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Morphological Deformities of Green Toad (*Bufo viridis*) Tadpoles Caused by Petroleum Products

Abstract: Environmental pollution with oil and petroleum products leads to a decrease in animal biodiversity and human diseases. Due to the intense pollution of Kazakhstan's water bodies located on the territory of oil producing regions, the purpose of this study was to study the effect of different concentrations of water-soluble fraction of oil (WSFO) on the growth and development of green toad (*Bufo viridis*). This species of anuran amphibians is widespread in Kazakhstan, which is especially important given the aridity of the lands of the oil-producing regions. A subchronic and chronic exposure to three concentrations of WSFO on the tadpoles of the green toad (*Bufo viridis*) was carried out. The results of the study revealed suppression of growth (size and weight) and a developmental delay in tadpoles from experimental groups by 1.6-1.8-fold. Moreover, developmental malformations such as axial curvature, edema,tail malformation, head malformations, pigmentation alteration were observed. Thus, exposure to WSFO suppresses the growth and development of the green toad *Bufo viridis).*

Keywords: water-soluble fraction of oil, *Bufo viridis*, growth, development, morphological deformities

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Introduction. Decline in amphibian populations has become a global problem and attention of many researches is focused on search of the causes of this decline [1]. The reasons may be various from habitat deterioration to the increase in the level of UV radiation [1, 2]. Anthropogenic activities such as oilproductionresult in contamination of natural habitats [3]. Petroleum hydrocarbons have negative effects on ecosystems including aquatic habitats [4]. The economy of Kazakhstan is based on oil production [5] and development of this industry leads to deterioration of the ecosystems [3].Petroleum contamination is one of the reasons of thedecreases in animal populations and biodiversity [6]. National ecological reports [7] show significant pollution of many water bodies of Kazakhstan with petroleum hydrocarbons. However, toxicological studies of oil and petroleum products in Kazakhstan are usually limited to investigation of rodents [8-12]. Thus, there is a need in studying effects of petroleum contamination on animals of the aquatic ecosystems, such as amphibians. They are a convenient model for ecotoxicological studies as they live both in water and on land during their life cycle[13, 14]. Moreover, their biological features make them sensitive to chemical contamination especially in their embryonic and larval stages [15]. The green toad (*Bufo viridis*) is one of the widespread amphibian species in Kazakhstan. It inhabits most areas of Kazakhstan, including oil producing regions [16].Its wide distribution on Kazakhstan territory makes it suitable model for assessing environmental effects of petroleum products.

There are many studies worldwide devoted to investigation of oil and petroleum influence on amphibian larvae.Various chemicals affect survival and growth rate, and induce malformationsin developing amphibians [17]. High mortality rates and significant delay in growth and development of *L. sylvaticus* was observed following exposure to naphthenic acids [18]. Exposure to fluoranthene (PAH) resulted in occurrence of severe malformations and low survival rate in *X. laevis* and *L. pipiens*[19]. However, there are few studies on the effects of petroleum products on larvae of green toad (*Bufo viridis*). Further, little research was conducted to determine the effects of oil from Kazakhstan on amphibians.

The aim of this study was to examine the growth and development of green toad (*Bufo viridis*) in water contaminated with petroleum products by means of subchronic and chronic exposure to various concentrations of water-soluble fraction of crude oil.

Materials and methods

Preparation of Water-Soluble Fraction of Oil. Preparation of water-soluble fraction of oil (WSFO)was carried out according to [20], taking into account the recommendations of [21].Crude oil from the Zhanazhol oil field (Aktobe region of the western part of the Republic of Kazakhstan) was mixed with water in a 1:9 ratio (100 ml oil per 900 ml distilled water).The resulting mixture was placed in a 1 L flask with a tightly closed stopperand stirred on a magnetic stirrer in the dark for 18 hr avoiding deterioration of the oil film integrity and emulsification.After stirring, the resulting mixture was allowed to stand at room temperature for 6 hr. Further, the water-soluble fraction was extracted using a separatory funnel and stored at $4\,^0$ C. Before use in exposure experiments, the water-soluble fraction was acclimated to room temperature (21-23 ° C).

Obtaining the eggs of B. viridis. Six mature specimens of B. viridis (4 males and 2 females) were caught from the river Emba (Aktobe region, The Republic of Kazakhstan) and brought to the Ecotoxicology Laboratory of the Faculty of Biology and Biotechnology of the Al-Farabi Kazakh National University. Adult males and females were placed separately in 100 L aquaria with dry surface and smaller reservoir with water. The air temperature was maintained at $24 \pm 2\,^\circ$ C. Toads were fed crickets and kept for two weeks to acclimatize before induction of spawning. For this purpose both males and females were injected (5 μl/g of body weight) with the AMPHIPLEX mixture of a gonadotropin-releasing hormone agonist (0.4 μg/g) and metoclopramide (10 μg/g) according to the procedure described by Trudeau et al. [22] except drugs were dissolved in saline. Adult frogs were divided into groups of 3 individuals (2 males and 1 female) into separate 50 L aquaria with algae and branches to simulate natural spawning conditions (2 aquaria in total). After 2-3 days, the fertilized eggs were obtained. For experiments on embryotoxicity of WSFO, the eggs were placed in Petri dishes after they reached the Gosner stage (Gs) 8-11. For a chronic experiment, the tadpoles were moved to the 18 L aquaria, when they all began to feed and swim independently (Gs 26).

