

# Regulatory Mechanisms in **Biosystems**

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## The influence of the synthetic food colourings tartrazine, allura red and indigo carmine on the body weight of *Tenebrio molitor* (Coleoptera, Tenebrionidae) larvae

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Oles Honchar Dnipro National University, Gagarin Ave, 72, Dnipro, 49010, Ukraine. Tel.: + 38-050-939-07-88. E-mail: martinov123456789@gmail.com, brigad@ua.fm Martynov, V. O., & Brygadyrenko, V. V. (2018). The influence of the synthetic food colourings tartrazine, allura red and indigo carmine on the body weight of Tenebrio molitor (Coleoptera, Tenebrionidae) larvae. Regulatory Mechanisms in Biosystems, 9(4), 479–484. doi:10.15421/021871

Substances for protecting plants often contain colourings, the impact of which on invertebrates has been studied insufficiently. The addition of food colourings in different concentrations to the diet of saprophage beetles can affect their metabolism, causing loss of body weight. In the experiment, we determined the impact of tartrazine, allura red and indigo carmine on the body weight of *Tenebrio molitor* Linnaeus, 1758 larvae. The substances were added to their fodder at five concentrations (1, 0.1, 0.01, 0.001 and 0.0001 g/kg of dry fodder) during a 21-day experiment. Statistically significant data on changes in the body weight of *T. molitor* larvae were received after adding 1 g/kg concentration of indigo carmine and 0.1 and 1 g/kg concentrations of tartrazine. In the other variants of the experiment, no statistically significant differences were determined. Tartrazine, allura red and indigo carmine cause decrease in the body weight of *T. molitor* larvae, depending on the concentration of the colouring. The toxic effect of synthetic food colourings on living organisms and the low number of studies devoted to such impact on insects indicate the relevance and necessity for further research in this sphere.

Keywords: food additives; azo compound colourings; saprophages; ecotoxicology; healthy diet; substances for protecting plants

#### Introduction

Food additives are widely used in developed countries for improving different properties of food products: taste, colour, aroma, texture, duration, fitness for consumption, etc. (Himri et al., 2011). Synthetic food colourings have an important place among the main food additives. They are mainly used for improving the natural colour of food products in order to attract customers. These substances are used to mark combined substances for protecting plants, which is facilitated by their low cost and absence of formal obstacles for using these substances in the environment. Due to their high stability, uniformity of colour, low microbiological contamination and the relatively low costs for their production, synthetic colourings are used widely compared to natural colourings (Wang et al., 2014). At the same time, in the course of prolonged use, many synthetic colourings cause digestive disorders, allergic reactions, damage to the brain, liver, kidneys, and cause pathological abnormalities among children (Mannell et al., 1962; Ashida et al., 2000; Kroes & Kozianowski, 2002; Moutinho et al., 2007).

Food colourings and other additives enter the environment due to various factors and become food for saprophage invertebrates. These substances used in human food, occur in municipal sewage water treatment plants, not used – on solid waste landfills, and therefore become included in food of aboveground and aquatic saprophages (Kroes & Kozianowski, 2002).

One of the most commonly used synthetic substances is tartrazine, an orange azo coloring also known as  $E_{102}$ , C.I. 19140, FD&C Yellow 5, Acid Yellow 23, Food Yellow 4. It is used all around the world as a food additive for colouring food products, medical and cosmetic preparations. It is also used for preparing food in many developed countries as a saffron substitute. The permissible daily intake (ADI) for humans is 7.5 mg/kg of body weight. Tartrazine is often a cause of allergic reactions among humans (Stenius & Lemola, 1976; Neuman et al., 1978; Devlin & David, 1992). Some studies mention a carcinogenic and mutagenic effect (Patterson & Butler, 1982; Maekawa et al., 1987; Borzelleca & Hallagan, 1988a, 1988b; Collins et al., 1992; Reyes et al., 1996).

Allura red is a red azo colouring which has several names, including:  $E_{129}$ , Food Red 17, C.I. 16035, FD&C Red 40. It is broadly used for colouring non-alcoholic beverages, sweets, ice cream, sweet baked products and cosmetic substances. The permissible daily intake (ADI) for humans is 7 mg/kg of body weight. It has been reported that toxic properties of the colouring have been manifested among rats due to decomposition of intestinal microflora into mutagenic aroma amines (Shrestha et al., 2006). Due to the potential risks, allura red is not recommended for consumption by children in a number of countries of European Union: it is currently forbidden in Denmark, Belgium, France, Germany, Switzerland, Sweden, Austria, and Norway (Abramsson-Zatterberg & Ilbäck, 2013).

Indigo carmine is a synthetic colouring known also as  $E_{132}$ . It is used for preparing ink, and is used as a food colouring for non-alcoholic beverages, sweet baked products and medicines. The permissible daily intake (AID) for humans is 5 mg/kg of body weight. It has been noted that Indigo carmine is poorly absorbed and concerns have arisen regarding its genotoxicity. Research on chronic toxicity has found no side effects when daily doses do not exceed 500 mg/kg of body weight (EFSA, 2014).

