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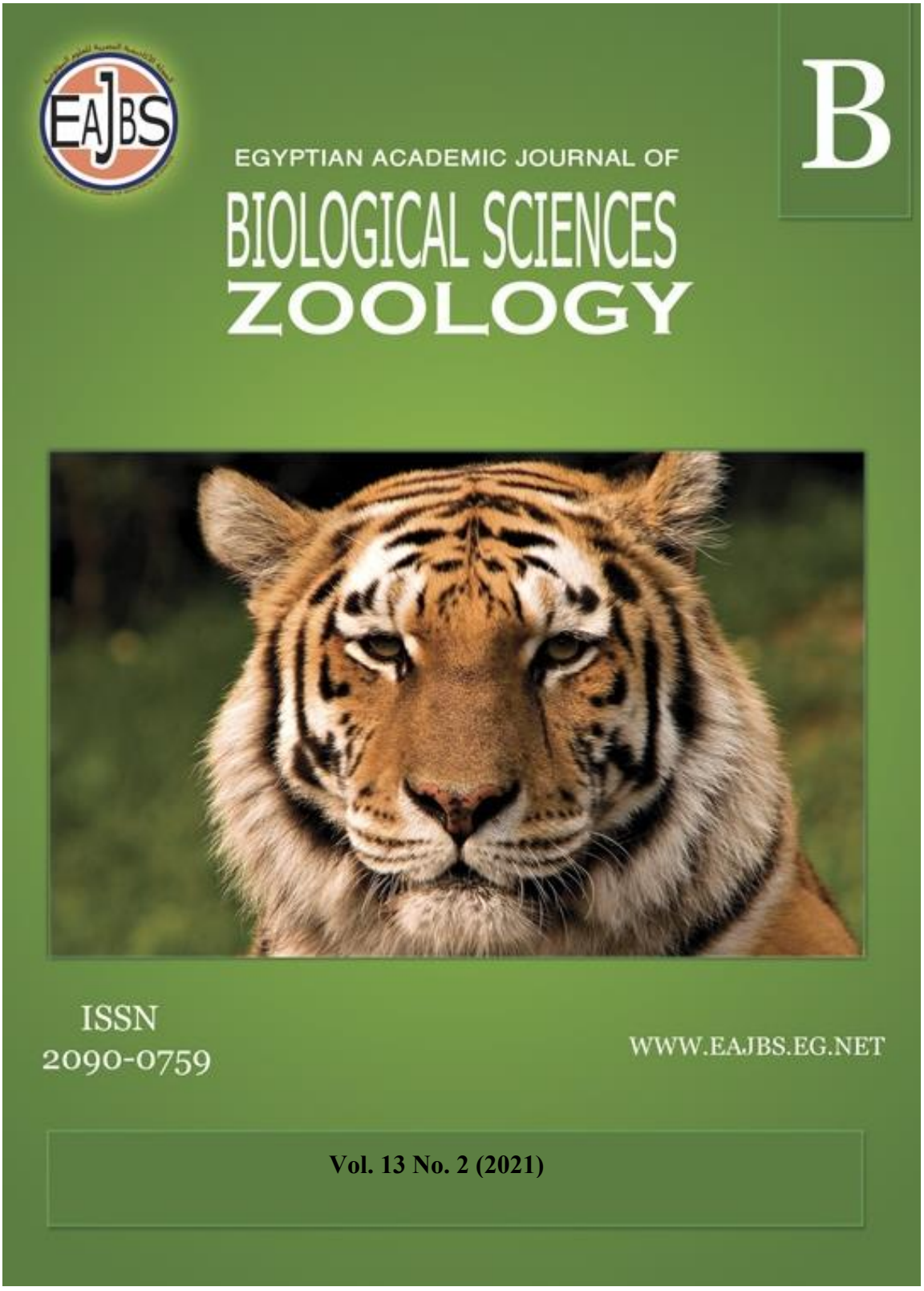

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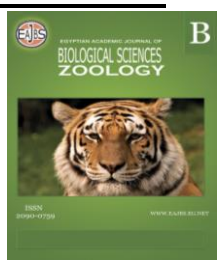
Citation: *Egypt. Acad. J. Biolog. Sci. (B. Zoology) Vol. 13(2) pp: 173-183(2021)*
DOI: 10.21608/EAJBSZ.2021.203498

