin (3) (Barron and Ibrahim,1996), lupalbigenin (4) (Rao *et al*., 2007), scandinone (5) (Rao *et al*., 2007), scandenin A (6) (Rao *et al.*, 2007), scandenin (7) (Rao *et al*.,2007) , sphaerobioside (8) (Markham and Mabry,1968) and genistein (9) (Guanga *et al*.,1998).

Insect collection and rearing

The test insect species, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) were obtained from laboratory cultures, which were fed and maintained on the whole wheat flour (*Triticum aestivum* L.) for *T. castaneum* and milled jowar (*Sorghum bicolor* L. Moench) for *C. cephalonica* in the laboratory of CSIR-Indian Institute of Chemical Technology (CSIR-IICT), Hyderabad, India and the rearing conditions were maintained at 25 ± 2°C, 60 ± 5% relative humidity and 16:8h (L:D) photoperiod.

Contact toxicity of test compounds

Insecticidal effects of test compounds, isolated from the root extract of *D. scandens* were evaluated against 12-15 day old larvae of *T. castaneum* and *C. cephalonica* in flour treatment method by contact. In this bioassay, test diet used for *T. castaneum* is wheat flour and milled jowar for *C. cephalonica*. Test diets were treated with aliquots of 1.0 ml of various concentrations of test compounds at 100, 150, 200, 300, 400 µg/g diets (for *T. castaneum*) and 100, 200, 400, 500, 600 µg/g diets (for *C. cephalonica*) in acetone, control diet was treated with the same volume of the solvent only. The solvent was allowed to evaporate in fume hood after treatment, at 30 ± 1 ºC and 70 ± 5% RH for 3 hrs, and the diet was placed in a glass container (4.5 cm diameter x 5 cm height). A group of 10 larvae of *T. castaneum* and *C. cephalonica* were released into each container having their respective diet. These were placed in incubators set at 25 ± 2ºC and 60 ± 5% RH. Mortality was determined after every five days of treatment. Each set of experiments consisted of 30 replicates and all the experiments were performed in similar conditions and were repeated 3 times.

Topical application bioassay

To remove the possibility of insects dying by feeding on different test compounds in flour treatment method, another set of experiments was conducted to determine the contact toxicity of the test compounds against 15 day old, *T. castaneum* and *C. cephalonica* larvae by topical application method. The experiments were conducted according to the method described earlier by Jamil *et al.* (1984) with some modifications by Huang *et al.* (2000). Test concentrations of compounds extracted from root were applied to the dorsal thoracic region of each larva as doses of 1, 2 4, 6, 8 or 10 µg/larva with a Hamilton micro-syringe (1 µL volume/larva). Controls were treated with solvent alone. After treatment, insects were placed into plastic containers (4 cm diameter x 2 cm height) containing their respective food and held at 25 ± 2°C, 60 ± 5% relative humidity and 16:8 h (L:D) photoperiod. Each set of experiments consisted of 30 replicates and all the experiments were performed in similar conditions and were repeated 3 times. Mortality of insects was recorded daily for 21 days. Percentage mortality was calculated using the corrected formula of Abbott's for natural mortality in untreated controls (Abbotts, 1925).

Nutritional and feeding deterrent activity of test compounds
A flour disc bioassay was used to detect the nutritional and feeding deterrent effects of isolated compounds from *D.*

scandens against *T. castaneum* and *C. cephalonica* according to the method described by Huang *et al.* (2000). Flour discs (80 ± 7 mg/disc) were prepared using 200 µL of a stored suspension of wheat flour (*T. castaneum*) and jowar flour (*C. cephalonica*) in water (50g in 100 mL) separately. Test solutions were prepared using acetone as solvent; each compound was applied at 50 and 75 µg/disc for *T. castaneum* and 75 and 100 µg/disc for *C. cephalonica*. Controls received solvent alone. After the solvent was allowed to evaporate for 24 hrs in a fume hood, two flour discs of the same treatment were weighed and placed in a plastic container (4 cm diameter x 2 cm height) per treatment. Then 10 pre-weighed larvae of *T. castaneum* and *C. cephalonica* were released into each container with treated discs. A total of 30 replicates were set up for each test compound and control. After 7 days, the flour discs and live insects were weighed again. Nutritional indices were calculated as previously described by Huang *et al.* (2000) as follows:

Relative growth rate (RGR) = (A–B)/B x day^{-1}

Relative consumption rate (RCR) = D/B x day^{-1}

Efficiency of conversion of ingested food (ECI) (%) = (RGR)/(RCR) x100

where, A- Weight of live insects on the seventh day (mg)

B- Represents the original weight of insects (mg)

D- Biomass ingested (mg)

The feeding deterrence index (FDI) (%) = [(C–T)/C] x 100

where, C - consumption of control discs and T-consumption of treated discs.