Darkling beetles are usually saprophages or phytophages (Brygadyrenko & Nazimov, 2015; Brygadyrenko, 2016) which consume a variety of types of food, including those which contain food colourings. Most species of this family are adapted to living in conditions of insufficient moisture, and feed on dry fodder with a minimum moisture content. The widespread distribution of many species of Tenebrionidae, their slow larvae development and ability to accumulate toxicity from food in their body, makes them a suitable object for ecotoxicological studies. The possibility of toxic impact of synthetic food colourings on living organisms indicates the necessity for detailed study of this issue. The objective of this study was to determine the influence of food colourings on the body weight of larvae of *Tenebrio molitor* Linnaeus, 1758 (Coleoptera, Tenebrionidae).

#### Materials and methods

In the experiment, we used third age *T. molitor* larvae. Before the experiment, the larvae were maintained in the same general container and had the same diet composed of dry and moist food (vegetables). For two weeks before the experiment, the larvae consumed only dry food (rolled oats). The experiment was conducted in plastic cups of 0.2 L capacity, which had 40 g of dry rolled oats in each. The fodder and larvae were weighed to 0.1 mg accuracy using analytic scales. The fodder was uniformly moistened from a pipette by solutions of the studied synthetic colourings. In the experiment, we used five concentrations of solutions. In the results, the concentration of the active substance in the food substrate was 1, 0.1, 0.01, 0.001, 0.0001 g/kg of dry fodder. In the control group, we added distilled water in the same volume. Then the fodder was dried to eliminate the excess moisture and prevent the growth of fungi and mixed again for eliminating aggregates of rolled oats which had formed after addition of water.

We aimed at comparing the effect of different concentrations of food colourings and determining the changeability of reaction of *T. molitor* larvae. In our preliminary 14 days study, we observed a tendency towards increase in the body weight during consumption of food which contains 0.35 g/kg of allura red, and a tendency towards decrease in

weight during consumption of tartrazine and indigo carmine. In the 21-day experiment described in this article, we used 5 concentrations of the substances.

In each variant of the experiment, we exposed 10 cups with one individual of *T. molitor* in each. A total of 160 larvae were used in all variants of the experiment. The cups were placed randomly on the tables in the laboratory with the same temperature and light, out of the reach of direct sunlight. The 24 hour temperature fluctuations were not higher than 2 °C (24–26 °C), the duration of a solar day between July 11 and August 1 was 1500–1550 h and was prolonged to 17 hours a day by artificial light. Before and after the 21 day experiment, all *T. molitor* individuals were weighed. The average initial body weight of the larvae was 69.6 ± 12.5 mg (x ± SD, n = 128), 21 days after the beginning of the experiment, the animals' body weight had increased to 102.7 ± 28.1 mg. Also, after the end of the experiment, we calculated the number of moultings of each larva and the number of individuals which had transformed into pupae or imagines.

The results were statistically analyzed in Statistica 8.0 (StatSoft Inc., USA) program pack. The differences between the selections were considered statistically reliable at P < 0.05 (Tukey's test).

#### Results

During the experiment, no deaths were observed among the larvae. The number of moults and individuals which reached another stage of development for different concentrations of the studied substances is presented in the Table. As the concentration of colourings increased, the larvae manifested a tendency towards increase in the number of moults (some individuals were observed to undergo two moults during the experiment).

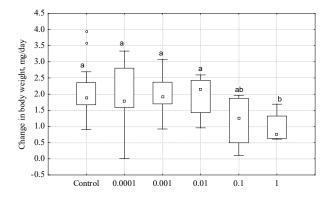
#### Table

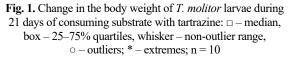
The number of moults and individuals which reached another phase of development during the experiment (n = 10)

Group	Control	Tartrazine					Allura red					Indigo carmine				
Concentration	0	0.0001	0.001	0.01	0.1	1	0.0001	0.001	0.01	0.1	1	0.0001	0.001	0.01	0.1	1
Moult	7	2	3	5	7(2*)	$5(1^{*})$	3	4	6	$6(1^{*})$	7	$5(1^{*})$	6	6	11(4*)	$10(3^{*})$
Pupa	_	_	2	1	2	2	1	-	_	_	_	1	-	-	2	1
Imago	_	_	1	1	1	_	1	-	_	-	_	1	2	_	1	_
Colouration of the intestine	-	-	1	_	-	1	_	_	-	2	5	_	_	-	4	4

Note: \* - the number of individuals which moulted twice.

Changes in the body weight of T. molitor larvae in the 21-day laboratory experiment with addition of synthetic food colourings to the fodder substrate are presented in Figures 1–3.





The most clearly manifested differences from the control in change in body weight were observed among larvae which consumed substrate with tartrazine (decreased 1.9 to 0.5 mg/day compared to the control, P <0.01) and indigo carmine (1.9 to 0.7 mg/day, P < 0.01) in a concentration of 1 g/kg of dry fodder. A statistically reliable decrease in the body weight was observed after adding tartrazine in a concentration of 0.1 g/kg (1.9 to 0.7 mg/day, P < 0.05) and indigo carmine (1.9 to 1.1 mg/day, P < 0.05). No significant differences were observed for the other variants of the experiment.