Egypt. Acad. J. Biolog. Sci., 13(2): 173-183 (2021)

Egyptian Academic Journal of Biological Sciences
B. Zoology
ISSN: 2090 – 0759
http://eajbsz.journals.ekb.eg/



Effects of Dietary Coffee on Feeding Parameters and Growth of Terrestrial Isopod, *Porcellio laevis*

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ARTICLE INFO

Article History
Received:2/9/2021
Accepted:26/10/2021

Keywords:
Caffeine, Pharmaceutical residues, terrestrial isopods, ecotoxicology

ABSTRACT

Caffeine is considered the most widely consumed pharmacologically active drug. It is probably found in popular beverages (coffee, tea, soft drinks), as well as cocoa or chocolates-based goods. However, it has received a lot of attention there is still much to be learned with respect to its toxicology in animals. Terrestrial isopods are considered one of the most investigated invertebrate groups in soil ecotoxicology. The aim of this study was to evaluate the effect of different concentrations of coffee on feeding parameters and the growth of terrestrial isopod *Porcellio laevis*. Feeding parameters and growth efficiency were calculated and compared among treated isopod groups. The results revealed decreasing in food parameters such as consumption ratio, assimilation ratio, and egestion ratio in *P. laevis* treated with different concentrations of coffee. Also, decreasing growth efficiency for this isopod was noticed after exposure to different concentrations of coffee. These results indicate that high levels of caffeine may have toxic effects on *P. laevis* after a long period of exposure. Further research about the toxic effect of caffeine on isopods is needed.

INTRODUCTION

Terrestrial isopods play an important role in the decomposition of leaf litter and so are making a significant contribution to nutrient cycling and soil ecosystem services. They are considered one of the most investigated invertebrate groups in soil ecotoxicology, both in laboratory toxicity tests and in-field monitoring and bioindication studies.

Recently, Pharmaceutical and personal care products (PPCPs) have gained great attention due to their continuous discharge in natural waters (Archana *et al*., 2017; Dafouz *et al*., 2018 and Korekar *et al*., 2020) and have been widely detected in the environment worldwide (Archana *et al*., 2017; Li *et al*., 2017; Dafouz *et al*., 2018). PPCPs can spread systemically and can ultimately pose toxic effects in higher plants (Sun *et al*., 2018). Furthermore, Melvin *et al*., (2016) reported that PPCPs (fluoxetine, diazinon, and triclosan) can alter diurnal activity patterns of mosquitofish (*Gambusia*

holbrooki) and scale up to influence performance including foraging success and predator avoidance. PPCPs are released into the environment through sewage treatment and their persistence in the environment has impacted flora, animals, and human being worldwide.

Caffeine is one of the most representative pharmaceutical residues (PPCPs) pollutant abundance in the environment (Li *et al.*, 2020). It is a purine alkaloid, with the formula of C8 H10N4 O2 and chemically known as 1,3,7-trimethylxanthine (Bruton *et al*., 2010; Martínez-Hernández *et al*., 2016). The main sources of caffeine in the environment are generally accepted as the excretory residues of consumed caffeine, the treatment of unconsumed caffeinated drinks by rinsing coffee cups or by disposing of them down the sink, inappropriate deposition of expired or unwanted caffeine that contains pharmaceutical products, manufacturing plant wastes, hospital wastes, and so on (Hillebrand *et al*., 2012). Coffee and tea are the principal natural dietary sources of caffeine accounting for up to 90% of total caffeine consumption. As well as, the presence of caffeine in both coffee, tea, and energy drinks, it is also naturally present in cocoa beans and thus in chocolate, also it is naturally found in the fruits, seeds, and leaves of many plants (Azam *et al*., 2003). In some geographies, coffee which is extracted from roasted beans of the coffee plant contains the highest levels of caffeine per unit weight among natural sources. 250 ml (8 ounces) One cup of either black or green coffee provides roughly 100 mg of caffeine, depending on the variety and brewing method (Temple *et al*., 2017). The caffeine content in extracts of the green coffee bean was in the range between 34.1 – 81.6 g kg^{-1} dry mass (Jeszka-Skowron *et al*., 2016).