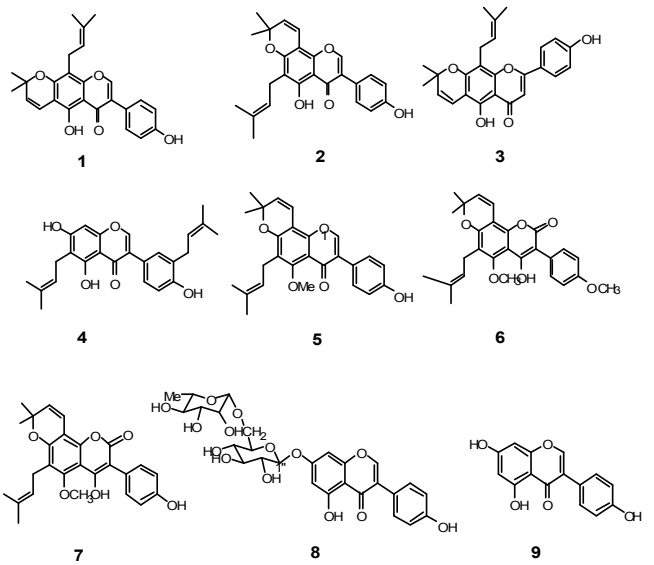
Statistical analysis

The toxicity data of different concentrations of each compound was calculated using the corrected formula of Abbott's (1925) for natural mortality in untreated controls and the percentage mortality data subjected to probit analysis (Finney, 1971.) using AnalystSoft, Biostat analysis program (Biostat, 2008) to determine LC_{50} representing the concentrations that caused 50% mortality with 95% fiducial limits. All experimental data were subjected to a one-way ANOVA to determine differences between samples, using the statistical software Sigmastat v 3.5. Means were separated using the Tukey's HSD test at the 5% level.

RESULTS AND DISCUSSION

Contact toxicity evaluated by the flour application method revealed that compounds derived from *D. scandens* (1-9) (Fig. 1) produced significant mortality among the treated larvae. However, the effect was not immediate because the mortality occurred only several days after the application. Test

Figure 1. Compounds isolated from *Derris scandens* Benth.



compounds 2, 4, 5, 8 and 9 produced mortality (> 40%) after 5th day and the toxic action increased thereafter with time. Mortality of 100% among the treated *T. castaneum* larvae occurred only after 10th day with contact application at 400 µg/g diet (Table 1). *Corcyra cephalonica* larvae showed 100% mortality with the treatment of compounds after 15th day at 600 µg/g (Table 2). At the end-point of the experiment (15 days) toxic action of compounds 2, 4, 5, 8 and 9 were showing greater toxicity than the other test compounds against both the larvae tested. Compounds 2, 4, 5, 8 and 9 had contact toxicity, adequate to kill 50% of *T. castaneum* at a concentration between 211.5 to 249.4 µg/g diets. The same compounds required higher concentrations to produce 50% mortality (LC_{50} values from 334.9 to 444.0 µg/g of diet) against *C. cephalonica.*

From our studies, it is concluded that, *T. castaneum* was more susceptible to the *D. scandens* derivatives than *C. cephalonica* at lower concentrations in flour treatment method. Prates *et al*. (1998) reported that the monocyclic monoterpenes 1, 8-cineole and R-(+)-limonene have been considered economically important contact toxicants against *T. castaneum*. In this study, we noticed for the first time that these isolated prenylated isoflavones (1-9) had contact toxicity against stored pests. Earlier studies of these compounds demonstrated antifeedant activity towards two major agricultural pests, *S. litura* and *A. janata* (Sreelatha *et al.*, 2010). In an earlier report (Singhal *et al.*, 1980), the insecticidal properties of seed extracts of *Millettia pachycarpa* Bentham (Fabaceae) against insect pests having prenylated isoflavones content (Pemonge *et al.*, 1997).

