БИОЛОГИЯ, БИОХИМИЯ, ГЕНЕТИКА

УДК 57.577.15:595.773:632.95.025.8
DOI: 10.31040/2222-8349-2024-0-3-38-43

CATALASE ACTIVITY IN *MUSCA DOMESTICA* L. ADULTS OF THE FIELD POPULATION IN COMPARISON WITH THE LABORATORY STRAIN

© K.Yu. Maslakova, L.Ya. Yangirova

The antioxidant system plays an important role in the life of organisms. Its primary function is to protect against the damaging effects of excessive free radicals that lead to a state of oxidative stress. Enzymes of the insect antioxidant system may play a significant role in insecticide resistance. For insects, a number of antioxidant enzymes have been characterized, including catalase, superoxide dismutase, glutathione transferase and glutathione reductase. The role of catalase in this system is to break down hydrogen peroxide to form water and oxygen, thereby preventing membrane lipid peroxidation and cell damage. The aim of this study was to investigate catalase activity in adults of *Musca domestica* L. in field population. During homogenate production a comparative evaluation of the two-extraction media on catalase activity in adults of the laboratory strain of the house fly was also conducted. We found a statistically significant 1.12-fold increase in enzyme activity in adults of the field population compared to specimens of the laboratory strain of *M. domestica*. The catalase activity in homogenates prepared on 0.9% sodium chloride solution was statistically higher by 1.77-fold compared to the enzyme activity in homogenates on sodium phosphate buffer (pH 7.6) with Triton-X 100. The study of the insect antioxidant system may be useful in addressing the problem of insect resistance to insecticides, thus allowing the regulation of insect populations.

Keywords: housefly, homogenate, enzyme activity, antioxidant system, free radicals, oxidative stress, insecticide resistance.

Introduction. *Musca domestica* Linnaeus (Diptera: Muscidae) is one of the most common insects worldwide; it is a synanthropic pest since it lives in close contact with humans and domestic animals and is often observed in a variety of human activities. *M. domestica* can be a serious nuisance as it serves as a mechanical vector for a variety of human and animal pathogens including parasites, bacteria, fungi and viruses (Zahn and Gerry, 2020). Globally, insecticides are largely used to control house flies, the inappropriate use of which leads to the emergence of resistant populations. Insecticide resistance is one of the major issues in pest management. Resistance is defined as the inherited ability of a population to tolerate a dose of insecticide that is lethal to the majority of specimens in a susceptible population of the same species (Abobakr et al., 2022). The spread of resistant insect populations remains a pressing problem (Ben'kovskaja and Nikonorov, 2018). The resistance development poses severe environmental hazards such as adverse impacts on non-target organisms and environmental contamination (Abobakr et al., 2022).

Catalase (EC 1.11.1.6) is an enzyme from the oxidoreductase family; it is a tetramer comprised of four iron-containing heme groups that allow the enzyme to react with hydrogen peroxide. Catalases are categorized into three groups: monofunctional heme-containing catalases, heme-containing peroxidase catalases, and manganese-containing catalases (Loncar and Fraaije, 2015). Catalase (CT) is found in almost all aerobic living organisms and is one of the key enzymes of the antioxidant system, which together with its other components protects cells from oxidative damage caused by reactive oxygen species (Ighodaro and Akinloye, 2018).

Insects have developed a complex and efficient network of enzymatic antioxidant systems for self-defense against reactive oxygen species (ROS) (Barbehenn, 2002). Catalase performs the decomposition of hydrogen peroxide to water and molecular oxygen, thereby preventing the formation of the highly active hydroxyl radical that plays a key role in membrane lipid damage (Ighodaro and Akinloye, 2018). The aim of this study was to investigate catalase activity in adults of *Musca domestica* L. in field population. The effect of the

МАСЛАКОВА Ксения Юрьевна, Тюменский научный центр СО РАН, e-mail: k.y.maslakova@gmail.com
ЯНГИРОВА Лиана Януровна, Тюменский научный центр СО РАН, e-mail: lianayangirova137@gmail.com

formulation of the homogenization medium on the tested enzyme activity was also studied.