Caffeine is being consumed by more than 80% of the World and up to 89% of the United States population (Heckman, *et al*., 2010, El Gohar *et al*., 2013, Verster and Koenig, 2018). Whilst, millions of humans enjoy drinking coffee every day (Santo and Lima 2009), it has severe effects on some animals, including insects and spiders (Araque *et al.*, 2007 and Foelix 2010). Other studies, reported that exposing eggs, larvae, or adults of dengue mosquitoes to either caffeine or coffee might affect reproductive traits, longevity (Laranja *et al*., 2003), oviposition behavior (Satho *et al*., 2015) and the life span of the vector (Dieng *et al*., 2017). Coffee alkaloids, notable caffeine, are lethal to many insect species at high doses (Nathanson 1984; Wink 1992). The present study aimed to evaluate the effect of coffee on feeding parameters and the growth of terrestrial isopod *Porcellio laevis*.

MATERIALS AND METHODS

Test Organisms:

Adult specimens of the isopod *P. laevis* were collected in November 2020 under the litter layer at an uncontaminated location in Assiut city, Egypt. The animals were kept under lab conditions for at least three months before use in the tests. For the experimental tests, healthy adult males and non-gravid females were used.

Experimental Setup:

For the experiment food was prepared to contain dry mango leaves, rabbit food, and different concentrations of green coffee obtained from the local market (Assiut city, Egypt). The present study has used green coffee because it may contain slightly more caffeine than black coffee (Bauer et al., 2018). Mango leaves were collected from an uncontaminated area on the farm of Assiut university and stored at room temperature. At the beginning of the experiment, four types of food were prepared which contain different amounts of coffee. First control food contains a mixture of mango leaves and

rabbit food without coffee. Then the rest prepared foods contain a mixture of mango leaves and rabbit food with 25%, 50% and 75% coffee of the total food weight.

Adult isopods, *P. laevis* were starved for 48 hours before the experiment to allow evacuation of the contents of the gut. Isopods were checked for pregnancy at the beginning of the test although there is no possibility to know if females will develop or not a future pregnancy. All isopods were within a weight range (98 ± 0.015) mg, so they were assumed to belong to the same age stage.

During the experiment, 40 animals of isopods were divided into four groups. Isopods were kept individually isolated during the duration of the experiment. The first group fed on mango leaves and rabbit food only (control). The second group fed on 25 % coffee food. The third group fed 50% coffee food. The fourth group fed on 75% coffee food.The experiment lasted for 4 weeks with daily checks. Every week, the animals and the new food were weighted. The remaining food and the fecal pellets were separated and weighted after drying. Feces were collected, weighted, and put in plastic bags weekly to avoid coprophagy.

Feeding Parameters Calculations:

Isopod consumption ratio, assimilation ratio, egestion ratios and assimilation efficiency and growth efficiency were calculated weekly according as following equations:

$$CR = (W_{f.wt} - W_{r.f.wt}) / W_{isop} * day$$

$$A_R = ((W_{f.wt} - W_{r.f.wt}) - Fp.wt)) / W_{isop} * day$$

$$AE = ((W_{f.wt} - W_{r.f.wt}) - Fp.wt)) / (W_{f.wt} - W_{r.f.wt}) * 100$$

$$ER = Fp.wt / W_{isop} * day$$

$$GE = G/(W_f wt - Wr_f wt)$$

Where, W.wt= food weight , Wr.f.wt= remaining food weight , Wisop = animal weight, Fp.wt = fecal pellets weight, G is the increase in dry weight of the isopods, CR = Consumption rate , AR= assimilation rate, AE= assimilation efficiency and ER= egestion rate, GE= growth efficiency.

Data Analysis:

Analysis of Variance on SPSS software package (version 20) (SYSTAT statistical program) was used to test the present data. In the case of significant differences, the Multiple Range Comparisons (Duncan test) were selected from the PostHoc window on the same statistical package to detect the distinct variances between means.