Table 1. Peroral insecticidal activity of isolated compounds from *D. scandens* against *T. castaneum* larvae.

Compounds	Toxicity (%)[a] (400 µg/g diet)			LC_{50}(95% CL[b]) (µg/g diet)	χ^2 (Df)	P-level
	5DAT	10DAT	14DAT			
1	15.3 ± 2.2a	27.6 ± 2.7c	48.2 ± 1.3	> 400	0.82(4)	0.843
2	40.4 ± 3.0d	100 ± 0.0a	-	229.6 (220.5-238.7)	5.00(4)	0.171
3	7.2 ± 1.3	25.8 ± 5.3c	31.0 ± 0.9	> 400	1.44(4)	0.694
4	51.8 ± 2.7c	100 ± 0.0a	-	211.5 (200.9- 220.0)	5.91(4)	0.115
5	45.2 ± 2.0	100 ± 0.0a	-	219.7 (209.1- 226.1)	10.1(4)	0.017
6	13.3 ± 2.6a	38.2 ± 3.6b	45.6 ± 1.6	> 400	0.60(4)	0.896
7	11.2 ± 1.8a	33.6 ± 3.0b	51.5 ± 2.5	> 400	0.51(4)	0.902
8	39.4 ± 3.5d	100 ± 0.0a	-	249.4 (239.9- 259.0)	5.93(4)	0.113
9	50.2 ± 3.3c	100 ± 0.0a	-	224.0 (213.0- 235.0)	8.03(4)	0.045
Control	0 ± 0.0	0 ± 0.0	0.0 ± 0.0			

[a] Values are mean ± SD, [b] Confidence Level after 10 days of treatment, because maximum activity was observed.
[b] Each column followed by different letters are significantly different from another (One-Way ANOVA; Tukey test at, < 0.05; n= 150). DAT- days after treatment

Table 2. Peroral insecticidal activity of isolated compounds from *D. scandens* against *C. cephalonica* larvae.

Compounds	Toxicity (%) [a] (600 µg/g diet)			LC_{50} (95% CL[b]) (µg/g diet)	χ^2 (Df)	P-level
	5DAT	7DAT	14DAT			
1	0 ± 0.0b	20.8 ± 1.8d	39.4 ± 2.6	> 600	4.19(4)	0.214
2	29.3 ± 1.6c	95.2 ± 3.0a	100 ± 0.0	416.1 (397.4- 449.8)	3.36(4)	0.338
3	0 ± 0.0b	22.0 ± 2.1d	46.3 ± 2.9	> 600	5.02(4)	0.169
4	33.0 ± 1.4	82.1 ± 3.6b	100 ± 0.0c	334.9 (312.5- 357.4)	7.46(4)	0.050
5	26.6 ± 2.1	80.0 ± 4.0b	100 ± 0.0c	372.0 (349.5- 391.0)	4.31(4)	0.229
6	3.3 ± 0.9	29.0 ± 3.3c	31.0 ± 2.2	> 600	4.07(4)	0.253
7	0 ± 0.0b	27.6 ± 5.1c	52.2 ± 3.1	> 600	6.50(4)	0.089
8	13.7 ± 1.3	75.4 ± 5.4c	100 ± 0.0c	444.0 (419.7- 468.3)	10.4(4)	0.015
9	30.4 ± 1.7c	80.8 ± 1.7b	100 ± 0.0c	368.0 (346.6- 389.8)	12.6(4)	0.005
Control	0 ± 0.0b	0.0 ± 0.0	0.0 ± 0.0			

[a] Values are mean ± SD, [b] Confidence Level after 10 days of treatment, because maximum activity was observed.

[b] Each column followed by different letters are significantly different from another (One-Way ANOVA; Tukey test at, < 0.05; n= 150). DAT- days after treatment.

Table 3. Contact insectidicidal activity of isolated compounds from *D. scandens* against larval *T. castaneum* and *C. cephalonica*.