Materials and methods. ***Insects.*** The study was conducted on laboratory culture and field population of *M. domestica*. Laboratory cultures were obtained from Novosibirsk State Agricultural University in 2009 (Tyumen strain) and from the Institute of Biochemistry and Genetics UFRC RAS in 2023 (Ufa strain). The field population was collected in the summer of 2023 in the village of Nikulino, Tyumen Oblast. CT activity was evaluated in adults of the house fly at the age of 4-6 days without separation by sex. Insect cultivation was based on the rules outlined by Ben'kovskaja G.V. (Ben'kovskaja, 2017). Adult specimens of *M. domestica* were kept in 25×25×25 cm metal frame cages covered with mesh fabric. Each cage contained a drinker with absorbent cotton filter and clean water, as well as a container of milk powder as feed. Plastic cups with a substrate, that consisted of heat-treated bran with the addition of baker's yeast and water, were used for egg laying. The optimal temperature (23–27°C) and relative humidity (50–60%) were established in the space where the cages were located.

Homogenate preparing. Prior to enzymatic activity determination, adults were weighed and frozen at -80°C; subsequently, 5% homogenates were prepared from the specimens. Homogenates were prepared using a laboratory homogenizer with active cooling function at +4°C (Bioprep-24R, Allsheng, China). The extraction media that were used: 0.9% NaCl physiological solution; 0.1M phosphate buffer pH 7.6, containing 1mM EDTA, 1mM PTU, 1mM PMSF, 1mM DTE and Triton-X. The acquired homogenates were centrifuged at 12.000 g for 2 minutes, and then the supernatant was collected and used for determination of enzyme activity and protein quantitative content.

Assay of enzyme activities. The method of Korolyuk M.A. (Korolyuk et al., 1988) with some minor modifications was adopted as the basis for the determination of CT activity. This method is based on the ability of hydrogen peroxide to form a persistent colored complex with molybdenum salts. Enzyme activity determination was performed on 96-well microtitration plates (MiniMed, Russia) on a Multiskan FC microplate photometer (Thermo Fisher Scientific Inc., Finland) at a wavelength of 405 nm. Protein levels were determined photometrically according to the Lowry O.H. method (Lowry et al., 1951), using bovine serum albumin solutions for calibration graph plotting.

Data analysis. Statistical processing of the study results included: calculation of mean value, standard error of mean, median, mode, standard deviation and sample variance using descriptive statistics from the data analysis section in Excel. When performing data analysis, we used Welch's T-test, which allows us to compare two groups of data, assuming that the data in both groups follow a normal distribution, but do not require the same variance between groups. By assuming that the variance differs between groups (laboratory group and field group, as well as laboratory group in buffer and laboratory group in physical solution), we use a two-sample t-test with different variances.

Results. According to the obtained results, the sample variance obtained from specimens of the laboratory strain in buffer is significantly different from both the sample obtained from the same specimens in physical solution (Fig. 1) and from the sample obtained from individuals of the field population in buffer solution (Fig. 2). All samples also conform to a normal distribution, but their variances are different, suggesting that we should use Welch's t-test to statistically assess the significance.

It was observed that the catalase activity in adults of *M. domestica* when using 0.9% NaCl solution as a homogenization medium was statistically higher (1.77-fold) compared to the enzyme activity in homogenates on phosphate buffer with detergent (Table 1 and Fig. 3). The enzyme activity in homogenates on phosphate buffer is 1.12 times lower for the laboratory strain compared to the field strain.

Discussion. A rather early work on catalase activity in *Drosophila* (Samis et al., 1972) provides data that can also be applied to *M. domestica*. The majority of catalase activity in both male and female *Drosophila* is found in the abdomens of flies. Moreover, the enzyme activity in the head, thorax and abdomen of male flies is higher than in the respective body parts of females. Samis H. V. (Samis et al., 1972) found that spectrophotometrically determined catalase activity in *Drosophila* is directly proportional to enzyme concentration, inversely proportional to substrate concentration, and independent of wide ranges of phosphate ion concentration. The optimum pH for Drosophila catalase activity ranges from 7.0 to 8.0. Enzyme activity peaks at pH 7.5 and decreases with increasing substrate concentration.

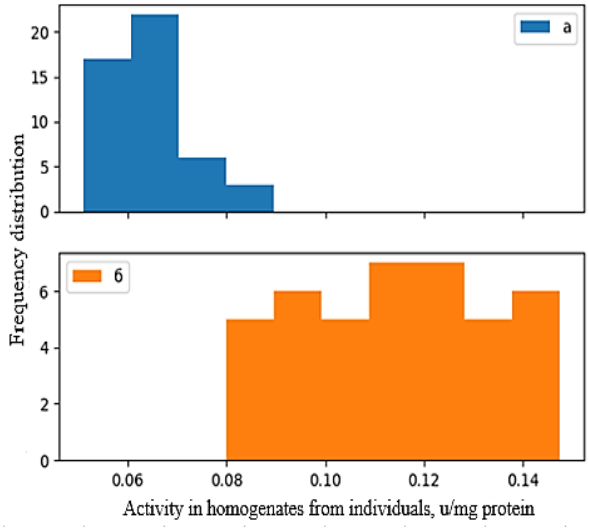

Fig. 1. Catalase activity frequency distribution in adults of *M. domestica* of the laboratory strain during homogenate preparation in different media: a) buffer solution (0.1M phosphate buffer pH 7.6 containing 1mM EDTA, 1mM PTU, 1mM PMSF, 1mM DTE and Triton-X), b) NaCl 0.9%