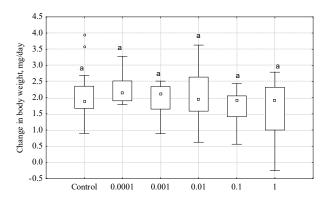
#### Discussion

The obtained results prove that high concentrations of the studied synthetic colourings in the diet of *T. molitor* larvae cause decrease in their weight, depending on dose. This demonstrated the inhibiting effect of high concentrations of colourings on saprophage insects. Perhaps, consuming food which contains synthetic additives leads to disorders in the animals' metabolism and has a negative effect on the bacterial microflora of their intestine, causing disorders in the function of gastrointestinal tract. The obtained results partly coincide with our previous study (Martynov & Brygadyrenko 2017), and confirm that tartrazine and indigo carmine lead to decrease in the body weight of *T. molitor* larvae.

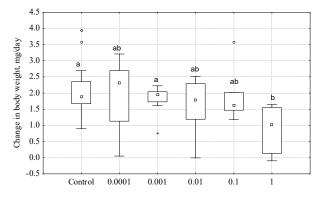
There are very few studies devoted to the impact of synthetic food colourings on the changeability of body weight and other characteristics of insects. Most publications cover the toxic effect of food additives on rats, mice and some other vertebrates.

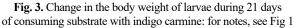
Himri et al. (2011) conducted a subchronical study on toxicity of tartrazine for Wistar rats. The animals were divided into groups which received the colouring in the amount of 5.0, 7.5 and 10.0 mg/kg of body weight. The consumption of tartrazine did not influence the death rate, consumption of food and body weight of rats compared to the control group. A statistically significant decrease was observed in the mass of the right kidney and increase in the mass of liver due to consumption of the colouring in a concentration of 10 mg/kg of body weight. Morphological changes were found in the form of erythrocytes, increase in the number of thrombocytes among rats which consumed 7.5 mg/kg of

tartrazine. Increases in the levels of glucose and creatinine were observed in all groups, and increases in cholesterol, triacyglyceride, aspartate aminotransferase and total protein were observed in the animals which consumed the colouring in the amount of 7.5 and 10.0 mg/kg, which coincides with studies by Mekkawy et al. (1998) and Aboel-Zahab et al. (1997). Histopathological studies found lymphoid infiltrations in the jejunum, fatty degeneration of the liver, dilatation of glomerular capillaries, intercapillary sclerosis and atrophy of glomeruli.



**Fig. 2.** Change in the body weight of *T. molitor* larvae during 21 days of consuming substrate with allure red: for notes, see Fig. 1





El-Wahab & Moram (2012) studied the toxic effect of some synthetic colourings, including tartrazine, on Spargue Dawley rats. The animals consumed the colouring in the concentration of 75 mg/kg of fodder during 42 days. A significant decrease in body weight was found when there was a significant increase in the consumption of fodder compared to the control group. Tartrazine induced change in the function of kidneys, which was manifested in the increase in the level of creatinine and urine in the blood serum. This could indicate acute kidney dysfunction, which coincides with the results obtained by Shousha et al. (1992). There was observed a significant decrease in the level of glutathione, glutathione-S-transferase and superoxide dismutase in the blood and liver, and also increase in the level of alanine transaminase, aspartate aminotransferase, activity of alkaline phosphatase, bilirubin and total protein compared to the control. The study by Amin et al. (2010) showed an increase in the total protein in the blood serum during consumption of low and high doses of tartrazine (15 and 500 mg/kg of body weight respectively). In his study on the toxicity of tarzanine in the amount of 5% of the rats' diet, Ershoff (1977) observed delayed growth, randomized loss of hair and death rate of 50% and over during the experiment which lasted 14 days.

Borzelleca & Hallagan (1988b) studied the chronic toxicity and carcinogenicity of tartrazine for CD Charles rats and Charles River mice. The colouring was added in the amount of 0.0, 0.01, 0.1, 1.0, 2.0 and 5.0% of fodder mass. Even at maximum concentrations, no toxic effects were observed. Davis et al. (1964) studied chronic toxicity of tartrazine on rats and dogs. Osborne-Mendel rats received the colouring in 0.0,

0.5, 1.0, 2.0 and 5.0% concentrations during two years. Tartrazine did not affect their body weight, vital and hematological parameters. At the maximum concentration, diarrhea and formation of stones in the pelvis was observed. The dogs which received tartrazine in the amount of 0, 1 and 2% had no symptoms of toxicity and hematological anomalies. Maekawa et al. (1987) studied the carcinogenicity of tartrazine on rats which consumed the colouring in 0.1 and 2% doses over two years: no symptoms of toxicity were observed, and the 1% increase in the number of tumours was not related to the colouring, according to the authors.

Moutinho et al. (2007) studied the effect of tartrazine on the stomach of Wistar rats. The animals consumed the colouring in the amount of 7.5 mg/kg daily with water during 10 months: there was observed a significant increase in the number of lymphocytes and eozinophiles in the mucous membrane of the stomach. During the study, no carcinogenic changes in the stomach were observed. In their study of 39 chemical substances used as food additives, Sasaki et al. (2002) determined that tartrazine induced damage to DNA in the stomach, large intestine and bladder at doses of 10 and 2000 mg/kg of body weight. All studied food colourings, including allura red and tartrazine, caused damage to DNA in the organs of the gastrointestinal tract at 10 and 100 mg/kg doses.