Compounds	*Tribolium castaneum*			*Corcyra cephalonica*		
	Toxicity (%) [a] (10 µg/larva)	LC_{50} (95% CL [b]) (µg/larva)	χ^2 (P-level)	Toxicity (%) (10 µg/larva)	LC_{50} (95% CL [b]) (µg/larva)	χ^2 (P-level)
1	34.4 ± 1.5 e	>10	62.2 (0.000)	21.6 ± 1.8h	>10	0.23(0.964)
2	91.2 ± 2.8 b	5.2 (4.7- 6.3)	37.8 (0.000)	61.0 ± 4.7d	8.0 (7.3- 8.6)	21.8 (0.000)
3	48.0 ± 3.1 a	>10	17.6 (0.000)	39.8 ± 2.3g	>10	11.3 (0.010)
4	94.0 ± 3.9 b	4.5 (3.9- 5.1)	18.4 (0.000)	80.8 ± 4.6a	5.7 (5.2- 6.3)	13.4 (0.003)
5	87.0 ± 4.1 cb	5.4 (4.8- 5.8)	27.7 (0.000)	74.2 ± 3.4b	7.0 (6.5- 7.4)	16.5 (0.000)
6	52.6 ± 2.8 a	9.4 (8.6- 10.0)	8.16 (0.042)	48.8 ± 3.8f	>10	4.11 (0.248)
7	51.6 ± 2.0 a	>10	17.8 (0.000)	50.2 ± 5.2e	>10	10.0 (0.018)
8	89.2 ± 2.7 cb	6.5 (6.2- 7.0)	28.3 (0.000)	61.0 ± 2.8d	8.9 (8.1- 9.2)	21.0 (0.000)
9	84.6 ± 3.3 c	6.9 (6.6- 7.7)	4.91 (0.178)	68.6 ± 6.1c	8.0 (7.7- 8.6)	3.46 (0.328)
Control	0.0 ± 0.0			0.0 ± 0.0		

[a] Values are mean ± SD, test concentrations 1, 2, 4, 6, 8 and 10 µg/larva
[b] Confidence Level, after 14 days of treatment. df= 5.
[b] Each column followed by different letters are significantly different from another (One-Way ANOVA; Tukey test at, < 0.05; n= 150).

In the studies conducted to confirm whether the mode of toxicity is confined to contact effect, the compounds were topically applied to the larvae of both the test insects. The percentage mortalities of *C. cephalonica* and *T. castaneum* after 14 days of treatment are given in Table 3. Compounds 2, 4, 5, 8, and 9 produced significant toxicity (> 80%) in *T. castaneum* larvae at a concentration of 10 µg/ larva after 14 days of treatment (Table 3). However, only the compounds 4 and 5 produced significant mortality (>70%) in *C. cephalonica*. The other derivatives failed to cause toxicity in the insects tested. Application of compounds 2, 4, 5, 8 and 9 produced significant ($P < 0.05$) mortality to *T. castaneum* larvae with the LC_{50} being 4.5-6.9 µg/larva after 14 days of treatment. The LC_{50} value of the above compounds was 5.7-8.9 µg/larva to *C. cephalonica* after 14 days of treatment. Similar results were reported against *T. castaneum* with topical application of *Trigonelle foenum-graecum* L., eudesmane and eremophilane sesquiterpenes extracts (Pemonge *et al*., 1997; Garcia *et al.,* 2003).

The nutritional and feeding deterrence indices of isolated compounds from *D. scandens* against two stored product pests are shown in Table 4 and 5. Compounds 2, 4, 5, 8, and 9 reduced the growth rate (RGR), food consumption (RCR) and food utilization (ECI) of *T. castaneum* and *C. cephalonica* larvae at all test concentrations in flour disc bioassay. The food utilization of larvae was significantly reduced with compounds 2, 4, 5, 8 and 9 at even at lower concentrations (30 µg/disc to *T. castaneum* and 60 µg/disc to *C. cephalonica*) in comparison with other test compounds after 7 days of treatment (Table 4 and 5). The above compounds exhibited a high rate of feeding deterrence activity (FDI% = > 75%) at a concentration of 75 µg/disc with the *T. castaneum* larvae, after 7 days of treatment (Table 4). However, higher concentrations (100 µg/disc) of compounds 2 and 4 were required into get similar activity against *C. cephalonica* (Table 5).