Subchronic Exposure Experiments. To examine the effects of petroleum products on early life stages of *B. viridis* its eggs were exposed to three concentrations of WSFO (0.05 mg/L, 0.5 mg/L, and 1.5 mg/L). The concentrations were chosen according to maximum permissible concentrations of oil hydrocarbons in water (MPCW) accepted in the Republic of Kazakhstan [23]. In our study, we used concentrations equal to MPCW (0.05 mg/L), and exceeding it by 10-fold (0.5 mg/L) and 30-fold (1.5 mg/L). *B. viridis* embryos were divided into 8 groups: control (declorinated water), WSFO and o-xylene at concentrations of 0.05 mg/L, 0.5 mg/L, and 1.5 mg/L. Each group consisted of 15 eggs at the Gs 8-11 stage in a Petri dish containing 15 ml of the corresponding medium: declorinated water (control), ethanol (solvent control), WSFO. Four replicates were set for each group (60 embryos in total for each group of the experiment). Embryos in each group were incubated at $23 \pm 2\,^\circ$ C for 7 days. The dishes were examined every 24 hours to identify and remove dead embryos and larvae, and to replace the media. The mortality was recorded daily. On day 7, the surviving larvae were euthanized in a buffered solution of the MS-222 anesthetic (Tricaine Methane Sulfonate; Sigma Aldrich) and fixed in formalin, and then examined for developmental disorders using a stereoscopic microscope (Motic DM 143, China).

Chronic Exposure Experiments. Chronic effects of WSFO on tadpoles of *B. viridis* were performed for 60 days. For the experiment, tadpoles capable of independent swimming and feeding were selected which corresponds to Gs 26 stage. Tadpoles were exposed to control (dechlorinated water)and 0.05 mg/L, 0.5 mg/L, and 1.5 mg/L WSFO. There were three replicates for each treatment group, each containing fifteen tadpoles. The tadpoles were placed into 18 L aquaria filled with 15 L of aerated dechlorinated water (t=$23 \pm 2\,^\circ$ C), and the appropriate concentrations of WSFO were added. The tadpoles were fed boiled lettuce and seaweed each day, *ad libitum*, and feces and food waste were removed daily. The water was replaced every two days, followed by the addition of appropriate concentrations of WSFO and o-xylene. At the end of the experiment (60 days), tadpoles were euthanized in a buffered solution of the MS-222 anesthetic (Tricaine Methane Sulfonate; Sigma Aldrich), weighed, photographed using a stereoscopic microscope (Motic DM 143, China) to measure the morphometric parameters (snout-vent length (SVL), and total body length). In addition, the occurrence of morphological abnormalities was also noted.

Statistical analyses. Data were analyzed for statistical significance with Fisher's exact test and one-way ANOVA using SPSS version 23 (IBM Inc., Chicago, USA), with α set as 0.05. To compare the survival rates in the control and experimental groups the Fisher's exact test was used. The morphometric measurements (SVL, total body length), and developmental stage (Gs), at the time of sampling were analyzed with one-way ANOVA followed by post-hoc test. The data were normally distributed (Levene's test). All data are presented as mean $\pm$ SEM.

Results

SubchronicExposure to WSFO

Exposure to WSFO caused high mortality of *B. viridis* embryos (Figure1)compared to control. Mortality of embryos in groups 0.05, 0.5, and 1.5 mg/L at the end of the experiment (168 hr) was 23, 37 and 52%, respectively.