The articles by Collins et al. (1985, 1989) reported the teratogenic potential of concentrations of tartrazine at 0, 60, 100, 200, 400, 600 and 1000 mg/kg of body weight when it was given to pregnant rats during the first 19 days of pregnancy. Tartrazine caused no significant toxic effect on the health of females and the development of the fetus. The average daily food consumption among the females which received the highest dose was significantly higher, though this had no effect on their body weight. Gautam et al. (2010) studied the toxic impact of tartrazine on the fertility of white mice. The animals were given the colouring in concentrations of 200 and 400 mg/kg of body weight with food during 50 days. It was determined that a 30.5% increase in the body weight of mice occurred at low doses, and by 28.7% - at high doses, which indicates a link between consumption of tartrazine and the development of obesity. Also there was observed a significant decrease in the mass of gonads, motor functionality of spermatozoa and a dose-dependent increase in the number of abnormal spermatozoa. Mehedi et al. (2009) studied the impact of tartrazine on the reproductive function of male white mice. The animals received the colouring with water in 0, 0.1, 1.0 and 2.5% doses during 13 weeks. No changes in the body weight and mass of testicles were found. There was found a decrease in the number of spermatozoa, increase in the frequency of anomalies of the sperm in the group which consumed 2.5% solution of the colouring. Visweswaran (2012) studied the impact of tartrazine on the activity of antioxidant enzymes of rats' testicles. The introduction of tartrazine to the rats during 60 days significantly decreased the activity of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase in the testicles, a decrease was observed in the content of Cu, Zn, Fe and Mn - cofactors of the main antioxidative enzymes. The introduction of tartrazine led to decrease in the space between seminiferous tubules, causing drying-out of the Leydig cells and disorders in the differentiation of spermatogenetic cells. Tartrazine acts as a zinc-chelating agent and causes its deficiency. Presence of Zn at the cell level is important for growth and division of cells in the gonads (MacDonald, 2000). Deficiency of Zn can seriously affect the reproductive functions of males of most species. Lack of Zn caused atrophy of testicles and seminiferous tubules, decrease of libido and total inhibition of spermatogenesis.

Mohamed et al. (2015) studied the neurotoxic effect of tartrazine on rats after peroral administration of the dose of 500 mg/kg of body weight during 30 days: there was determined significant decrease in the concentration of the brain neurotransmitters, sharp deficiency in superoxide dismutase, catalase and glutathione, significant increase in the level of malondialdehyde, and numerous apoptotic cells, which indicated oxidative stress. Kamel & El-lethey (2011) studied the impact of tartrazine on the levels of hyperactivity, anxiety, depression and antisocial behaviour of rats. The animals received the colouring in 0.1% and 2.5% concentrations during 16 weeks. The results indicated that the consumption of tartrazine increased the level of anxiety, caused depression and alarm, and also lead to hyperactivity. Also there are reports on the significant effect of tartrazine on the behaviour of mice (Tanaka, 2006; Tanaka et al., 2008). Such studies provide an impression of the potential danger for psychological health of humans during prolonged consumption of food additives. The works by Schab & Trinh (2004) and McCann et al. (2007) describe hyperactivity of children, which was related to consumption of tartrazine.

Rus et al. (2010) studied the toxicity of tartrazine for guinea pigs in 1, 2 and 3% doses during 3 weeks. At 1% concentration of the colouring, there was observed perivascular edema of liver and apoptotic hepatocytes in the external third of the liver acini. The concentration of 2% caused clearly manifested capillary congestion of many acini and light atrophy. At the highest concentration, the same symptoms were observed which had a clear pattern. The results indicated that tartrazine has a hepatoxic and neurotoxic effect, causes congestion and edema in liver and kidneys, apoptosis and atrophy of hepatocytes and neurocytes.

Himri et al. (2013) studied the toxicity of tartrazine on *Caenorhabditis elegans* Maupas, 1900 nematodes and *Artemia salina* Linnaeus, 1758 crustaceans. The colouring caused no death of nematodes even at the highest concentration (3 mmol). A disruption in the life cycle of *C. elegans* was observed – slowing of larvae' hatching (by 20% on average). Tartrazine in 0.05 and 1 mmol concentrations caused no effect on the nematodes. *A. salina* were affected by tartrazine at concentrations of 10–100 µg/ml, which caused no symptoms of intoxication. However, metabolite of tartrazine – sulfanilic acid – causes total death of *A. salina* over 24 h at the concentration of 100 µg/ml.

Sadar et al. (2017) studied the toxicity of tartrazine for *Escherichia* coli (Migula, 1895) Castellani & Chalmers, 1919, *Staphylococcus epi*dermidis (Winslow & Winslow, 1908) Evans, 1916 and *Saccharomy*ces cerevisiae Meyen ex E. C. Hansen, 1883. The colouring inhibited the development of *E. coli* and *S. epidermidis* at 0.1, 1 and 10 mg/ml concentrations. Yeast cells were inhibited only at the concentration of 1 mg/ml and higher. In the studies by Mpountoukas et al. (2010), tartarzine caused broad DNA-binding and decrease in the mitotic index. Several reports mention the absence of mutagenic effect of tartrazine on *Salmonella typhimurium* and *E. coli* (Chung et al., 1981; Das & Mukherjee, 2004; Elhkim et al., 2007).