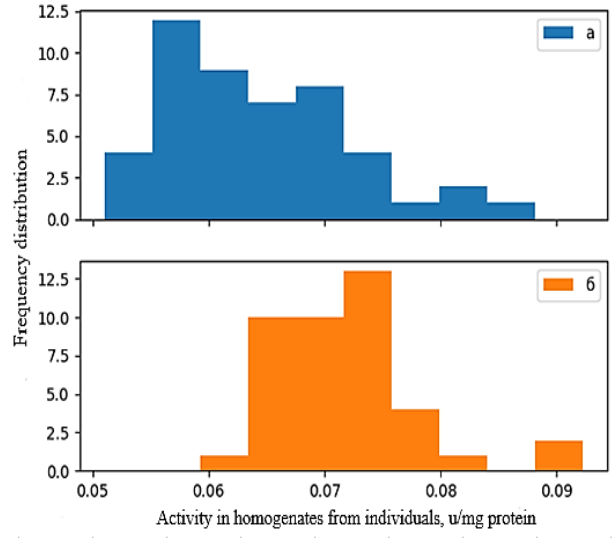

Fig. 2. Catalase activity frequency distribution in adults of *M. domestica* of the laboratory strain (a) and of the field population (b) during homogenate preparation in buffer solution (0.1M phosphate buffer pH 7.6 containing 1mM EDTA, 1mM PTU, 1mM PMSF, 1mM DTE and Triton-X)

Early studies on insect antioxidants under exposure to various chemical factors including pesticides (Barbehenn, 2002), as well as data on changes in antioxidant levels during ontogeny and their role in aging processes are available (Mockett et al., 2001). In a study by Sohal R.S. (Sohal et al., 1984) on the effect of aging on the activity of antioxidant enzymes (including catalase) in *M. domestica*, insects were homogenized using 0.1% Triton-X. Later Sohal R.S. (Sohal et al., 1990) investigated the effect of aging on the activity of the same enzymes in *Drosophila melanogaster*, where specimens were homogenized in 66 mM potassium phosphate buffer, with the application of 0.1% Triton-X. In a study of catalase in *Bombyx mori* by Yamamoto K. (Yamamoto et al., 2005), homogenates were prepared in 70 mM potassium phosphate buffer (pH 6.5) containing 0.1% Triton-X 100. As stated earlier, the inclusion of detergent in the composition of the medium for insect homogenization is essential. Therefore, the identification of catalase activity in adults of *M. domestica* was determined in homogenates prepared on phosphate buffer containing Triton-X. Adams D.H. and Burgess E.H. (Adams and Burgess, 1957) were the first to report the use of the non-ionogenic detergent Triton-X 100 to increase the observed catalase activity of mouse liver homogenates. The authors point out, unless Triton-X 100 is used, there is an inherent danger, that livers isolated from different experimental animals may be activated to varying degrees during homogenization. In our study, as shown in Figure 1 and Table 1, the detectable enzyme activity was lower when using triton buffer than when using sodium chloride. However, the characterized trait (enzyme activity) varied less in the sample with detergent than in the sample with saline solution, as indicated by the variance and standard deviation values (Table 1). Hence, we subsequently used a detergent buffer.