Probability values ≤ 0.05 were defined as significant throughout the present study; however, the values > 0.05 were defined as non-significant. Probability values between 0.05 and 0.01 (both are included) were evaluated as significant, whereas that less than 0.01 were defined as highly significant.

RESULTS

During the 4 weeks of exposure to different coffee concentrations, no mortality was observed in all treatments.

Effects of Coffee on Feeding Inhibition in Different Treatments:

The experimental procedure for the determination of the means and standard errors of the feeding parameters for treated groups *P. laevis* was shown in table (1). The highest mean value of consumption ratio was noticed in control animals and significantly decreased and recorded less value with 75% coffee group (Fig. 1a). The mean value of consumption ratio in the control was (0.244 ± 0.014 mg food/mg isopod), while it ranged

between (0.244 ± 0.014 mg food/mg isopod, (0.186 ± 0.014 mg food/mg isopod and (0.151 ± 0.015 mg food/mg isopod) for 25%, 50% and 75% coffee, respectively.

The highest mean value of assimilation ratio was noticed after treatment with 25% coffee. This value decreased significantly in isopods exposed to dietary 50% and 75% coffee (Fig.1b). The mean value of Assimilation ratio in control animals was (0.154 ± 0.010 mg food/mg isopod), while it ranged between (0.172 ± 0.010 mg food/mg isopod), (0.150 ± 0.010 mg food/mg isopod) and (0.119 ± 0.011 mg food/mg isopod) for the 25%, 50% and 75% coffee exposures, respectively.

Assimilation efficiency showed an increase after feeding with different concentrations of coffee more than the control one (Fig 1c). The highest mean value was noticed after exposure to 50% coffee. The mean value of assimilation efficiency in control animals was (61.195 ± 3.140 %), while it ranged between (71.167 ± 3.140 %), (79.168 ± 3.140 %) and (75.779 ± 3.310 %) for the 25%, 50% and 75% coffee, respectively.

Egestion ratio was significantly decreased for isopods exposed to high concentrations of coffee (Fig. 1d). In control animals, egestion ratio was (0.091 ± 0.008 mg faeces/mg isopod) then decreased respectively after feeding with 25 % coffee (0.063 ± 0.008 mg faeces/mg isopod), 50% coffee (0.036 ± 0.008 mg faeces/mg isopod) and 75 % coffee (0.032 ± 0.009 mg faeces/mg isopod).

Growth efficiency showed the highest mean value in the control animals and significantly decreased after exposure to different concentrations of coffee (Fig. 1e). The mean value of growth efficiency in control was (1.737 ± 0.609 %), while it ranged between (-0.895 ± 0.609 %), (- 0.291 ± 0.609 %), and (-1.227 ± 0.642 %) for 25%, 50 % and 75% coffee exposure.

Table1: Mean values ± standard errors (SE) of the feeding parameters for *P. laevis* treated with different concentrations of coffee.