Chung et al. (1981) and Combes & Haveland-Smith (1982) reported absence of mutagenic effect and genotoxicity of allura red in many tests on prokaryotic and eukaryotic cells. Abramsson-Zetterberg & Ilbäck (2013) mentioned that allura red is not genotoxic. Intraperitoneal injections of the colouring at concentrations of 100–2000 mg/kg of body weight caused no changes of blood parameters and proliferation of cells among mice. However, Jabeen et al. (2012) studied the genotoxicity of allura red in different concentrations for *S. cerevisiae* and determined that at 28 °C, no significant damage to DNA occurred at any concentration of the colouring even after a four-hour exposure. Significant damage to DNA was observed at 37 °C at 1250–5000 µg/mL concentrations of allura red.

Tsuda (2001) and Shimada et al. (2010) studied the teratogenic effect of allura red for rats. A teratogenic effect from single peroral doses of the colouring was observed even at the concentrations of 2000 mg/kg of body weight. During the evaluation of the DNA condition, specific damage among mice was determined at the dose of 10 mg/kg of body weight, but no DNA damage was found among rats. Collins et al. (1985, 1989) studied the teratogenic potential of allura red for Osborne-Mendel rats. The rats received the colouring in doses of 0, 30, 75, 150, 300, 600 and 1000 mg/kg of body weight daily in the first experiment, and 273.6, 545.7 and 939.3 mg/kg in the second. No changes in the body weight, food consumption by the females, vitality, mass, length of the body of the fetus and distribution of sexes were observed. Abramsson & Ilbäck (2013) reported that the intraperitoneal injections of allura red caused no depression, proliferation of cells and increase in the frequency of nuclei in polychrome erythrocytes. Vorhees et al. (1983) studied the toxicity of allura red for Sprague-Dawley rats which consumed the colouring in the amount of 0.0, 2.5, 5.0 and 10.0% of all food for two weeks. A significant decrease was observed in fertility, body weight, activity and survivability at doses up to 10%. In a similar study by Borzelleca et al. (1989), the animals received the colouring in the amount of 0.0, 0.37, 1.39 and 5.19% over the breeding period, pregnancy and lactation. No side-effects were observed, except the decrease in the body

weight of females with a high dose at the end of the study. The studies by Lison (1937, 1938) and Palm (1952) established that colourings are removed from the organism of insects mostly via the Malpighian tubule system. Also, depending on the colouring, its concentration and the species of insect, pericardial cells and neurons can be involved in the excretion. Nijhout (1975) studied the removal from the organism of 24 colourings from the organism of *Manduca sexta* Linnaeus, 1763 caterpillars. For withdrawal of colourings, caterpillars use two types of excretory organs. The first is situated in the distal and middle Malpighian vessels and has a affinity to the anionic colourings. The second removes the cationic colourings and is most active in the proximal vessels in the mesenteron. However, *M. sexta* larvae are unique, for they remove some colourings via the mesenteron only.

Maddrell et al. (1974) studied the transport and removal of indigo carmine from the organism of insects on the example of *Calliphora* flies and a bug of the Rhodnius family. The colouring quickly enters the Malpighian vessels of the insects, but its accumulation depends on the passive penetrability of the walls of the vessels and the speed of the fluid removal. *Calliphora* concentrates in the vessels 30 times more indigo carmine than *Rhodnius*. The speed of indigo carmine removal was determined at three different values of pH: 6.7, 7.0 and 7.3. The colouring was removed faster at high values of pH.

Hooson et al. (1975) studied chronic toxicity of indigo carmine. Mice were fed with food which contained 0.2, 0.4, 0.8 and 1.6% concentrations of the colouring during 80 weeks. Indigo carmine did not affect the death rate, body weight, mass of organs, and the histological parameters. The concentrations of 0.8 and 1.6% caused light anemia.

Gaunt et al. (1969) studied the impact of indigo carmine on pigs. The animals consumed the colouring at concentrations of 0, 150, 450 and 1350 mg/kg daily over 90 days. Indigo carmine caused no changes in the body weight and mass of organs, parameters of blood and urine. Three individuals which received the maximum dose (1350 mg/kg) had a decrease in the level of hemoglobin and the number of erythrocytes.

It is also worth mentioning the publications devoted to the study of biodegradation of food colourings. A number of works describe the biodegradation of indigo carmine after analyzing it using different microorganisms, including thermophilic (Nicholson & John, 2005), alkalophilic (Nakajima et al., 2005) and anaerobic bacteria (Nicholson & John, 2004). Campos et al. (2001) reported enzymatic degradation of indigo carmine over using laccase of *Trametes hirsuta* (Wulfen) Pilát, 1939 and *Athelia rolfsii* (Curzi) C. C. Tu & Kimbr. Ramya et al. (2007) studied the biodegradation of indigo carmine with usage of *Paenibacillus larvae* White 1906 bacteria. The maximum efficiency (100%) was observed at 30 °C over 8 h and at the level of pH which equaled 7 and 8. The products of indigo carmine degradation are Isotine-sulfonic and anthranilic acid – intermediary metabolites of tryptophan biosynthesis. Similar results were obtained using *Aeromonas hydrophila* (Chester, 1901) Stanier, 1943 and mixed cultures (Olusegun & Olajire, 2015).

#### Conclusion

Statistically reliable changes in the body weight of *T. molitor* larvae were observed when tartrazine and indigo carmine were added to their diet at the concentration of 1 g/kg of dry fodder (P < 0.01) and tartrazine was added at the concentration of 0.1 g/kg (P < 0.05) to the fodder. No reliable changes occurred in the other variants of the experiment. Tartrazine and indigo carmine cause decrease in the body weight of *T. molitor* larvae, depending on the concentration of the colouring. The toxic impact of synthetic food additives on living organisms and the insignificant number of studies focused on the effect of these additives on insects indicates the relevance and necessity for further research in this sphere.