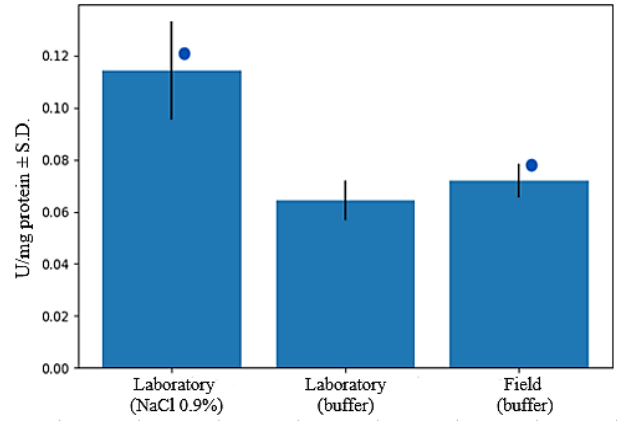

Fig. 3. Catalase activity in *M. domestica* adults of laboratory strain and field population. Note: n-sample; differences were considered statistically significant compared to activity in adults of the laboratory strain (buffer) at $p<0.05$ according to Welch's T-criterion

Table 1

Statistical parameters of compared samples of catalase activity values in M. domestica adults of laboratory strain and field population

Strain	Mean value	Standard error	Standard deviation	Variance	Sample volume	Reliability level (95%), compared to the laboratory strain (0.1M phosphate buffer pH 7.6)
Laboratory (0.1M phosphate buffer pH 7.6)	0.064	0.001	0.008	0.00006	48	–
Field (0.1M phosphate buffer pH 7.6)	0.072	0.001	0.006	0.00004	41	0.002
Laboratory (NaCl 0.9%)	0.114	0.003	0.019	0.00035	41	0.006

The field environment as opposed to the artificial laboratory environment is unstable. Field population exists in more complex living conditions, often encountering various environmental changes. Such factors as fluctuating environmental conditions, food availability, various diseases, insecticide exposure are stressful to insects. Any change in homeostasis leads to oxidative stress – an imbalance between the production of free radicals and their neutralization. In turn, this leads to the activation of the antioxidant system, which manifests itself in an increase in the activity of the corresponding enzymes. Based on the obtained results for catalase, it can be assumed that the total antioxidant activity of adult specimens of the field population of *M. domestica* is more pronounced compared to the laboratory population. The study of insect antioxidant system can be very useful when addressing the problem of insecticide resistance. Our future research will focus on studying other enzymes of the antioxidant system and investigating the catalase activity at different stages of the life cycle of *M. domestica*. This will allow for the most thorough characterization of the antioxidant defense system operation of *M. domestica*, which may subsequently prove to be a promising target for the development of methods to regulate the populations of both the house fly and insects in general.

Conclusion. The results of this study show that catalase activity in adults of the field population is statistically higher compared to specimens of the laboratory strain of *M. domestica*. The catalase activity in adults of *M. domestica* was statistically higher (1.77-fold) in 0.9% NaCl solution as a homogenization medium compared to that in homogenates on phosphate buffer with detergent. The enzyme activity in homogenates on phosphate buffer is 1.12 times lower for the laboratory strain compared to the field strain. Our further studies will be aimed at the investigation of *M. domestica* catalase activity during ontogenetic development, since this enzyme may prove to be a promising target for the development of methods to regulate the populations of both the house fly and insects in general.

The research was carried by the All-Russian Scientific Research Institute of Veterinary Entomology and Arachnology of the Tyumen Scientific Centre of Siberian Branch of the Russian Academy of Sciences within the framework of the state assignment of the Ministry of Science and Higher Education of the Russian Federation (theme No. FWRZ-2022-0022).

Литертура

1. Беньковская Г.В., Никоноров Ю.М. Молекулярные механизмы неспецифической устойчивости к инсектицидам у колорадского жука: активация транскрипции генов рецепторных и запасных белков // Известия Уфимского научного центра РАН. 2018. № 3-1. С. 36–40.

2. Беньковская Г.В. Принципы содержания лабораторных линий насекомых // Биомика. 2017. Т. 9. № 1. С. 24–32.

3. Королюк М.А., Иванова, Л.И., Майорова И.Г., Токарев В.Е. Метод определения активности каталазы // Лаб. дело. 1988. № 1. С. 16–19.

4. Abobakr Y., Al-Hussein F.I., Bayoumi A.E., Alzabib A.A., Al-Sarar A.S. Organophosphate Insecticides Resistance in Field Populations of House Flies, *Musca domestica* L.: Levels of Resistance and Acetylcholinesterase Activity // Insects. 2022. Vol. 13(2). 192 p. https://doi.org/10.3390/insects13020192

5. Adams D.H., Burgess E.H., Brit J. Cancer, 1957. Vol. 11. 310 p.

6. Barbehenn R.V. Gut-Based Antioxidant Enzymes in a Polyphagous and a Graminivorous Grasshopper // J. Chem. Ecol. 2002. Vol. 28. P. 1329-1347. https://doi.org/10.1023/a:1016288201110

7. Ighodaro O.M., Akinloye O.A. First line defense antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid // Alexandria J. Medic. 2018. Vol. 54(4). P. 287–293. https://doi.org/10.1016/j.ajme.2017.09.001

8. Loncar N., Fraaije M.W. Catalases as biocatalysts in technical applications: Current state and perspectives // Appl. Microbiol. Biotechnol. 2015. Vol. 99. P. 3351–3357. https://doi.org/10.1007/s00253-015-6512-6

9. Lowry O.H., Rosebrough N.J., Farr A.L., Randall, R.J. Protein measurement with Folin phenol reagent // J. Biol. Chem. 1951. Vol. 193(1). P. 265–275.