Treatment		Consumption ratio (mg leaf/mg isopod. Day)			Assimilation ratio (mg leaf/mg isopod. Day)			Assimilation efficiency (%)			Egestion ratio (mg faeces/mg isopod. Day)			Growth efficiency (%)		
Control	1st week	.400	±	.026	.288	±	.021	71.523	±	2.777	.112	±	.009	3.160	±	.926
	2nd week	.259	±	.028	.170	±	.020	70.340	±	6.313	.089	±	.018	.625	±	.821
	3rd week	.199	±	.028	.073	±	.019	38.574	±	6.180	.126	±	.018	4.302	±	1.439
	4th week	.120	±	.019	.084	±	.016	64.343	±	5.409	.036	±	.007	-1.140	±	1.104
	Total	.244	±	.014	.154	±	.010	61.195	±	3.140	.091	±	.008	1.737	±	.609
25% coffe	1st week	.337	±	.026	.282	±	.021	83.949	±	2.777	.055	±	.009	-1.874	±	.926
	2nd week	.232	±	.028	.152	±	.020	64.215	±	6.313	.079	±	.018	.940	±	.821
	3rd week	.192	±	.028	.122	±	.019	61.504	±	6.180	.069	±	.018	1.185	±	1.439
	4th week	.180	±	.019	.132	±	.016	74.998	±	5.409	.048	±	.007	-3.832	±	1.104
	Total	.235	±	.014	.172	±	.010	71.167	±	3.140	.063	±	.008	-.895	±	.609
50% Coffe	1st week	.187	±	.026	.146	±	.021	77.318	±	2.777	.041	±	.009	-.182	±	.926
	2nd week	.203	±	.028	.180	±	.020	88.345	±	6.313	.023	±	.018	1.500	±	.821
	3rd week	.179	±	.028	.140	±	.019	74.847	±	6.180	.040	±	.018	.047	±	1.439
	4th week	.175	±	.019	.134	±	.016	76.163	±	5.409	.041	±	.007	-2.530	±	1.104
	Total	.186	±	.014	.150	±	.010	79.168	±	3.140	.036	±	.008	-.291	±	.609
75% Coffe	1st week	.185	±	.027	.156	±	.022	85.069	±	2.928	.029	±	.010	-.698	±	.976
	2nd week	.178	±	.030	.150	±	.021	84.010	±	6.654	.028	±	.019	-.915	±	.865
	3rd week	.148	±	.029	.103	±	.020	65.614	±	6.514	.045	±	.019	-.104	±	1.517
	4th week	.093	±	.020	.068	±	.017	68.424	±	5.702	.025	±	.008	-3.191	±	1.164
	Total	.151	±	.015	.119	±	.011	75.779	±	3.310	.032	±	.009	-1.227	±	.642

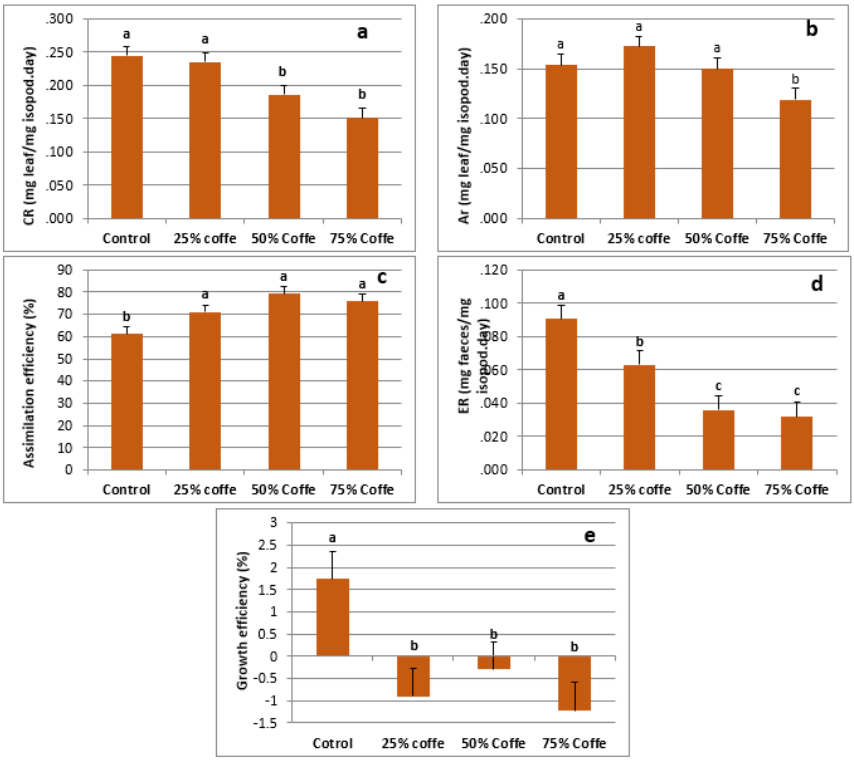

Fig. 1: Feeding parameters of *P. laevis* exposed to different concentrations of coffee during the one month of the study. Data is expressed as mean ± standard error. Mean values of different subscript letters (a,b) were significantly different ($p<0.001$).

Multivariate analysis of variance test (MANOVA) recorded highly significant differences between all parameters during the weeks of the experiment (Two-way MANOVA, $P<0.001$) **table 2.** Whilst, the interaction between treatments and weekly results for all animal groups showed significant differences for all parameters (Two-way MANOVA, $P<0.05$), except in growth efficiency showed no significant differences (Two-way MANOVA, $F=1.246$, $P=0.285$).