#### References

Aboel-Zahab, H., El-Khyat, Z., Sidhom, G., Awadallah, R., Abdel-al, W., & Mahdy, K. (1997). Physiological effects of some synthetic food colouring additives on rats. Bollettino Chimico Farmaceutico, 136, 615–627.

Abramsson-Zetterberg, L., & Ilbäck, N.-G. (2013). The synthetic food colouring agent Allura Red AC (E129) is not genotoxic in a flow cytometry-based micronucleus assay *in vivo*. Food and Chemical Toxicology, 59, 86–89.

- Amin, K. A., Abdel Hameid, H., & Abd Elsttar, A. H. (2010). Effect of food azo dyes tartrazine and carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats. Food and Chemical Toxicology, 48(10), 2994–2999.
- Ashida, H., Hashimoto, T., Tsuji, S., Kanazawa, K., & Danno, G. (2000). Synergistic effects of food colors on the toxicity of 3-amino-1,4-dimethyl-5Hpyrido[4,3-b]indole (Trp-P-1) in primary cultured rat hepatocytes. Journal of Nutritional Science and Vitaminology, 46(3), 130–136.
- Borzelleca, J. F., & Hallagan, J. B. (1988). A chronic toxicity/carcinogenicity study of FD & C Yellow No. 5 (tartrazine) in mice. Food and Chemical Toxicology, 26(3), 189–194.
- Borzelleca, J. F., & Hallagan, J. B. (1988). Chronic toxicity/carcinogenicity studies of FD & C Yellow No. 5 (tartrazine) in rats. Food and Chemical Toxicology, 26(3), 179–187.
- Borzelleca, J. F., Olson, J. W., & Reno, F. E. (1989). Lifetime toxicity/carcinogenicity study of FD & C Red No. 40 (Allura Red) in Sprague-Dawley rats. Food and Chemical Toxicology, 27(11), 701–705.
- Brygadyrenko, V. V. (2016). Influence of litter thickness on the structure of litter macrofauna of deciduous forests of Ukraine's steppe zone. Visnyk of Dnipropetrovsk University. Biology, Ecology, 24(1), 240–248.
- Brygadyrenko, V., & Nazimov, S. (2015). Trophic relations of *Opatrum sabulo-sum* (Coleoptera, Tenebrionidae) with leaves of cultivated and uncultivated species of herbaceous plants under laboratory conditions. ZooKeys, 481, 57–68.
- Campos, R., Kandelbauer, A., Robra, K., Cavaco-Paulo, A., & Gübitz, G. (2001). Indigo degradation with purified laccases from *Trametes hirsuta* and *Sclero-tium rolfsii*. Journal of Biotechnology, 89(2–3), 131–139.
- Chung, K.-T., Fulk, G. E., & Andrews, A. W. (1978). The mutagenicity of methyl orange and metabolites produced by intestinal anaerobes. Mutation Research/Genetic Toxicology, 58(2–3), 375–379.
- Collins, T. F. X., Black, T. N., O'Donnell, M. W., & Bulhack, P. (1992). Study of the teratogenic potential of FD & C Yellow No. 5 when given in drinkingwater. Food and Chemical Toxicology, 30(4), 263–268.
- Collins, T. F. X., Black, T. N., Welsh, J. J., & Brown, L. H. (1985). Study of the teratogenic potential of Fd&C Red No. 40 when given in drinking water. Toxicology and Industrial Health, 5(6), 937–948.
- Collins, T. F. X., Black, T. N., Welsh, J. J., & Brown, L. H. (1989). Study of the teratogenic potential of FD & C Red No. 40 when given by gavage to rats. Food and Chemical Toxicology, 27(11), 707–713.
- Combes, R. D., & Haveland-Smith, R. B. (1982). A review of the genotoxicity of food, drug and cosmetic colours and other azo, triphenylmethane and xanthene dyes. Mutation Research/Reviews in Genetic Toxicology, 98(2), 101–243.
- Das, A., & Mukherjee, A. (2004). Genotoxicity testing of the food colours amaranth and tartrazine. International Journal of Human Genetics, 4(4), 277–280.
- Davis, K. J., Fitzhugh, O. G., & Nelson, A. A. (1964). Chronic rat and dog toxicity studies on tartrazine. Toxicology and Applied Pharmacology, 6(5), 621–626.
- Devlin, J., & David, T. J. (1992). Tartrazine in atopic eczema. Archives of Disease in Childhood, 67(6), 709–711.
- Elhkim, M. O., Héraud, F., Bemrah, N., Gauchard, F., Lorino, T., Lambré, C., & Poul, J.-M. (2007). New considerations regarding the risk assessment on Tartrazine. Regulatory Toxicology and Pharmacology, 47(3), 308–316.
- El-Wahab, H. M. F. A., & Moram, G. S. E.-D. (2012). Toxic effects of some synthetic food colorants and/or flavor additives on male rats. Toxicology and Industrial Health, 29(2), 224–232.
- Ershoff, B. H. (1977). Effects of diet on growth and survival of rats fed toxic levels of tartrazine (FD&C Yellow No. 5) and sunset yellow FCF (FD&C Yellow No. 6). The Journal of Nutrition, 107(5), 822–828.
- Gaunt, I. F., Grasso, P., Kiss, I. S., & Gangolli, S. D. (1969). Short-term toxicity study on indigo carmine in the pig. Food and Cosmetics Toxicology, 7, 17–24.
- Gautam, D., Sharma, G., & Goyal, R. P. (2010). Evaluation of toxic impact of tartrazine on male Swiss albino mice. Pharmacologyonline, 1, 133–140.
- Himri, I., Bellahcen, S., Souna, F., Belmekki, F., Aziz, M., Bnouham, M., Zoheir, J., Berkia, Z., Mekhfi, H., & Saalaoui, E. (2011). A 90-day oral toxicity study of tartrazine, a synthetic food dye, in Wistar rats. International Journal of Pharmacy and Pharmaceutical Sciences, 3, 159–169.
- Himri, I., Guaadaoui, A. H., Souna, F., Bouakka, M., Melhaoui, A., Hakkou, A., & Saalaoui, E. (2013). Toxicity testing of tartrazine using the nematode *Caenorhabditis elegans*, brine shrimp larvae (*Artemia salina*) and KGN granulosa cell line. Journal of Applied Pharmaceutical Science, 3, 51–58.
- Hooson, J., Gaunt, I. F., Kiss, I. S., Grasso, P., & Butterworth, K. R. (1975). Longterm toxicity of indigo carmine in mice. Food and Cosmetics Toxicology, 13(2), 167–176.
- Jabeen, H. S., ur Rahman, S., Mahmood, S., & Anwer, S. (2012). Genotoxicity assessment of amaranth and allura red using *Saccharomyces cerevisiae*. Bulletin of Environmental Contamination and Toxicology, 90(1), 22–26.
- Kamel, M. M., & El-lethey, H. S. (2011). The potential health hazard of tartrazine and levels of hyperactivity, anxiety-like symptoms, depression and anti-social behaviour in rats. Journal of American Science, 7, 1211–1218.