10. Mockett R.J., Orr W.C., Rahmander J.J., Sohal B.H., Sohal R.S. Antioxidant Status and Stress Resistance in Long- and Short-Lived Lines of *Drosophila melanogaster* // Exp. Gerontol. 2001. Vol. 36. P. 411–630. https://doi.org/10.1016/s0531-5565(00)00258-8

11. Samis H.V. Baird M.B., Massie H.R. Renewal of catalase activity in *Drosophila* following treatment with 3-amino-1,2,4-triazole // J. Insect Physiol. 1972. Vol. 18(5). P. 991–1000. https://doi.org/10.1016/0022-1910(72)90036-4

12. Sohal R.S., Arnold L. and Orr W.C., Effect of age on superoxide dismutase, catalase, glutathione reductase, inorganic peroxides, TBA-reactive material, GSH/GSSG, NADPH/NADP+ and NADH/NAD+ in *Drosophila melanogaster* // Mech. Ageing. Dev. 1990. Vol. 56(3). P. 223–235. https://doi.org/110.1016/0047-6374(90)90084-s

13. Sohal R.S., Farmer K.J., Allen R.G., Cohen N.R. Effect of age on oxygen consumption, superoxide dismutase, catalase, glutathione, inorganic peroxides and chloroform-soluble antioxidants in the adult male housefly, *Musca domestica* // Mech. Ageing Dev. 1984. Vol. 24(2). P. 185–195. https://doi.org/10.1016/0047-6374(84)90070-8

14. Yamamoto K., Banno Y., Fujii H. Miake F., Kashige N., Aso Y. Catalase from the silkworm, *Bombyx mori*: Gene sequence, distribution, and overexpression // Insect Biochem. Mol. Biol. 2005. Vol. 35(4). P. 277–283. https://doi.org/10.1016/j.ibmb.2005.01.001

15. Zahn L.K., Gerry A.C. Diurnal Flight Activity of House Flies (*Musca domestica*) is Influenced by Sex, Time of Day, and Environmental Conditions // Insects. 2020. Vol. 11(6). 391 p.

References

1. Ben'kovskaja G.V., Nikonorov Ju.M. Molekuljarnye mehanizmy nespecificheskoj ustojchivosti k insekticidam u koloradskogo zhuka: aktivacija transkripcii genov receptornyh i zapasnyh belkov // Izvestija Ufimskogo nauchnogo centra RAN, 2018, no. 3-1, pp. 36-40.

2. Ben'kovskaja G.V. Principy soderzhanija laboratornyh linij nasekomyh // Biomika, 2017, vol. 9, no. 1, pp. 24-32.

3. Koroljuk M.A., Ivanova, L.I., Majorova I.G., Tokarev V.E. Metod opredelenija aktivnosti katalazy // Lab. delo, 1988, no. 1, pp. 16-19.

4. Abobakr Y., Al-Hussein F.I., Bayoumi A.E., Alzabib A.A., Al-Sarar A.S. Organophosphate Insecticides Resistance in Field Populations of House Flies, *Musca domestica* L.: Levels of Resistance and Acetylcholinesterase Activity // Insects, 2022, vol. 13(2), 192 p. https://doi.org/10.3390/insects13020192

5. Adams D.H., Burgess E.H., Brit. J. Cancer, 1957, vol. 11, 310 p.

6. Barbehenn R.V. Gut-Based Antioxidant Enzymes in a Polyphagous and a Graminivorous Grasshopper // J. Chem. Ecol., 2002, vol. 28, pp. 1329-1347. https://doi.org/10.1023/a:1016288201110

7. Ighodaro O.M., Akinloye O.A. First line defense antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid // Alexandria J. Medic., 2018, vol. 54(4), pp. 287-293. https://doi.org/10.1016/j.ajme.2017.09.001

8. Loncar N., Fraaije M.W. Catalases as biocatalysts in technical applications: Current state and perspectives // Appl. Microbiol. Biotechnol., 2015, vol. 99, pp. 3351-3357. https://doi.org/10.1007/s00253-015-6512-6

9. Lowry O.H., Rosebrough N.J., Farr A.L., Randall, R.J. Protein measurement with Folin phenol reagent // J. Biol. Chem., 1951, vol. 193(1), pp. 265-275.