Table 2: Two-way multivariate analysis of variance (MANOVA) for feeding parameters of *P. laevis* treated with different concentrations of coffee.

Parameters	Source	Sum of squares	Df	Mean square	F	P value
Consumption ratio	Week	0.389	3	0.13	22.05	< 0.001
	Week * treatment	0.227	9	0.025	4.292	< 0.001
	Error (week)	0.618	105	0.006		
Assimilation ratio	Week	0.33	3	0.11	32.573	< 0.001
	Week * treatment	0.185	9	0.021	6.104	< 0.001
	Error (week)	0.354	105	0.003		
Assimilation efficiency	Week	8553.512	2.239	3825.296	11.317	< 0.001
	Week * treatment	5734.237	6.708	854.821	2.529	0.023
	Error (week)	26452.69	78.261	338.004		
Egestion ratio	Week	0.021	1.972	0.011	4.395	0.016
	Week * treatment	0.035	5.915	0.006	2.4	0.037
	Error (week)	0.171	69.006	0.002		
Growth efficiency	Week	357.029	3	119.01	10.703	< 0.001
	Week * treatment	124.73	9	13.859	1.246	0.275
	Error (week)	1167.57	105	11.12		

Effects of Coffee on The Feeding Parameters in Different Weeks During A Month of Study:

In the present study consumption ratio recorded a high level in the first week and decreased regularly in the second, third, and fourth week, this model was observed in control and treated groups with 25% and 75% coffee. Whilst, its ratio showed the highest level in the second week and the lowest was in the fourth week for the animal group exposed to 50 % caffeine (Fig. 2a).

The assimilation ratio parameter showed irregular curves for different animal groups during the weeks of the experiment. The highest level of assimilation ratio in the control group was recorded in the first week and the lowest was recorded in the third week. While it showed the highest level in the first week and the lowest level in the fourth week in treated groups with 25% and 75 % caffeine. A different result was noticed for isopods exposed to 50% caffeine, where the highest level of assimilation ratio was noticed in the second week and the lowest was in the fourth week (Fig. 2b). Assimilation efficiency recorded the same curve for all animal groups, it was very high in the first week and low in the third week (Fig. 2c).