- Kroes, R., & Kozianowski, G. (2002). Threshold of toxicological concern (TTC) in food safety assessment. Toxicology Letters, 127(1–3), 43–46.
- Lison, L. (1937). Études histophysiologiques sur les tubes de Malpighi des insectes. I. Élimination des colorants acides par le tube de Malpighi chez les Orthoptères. Archives De Biologie, 48, 321–360.
- Lison, L. (1938). Études histophysiologiques sur les Tubes de Malpighi des Insectes. Zeitschrift Für Zellforschung Und Mikroskopische Anatomie, 28(2), 179–209.
- MacDonald, R. S. (2000). The role of zinc in growth and cell proliferation. The Journal of Nutrition, 130(5), 15008–15088.
- Maddrell, S. H. P., Gardiner, B. O. C., Pilcher, D. E. M., & Reynolds, S. E. (1974). Active transport by insect malpighian tubules of acidic dyes and of acrylamides. Journal of Experimental Biology, 61, 357–377.
- Maekawa, A., Matsuoka, C., Onodera, H., Tanigawa, H., Furuta, K., Kanno, J., & Ogiu, T. (1987). Lack of carcinogenicity of tartrazine (FD & C Yellow No. 5) in the F344 rat. Food and Chemical Toxicology, 25(12), 891–896.
- Mannell, W. A., Grice, H. C., & Allmark, M. G. (1962). Chronic toxicity studies on food colours: V. Observations on the toxicity of brilliant blue FCF, guinea green B and benzyl violet 4B in rats. Journal of Pharmacy and Pharmacology, 14(1), 378–384.
- Martynov, V. O., & Brygadyrenko, V. V. (2017). The influence of synthetic food additives and surfactants on the body weight of larvae of *Tenebrio molitor* (Coleoptera, Tenebrionidae). Biosystems Diversity, 25(3), 236–242.
- McCann, D., Barrett, A., Cooper, A., Crumpler, D., Dalen, L., Grimshaw, K., & Stevenson, J. (2007). Food additives and hyperactive behaviour in 3-year-old and 8/9-year-old children in the community: A randomised, double-blinded, placebo-controlled trial. The Lancet, 370(9598), 1560–1567.
- Mehedi, N., Ainad-Tabe, S., Mokrane, N., Addou, S., Zaoui, C., Kheroua, O., & Saidi, D. (2009). Reproductive toxicology of tartrazine (FD and C yellow No. 5) in Swiss Albino mice. American Journal of Pharmacology and Toxicology, 4(4), 130–135.
- Mekkawy, H. A., Ali, M. O., & El-Zawahry, A. M. (1998). Toxic effect of synthetic and natural food dyes on renal and hepatic functions in rats. Toxicology Letters, 95, 155.
- Mohamed, A. A.-R., Galal, A. A. A., & Elewa, Y. H. A. (2015). Comparative protective effects of royal jelly and cod liver oil against neurotoxic impact of tartrazine on male rat pups brain. Acta Histochemica, 117(7), 649–658.
- Moutinho, I., Bertges, L., & Assis, R. (2007). Prolonged use of the food dye tartrazine (FD&C yellow n° 5) and its effects on the gastric mucosa of Wistar rats. Brazilian Journal of Biology, 67(1), 141–145.
- Mpountoukas, P., Pantazaki, A., Kostareli, E., Christodoulou, P., Kareli, D., Poliliou, S., & Lialiaris, T. (2010). Cytogenetic evaluation and DNA interaction studies of the food colorants amaranth, erythrosine and tartrazine. Food and Chemical Toxicology, 48(10), 2934–2944.
- Nakajima, K. (2005). Alkalibacterium iburiense sp. nov., an obligate alkaliphile that reduces an indigo dye. International Journal of Systematic and Evolutionary Microbiology, 55(4), 1525–1530.
- Neuman, I., Elian, R., Nahum, H., Shaked, P., & Creter, D. (1978). The danger of "yellow dyes" (tartrazine) to allergic subjects. Clinical Experimental Allergy, 8(1), 65–68.
- Nicholson, S. K., & John, P. (2004). Bacterial indigo reduction. Biocatalysis and Biotransformation, 22(5–6), 397–400.
- Nicholson, S. K., & John, P. (2005). The mechanism of bacterial indigo reduction. Applied Microbiology and Biotechnology, 68(1), 117–123.
- Nijhout, H. F. (1975). Excretory role of the midgut in larvae of the tobacco homworm, *Manduca sexta* (L.). Journal of Experimental Biology, 62, 221–230.
- Olusegun, E. T., & Olajire, A. A. (2015). Toxicity of food colours and additives: A review. African Journal of Pharmacy and Pharmacology, 9(36), 900–914.
- Palm, N. B. (1952). Storage and excretion of vital dyes in insects with special regard to trypan blue. Arkiv för Zoologi, 3, 195–272.
- Patterson, R. M., & Butler, J. S. (1982). Tartrazine-induced chromosomal aberrations in mammalian cells. Food and Chemical Toxicology, 20(4), 461–465.
- Ramya, M., Anusha, B., & Kalavathy, S. (2007). Decolorization and biodegradation of indigo carmine by a textile soil isolate *Paenibacillus larvae*. Biodegradation, 19(2), 283–291.
- Reyes, F. G. R., Valim, M. F. C. F. A., & Vercesi, A. E. (1996). Effect of organic synthetic food colours on mitochondrial respiration. Food Additives and Contaminants, 13(1), 5–11.
- Rus, V., Gherman, C., Miclăuş, V., Mihalca, A., & Nadăş, G. C. (2010). Comparative toxicity of food dyes on liver and kidney in guinea pigs: A histopathological study. Annals of the Romanian Society for Cell Biology, 15, 161–165.
- Sadar, P., Dande, P., Kulkami, N., & Pachori, R. (2017). Evaluation of toxicity of synthetic food colors on human normal flora and yeast. International Journal of Health Sciences and Research, 7, 110–114.
- Sasaki, Y. F., Kawaguchi, S., Kamaya, A., Ohshita, M., Kabasawa, K., Iwama, K., & Tsuda, S. (2002). The comet assay with 8 mouse organs: Results with 39 currently used food additives. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 519(1–2), 103–119.