10. Mockett R.J., Orr W.C., Rahmander J.J., Sohal B.H., Sohal R.S. Antioxidant Status and Stress Resistance in Long- and Short-Lived Lines of *Drosophila melanogaster* // Exp. Gerontol., 2001, vol. 36, pp. 411-630. https://doi.org/10.1016/s0531-5565(00)00258-8

11. Samis H.V. Baird M.B., Massie H.R. Renewal of catalase activity in *Drosophila* following treatment with 3-amino-1,2,4-triazole // J. Insect Physiol., 1972, vol. 18(5), pp. 991-1000. https://doi.org/10.1016/0022-1910(72)90036-4

12. Sohal, R.S., Arnold, L. and Orr, W.C., Effect of age on superoxide dismutase, catalase, glutathione reductase, inorganic peroxides, TBA-reactive material, GSH/GSSG, NADPH/NADP+ and NADH/NAD+ in *Drosophila melanogaster* // Mech. Ageing. Dev., 1990, vol. 56(3), pp. 223-235. https://doi.org/110.1016/0047-6374(90)90084-s

13. Sohal R.S., Farmer K.J., Allen R.G., Cohen N.R. Effect of age on oxygen consumption, superoxide dismutase, catalase, glutathione, inorganic peroxides and chloroform-soluble antioxidants in the adult male housefly, *Musca domestica* // Mech. Ageing Dev., 1984, vol. 24(2), pp. 185-195. https://doi.org/10.1016/0047-6374(84)90070-8

14. Yamamoto K., Banno Y., Fujii H. Miake F., Kashige N., Aso Y. Catalase from the silkworm, *Bombyx mori*: Gene sequence, distribution, and overexpression // Insect Biochem. Mol. Biol., 2005, vol. 35(4), pp. 277-283. https://doi.org/10.1016/j.ibmb.2005.01.001

15. Zahn L.K., Gerry A.C. Diurnal Flight Activity of House Flies (*Musca domestica*) is Influenced by Sex, Time of Day, and Environmental Conditions // Insects, 2020, vol. 11(6), p. 391.

АКТИВНОСТЬ КАТАЛАЗЫ У ИМАГО *MUSCA DOMESTICA* L. ПРИРОДНОЙ ПОПУЛЯЦИИ В СРАВНЕНИИ С ЛАБОРАТОРНОЙ ЛИНИЕЙ

© К.Ю. Маслакова, Л.Я. Янгирова

Тюменский научный центр Сибирского отделения Российской академии наук,
2, улица Институтская, 625041, Тюмень, Россия

Антиоксидантная система играет важную роль в жизнедеятельности организмов. Основная функция – защита от разрушающего воздействия избыточного количества свободных радикалов, которые приводят к состоянию окислительного стресса. Ферменты антиоксидантной системы насекомых могут играть значительную роль в устойчивости к инсектицидам. Для насекомых охарактеризован ряд антиоксидантных ферментов, в числе которых каталаза, супероксиддисмутаза, глутатионтрансфераза и глутатионредуктаза. Роль каталазы в этой системе сводится к расщеплению пероксида водорода с образованием воды и кислорода, тем самым предупреждая перекисное окисление липидов мембран и повреждение клеток. Целью настоящего исследования стало изучение активности каталазы у имаго *Musca domestica* L. природной популяции. Также проведена сравнительная оценка двух сред выделения при получении гомогенатов на активность каталазы у имаго лабораторной линии комнатной мухи. Нами обнаружено статистически значимое увеличение активности фермента у имаго природной популяции по сравнению с особями лабораторной линии *M. domestica* в 1.12 раз. Активность каталазы в гомогенатах, приготовленных на 0,9% растворе хлорида натрия, статистически выше в 1.77 раз по сравнению с активностью фермента в гомогенатах на натрий-фосфатном буфере (pH 7,6) с Triton-X 100. Изучение антиоксидантной системы насекомых может быть полезным в решении проблемы устойчивости насекомых к инсектицидам, что позволит регулировать их численность.

Ключевые слова: комнатная муха, гомогенат, ферментативная активность, антиоксидантная система, свободные радикалы, окислительный стресс, инсектицидная резистентность.