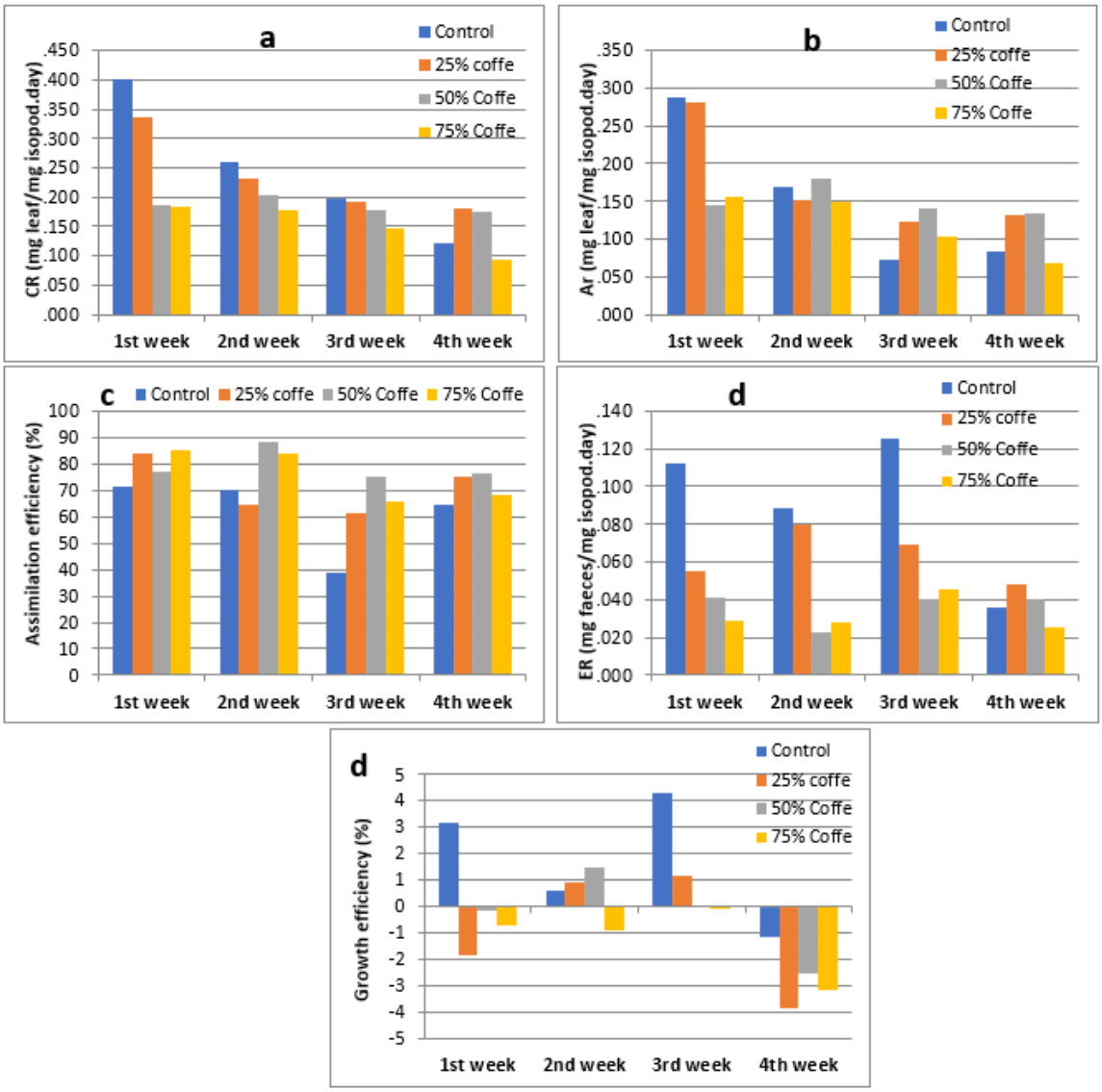

Fig. 2: the mean of feeding parameters of *P. laevis* exposed to different concentrations of coffee in each week during the one month of the study.

Egestion ratio recorded the highest value in the third week and the lowest level in the fourth week for both the control and treated group fed with 75% caffeine. However, it showed a high level in the second group and a low level in the fourth group treated group with 25 % caffeine. Egestion ratio for the treated group with 50% caffeine recorded high levels in the first and fourth week and low levels in the second week (Fig. 2d).

Growth efficiency showed a very high level in the 3rd week and was very low in the 4th week in both groups of control, 25% caffeine, and 75% caffeine. Moreover, it showed a high level in the second week and a low level in the fourth week for the treated group with 50 % caffeine (Fig. 2e).

DISCUSSION

Naturally, all animals favor the intake of rich energy food and avoid the consumption of toxic compounds. Therefore, the sense of taste allows animals, to evaluate and predict the quality of a food source, which may be potentially nutritious or harmful. Although millions of people enjoy drinking coffee every day, it is harmful to some animals (Santo and Lima 2009).

In the present study, coffee treatment induced a decrease in the consumption ratio of food for isopod *P. laevis*. This effect may be due to the toxic effect of the caffeine on the study animal, and this food may be less palatable. This avoidance behavior could be simply explained as low palatability of chemicals, or as physiological constraints reflected in the metabolism of isopod. Many alkaloids such as caffeine had an impact on modifying the insect's metabolism and reducing its survival (Ignacio *et al.,* 2020). Araque *et al.* (2007) reported that a high concentration of caffeine in food causes bees to consume less.

In this study, the clear results of coffee on food consumption of *P. laevis* would be explained as an effect of the modulation of its nervous system. Previous studies such as, (Coelho *et al.,* 2015; Bahrami *et al.,* 2018; Delgado *et al.,* 2019) submitted that ingestion of psychoactive substances, such as caffeine, led to functional and structural changes throughout the body, mainly through interference and suppressing in neurotransmitters. Meyer and Quenzer, (2005) mentioned that caffeine binds to metabotropic receptors for adenosine, furthermore blocking the gamma-amino-butyric acid neurotransmitter (GABAA). Terry *et al.,* (1995) discussed that neurotransmitters are closely linked to the central nervous system, and the binding of caffeine to its active sites can trigger various behavioral actions, as well as act on mood and food intake, which may have influenced the consumption of animals.