- Schab, D. W., & Trinh, N.-H. T. (2004). Do artificial food colors promote hyperactivity in children with hyperactive syndromes? A meta-analysis of doubleblind placebo-controlled trials. Journal of Developmental and Behavioral Pediatrics, 25(6), 423–434.
- Shimada, C., Kano, K., Sasaki, Y. F., Sato, I., & Tsudua, S. (2010). Differential colon DNA damage induced by azo food additives between rats and mice. The Journal of Toxicological Sciences, 35(4), 547–554.
- Shousha, M. A., Sakr, A. A., Hammam, M. A., & Abdel-Moein, N. M. (1992). Effect of synthetic banana food additives on energy and nucleic acids metabolism in brain, liver and kidney tissues of albino rats. Egyptian Journal of Applied Sciences, 7, 45–55.
- Shrestha, S., Bhattarai, B. R., Lee, K., & Cho, H. (2006). Some of the food color additives are potent inhibitors of human protein tyrosine phosphatases. Bulletin of the Korean Chemical Society, 27(10), 1567–1571.
- Stenius, B. S. M., & Lemola, M. (1976). Hypersensitivity to acetylsalicylic acid (ASA) and tartrazine in patients with asthma. Clinical Experimental Allergy, 6(2), 119–129.

- Tanaka, T. (2006). Reproductive and neurobehavioural toxicity study of tartrazine administered to mice in the diet. Food and Chemical Toxicology, 44(2), 179–187.
- Tanaka, T., Takahashi, O., Oishi, S., & Ogata, A. (2008). Effects of tartrazine on exploratory behavior in a three-generation toxicity study in mice. Reproductive Toxicology, 26(2), 156–163.
- Tsuda, S. (2001). DNA damage induced by red food dyes orally administered to pregnant and male mice. Toxicological Sciences, 61(1), 92–99.
- Visweswaran, B. (2012). Oxidative stress by tartrazine in the testis of Wistar rats. IOSR Journal of Pharmacy and Biological Sciences, 2(3), 44–47.
- Vorhees, C. V., Butcher, R. E., Brunner, R. L., Wootten, V., & Sobotka, T. J. (1983). Developmental toxicity and psychotoxicity of FD and C red dye no. 40 (Allura red AC) in rats. Toxicology, 28(3), 207–217.
- Wang, L., Zhang, G., & Wang, Y. (2014). Binding properties of food colorant allura red with human serum albumin *in vitro*. Molecular Biology Reports, 41(5), 3381–3391.