CONCLUSION

Our observations revealed that the control of *T. castaneum* and *C. cephalonica* was achieved with prenylated isoflavone compounds 2, 4, 5, 8, and 9 derived from the root extract of *D. scandens*. Our findings suggested that the above said compounds can be utilized as insecticidal and feeding deterrent agents against pests on stored grain. Future area of focus would be to investigate the cost effective formulations with improved efficacy, insecticidal potency and stability of these compounds against the stored product pests.

Table 4. Feeding deterrence and nutritional indices of isolated compounds from *D. scandens* against *T. castaneum* larvae in a flour disc bioassay.

Compounds (µg/disc)	FDI (%)[a]	Nutritional Index (50 µg)		ECI (%)[a]	FDI (%)[a]	Nutritional Index (75 µg)		ECI (%)[a]
		RGR (mg/mg/d)[a]	RCR (mg/mg/d)[a]			RGR (mg/mg/d)[a]	RCR (mg/mg/d)[a]	
1	38.77	0.011	0.012	88.88	42.57d	-0.004	0.009	-43.75
2	69.38	-0.004	0.006	71.11	78.21a	-0.021	0.004	-481.81
3	25.38	0.010	0.015	66.66	31.68e	-0.005	0.014	-39.13
4	74.48	-0.008	0.005	-149.33	82.17a	-0.024	0.003	-666.66
5	56.12	-0.005	0.009	-57.36	78.21a	-0.022	0.004	-504.54
6	11.22	0.007	0.018	38.31	19.80g	-0.005	0.016	-33.33
7	22.44	0.009	0.016	55.26	28.71f	-0.003	0.014	-22.22
8	59.18	-0.007	0.008	-86.01	64.35c	-0.019	0.007	-263.88
9	65.30	-0.003	0.007	-50.98	76.23b	-0.022	0.004	-458.33
Control	-	-	-	-	0.00	0.018	0.020	87.12

[a] Each datum represents mean of 15 replicates, each setup with 10 adults (n=150).
Means in a column followed by different letters are significantly different from another (One-Way ANOVA; Tukey test at, < 0.05). FDI- feeding deterrent index; RGR- relative growth rate; RCR- relative consumption rate; ECI- efficiency of conversion of ingested food.

Table 5. Feeding deterrence and nutritional indices of isolated compounds from *D. scandens* against *C. cephalonica* larvae in a flour disc bioassay.

(µg/disc)	Compounds FDI (%)[a]	Nutritional Index (75 µg)			FDI (%)[a]	Nutritional Index (100 µ		
		RGR (mg/mg/d)[a]	RCR (mg/mg/d)[a]	ECI (%)[a]		RGR (mg/mg/d)[a]	RCR (mg/mg/d)[a]	ECI (%)[a]
1	12.41	0.010	0.053	0.053	21.53d	0.001	0.039	4.32
2	59.44cb	-0.003	0.024	0.024	75.23a	-0.011	0.012	-94.68
3	17.31	0.007	0.050	0.050	20.81d	0.000	0.040	1.20
4	62.01cb	-0.006	0.023	0.023	75.83a	-0.010	0.012	-86.13
5	51.46c	-0.005	0.029	0.029	64.11b	-0.010	0.018	-56.73
6	8.84a	0.004	0.055	0.055	11.48e	0.001	0.044	3.08
7	6.47a	0.007	0.057	0.057	23.44d	0.002	0.038	7.18
8	52.31c	-0.003	0.029	0.029	56.33c	-0.011	0.022	-52.60
9	55.06b	-0.005	0.027	0.027	63.42b	-0.012	0.018	-69.80
Control			0.00	0.018 0.061	29.79			

[a] Each datum represents mean of 15 replicates, each setup with 10 adults (n=150).

Means in a column with followed by different letters are significantly different from another (One-Way ANOVA; Tukey test at, <0.05).

FDI- feeding deterrent index; RGR- relative growth rate; RCR- relative consumption rate; ECI- efficiency of conversion of ingested food.

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