The reduction in the level of assimilation ratio after treatment of *P. laevis* to a high concentration of coffee may be a result of the toxic effect of the alkaloid which forced the animal to consume little food. While increasing the level of assimilation efficiency for *P. laevis* was noticed after exposure to different concentrations of coffee. This enhancement might be explained by a slower passage of food through the isopods' guts, which consequently decreased fecal production. As longer the food stays in the gut this means the high assimilation of food and might be related to food characteristics. Loureiro *et al.,* (2006) and Mohammad *et al.,* (2021) showed that terrestrial isopods displayed high assimilation efficiencies in animals fed with high contaminated leaves more than animals fed on uncontaminated leaves.

The egestion ratio, or feces production, was the most sensitive ecologically relevant parameter because fecal production occurs as a direct consequence of litter fragmentation, included in the primary step in the leaf decomposition process. Since it is

well known that feces acquire more fungi and bacteria than decay leaves, thereby accelerating decomposition. In the present study egestion ratio diminished in animals treated with high concentrations of caffeine. Since isopods consumed less food in high concentrations of coffee, their egestion ratio was obviously decreased. The reduction of egestion may be as a result of the movement of the gut which could be affected by the toxicity of coffee. The same result was recorded by (Mohammad *et al.,* 2021) recorded the inhibition of egestion ratio for *P. laevis* exposed to food contaminated with cd.

The decrease in Growth efficiency for *P. laevis* exposed to different levels of coffee may be as a result of a decrease in the consumption ratio of food. The energy saved in this case could have been responsible for the increased feed efficiency and the use of body fat reserves for energy could result in a net saving of food. Carvalho *et al.* (2012) recorded lower mean levels for weight gain, specific growth, and protein efficiency when animals were fed with 150 g of the cocoa meal (fruit, which presents caffeine in its composition). Dullo *et al.,* (1989) concluded that the demonstration of animals with high doses of caffeine has a reducing effect on body fat and body weight. Chatifotis, *et al.* (2008) showed that caffeine adversely affected sea-bream growth at a concentration higher than 1 g /kg diet, judging by the reduced specific growth rate of fish. In addition, Veirira *et al.*, (2018) concluded that caffeine levels, up to and including 1 g/3 kg of diet, positively affected weight, total length, standard length, height, weight gain, feed intake, and specific growth rate for Nile Tilapia. Laranja *et al.,* (2003) and Guirado & Bicudo (2016) concluded that caffeine has been shown to have toxic effects on *Aedes aegypti* larvae, interfering with development and preventing them from reaching adulthood.

Another explanation of the decrease in growth efficiency in *P. laevis* may be a direct effect of the binding of caffeine to neurotransmitters in the central nervous system, which may have influenced on body growth of animals. The binding of caffeine to neurotransmitters in the central nervous system of *P. laevis* would certainly affect the growth hormones of animals, which need further configurations. Saldanha, (2012) confirmed that caffeine increases the synthesis of catecholamines, the neurotransmitters that stimulate lipolysis. Mello *et al.* (2007) reported that caffeine triggers an increase in the mobilization of free fatty acids in the tissue with less oxidation of carbohydrates and greater oxidation of muscle fat. Cunningham, (1968) suggested that high levels of caffeine were also found to increase the total CO_2, exhaled by pigs, so it is probable that some of the extra energy derived from the mobilization of body fat was squandered rather than directed to the growth and development of new tissue.

In conclusion, decreasing of food parameters such as consumption ratio, assimilation ratio, and egestion ratio were observed in *P. laevis* treated with different levels of coffee. Moreover, decreasing growth efficiency for this isopod was noticed. High levels of caffeine may have toxic effects on the above feeding parameters and growth efficiency after a long period of exposure (4 weeks). Further studies are necessary to establish the effect of caffeine on soil animals and terrestrial isopods.

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