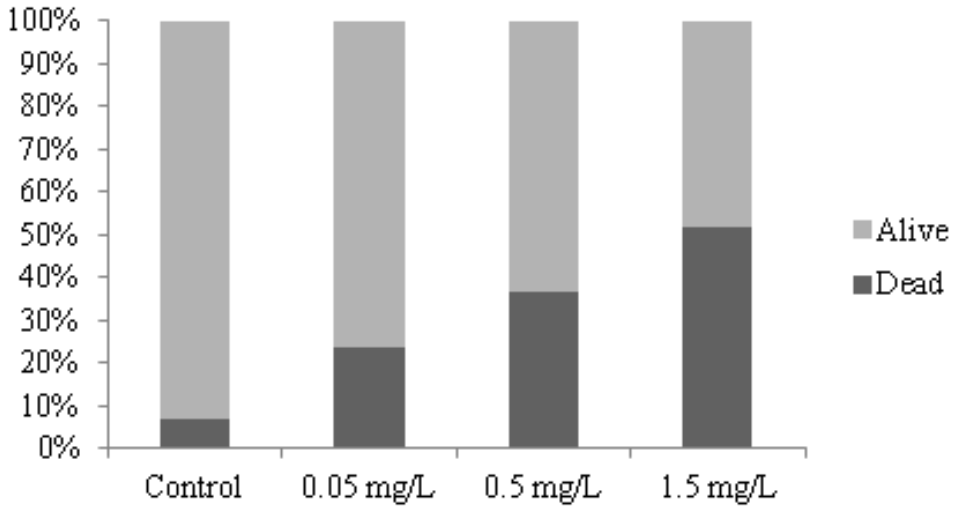

Figure 1 – **Survival rate of *B.viridis* embryos exposed to WSFO for 7 days**

Survived tadpoles in treatment groups had multiple morphological alterations (Figure 2). Dose-dependent effect was observed where the number of tadpoles with malformations raised from low (0.05 mg/L) to medium (0.5 mg/L) and high (1.5 mg/L) concentration groups (Figure 3). The most frequent observed malformations were axial curvature and edema.

Tadpoles from 0.05 mg/L group had the lowest level of abnormalities among treatment groups, and that level did not differ markedly from control. In 0.5 mg/L treatment group 38% of tadpoles had axial curvature, 21% of which also had edema and 7% had gut malformations, 13% had visceral edemas, 11% had different types of head malformations, including microphthalmia, mouth malformation, and eye malformation. There were also a few tadpoles with pigmentation alteration (Figure 2f). In 1.5 mg/L treatment group 14% of tadpoles had no malformations, 57% had axial curvature combined with edemas, 23% of which also had gut malformations, 19% had head malformations.

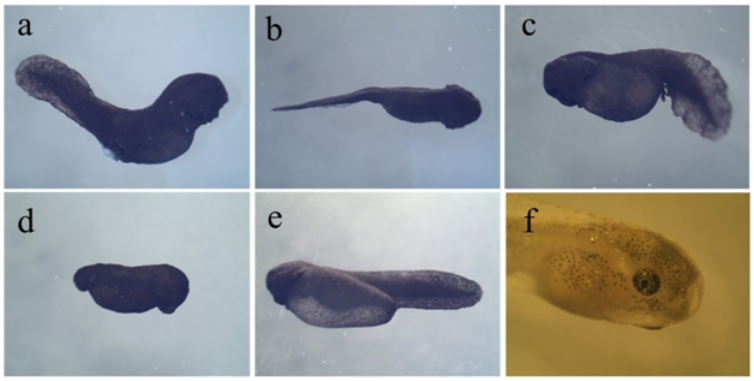

FIGURE 2 – **Morphological abnormalities in *B. viridis* tadpoles subchronically (7 days) exposed to WSFO: a -axial curvature (0.5 mg/L); b - axial curvature (0.05 mg/L); c - axial curvature and edema (1.5 mg/L); d – tail malformation and edema (1.5 mg/L); e – severe visceral edema (0.5 mg/L); f - pigmentation alteration (1.5 mg/L)**

ChronicExposure to WSFO

Survival rate was high among all experimental groups, except the highest concentration group (1.5 mg/L). Mortality was found to be 3% in control, 5% in 0.05 mg/L, 9% in 0.5 mg/L, and 22% in 1.5 mg/L. However, there were alterations in weight, SVL, total body length, developmental stage between treatment groups and control, especially when high concentration groups.

Similarly to results of subchronic experiments tadpoles from 0.05 mg/L WSFO group were not markedly different from controlin all examined parameters. Tadpoles exposed to 0.5 or 1.5 mg/L WSFO during 60 days demonstrateda significantly lower weight compared to control (Figure 3).

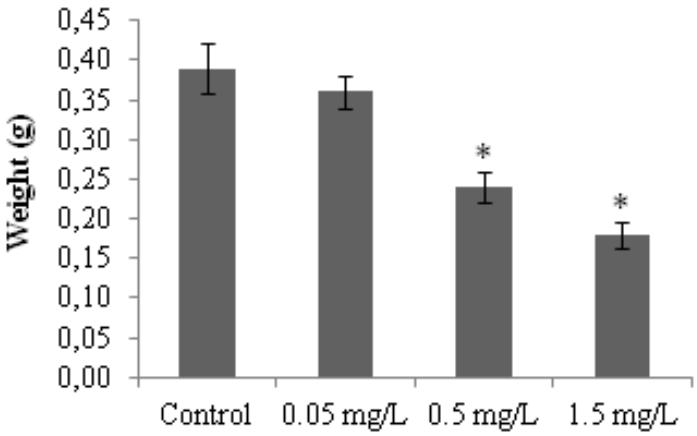

FIGURE 3 – **Weight of *B. viridis* tadpoles chronically (60 days) exposed to WSFO. Stars indicate $p < 0.01$**

SVL and total body length measurements (Figure 4a, b) exhibited a noticeable decrease in the WSFO compared with control. Tadpoles from0.5 or 1.5 mg/Lgroups were markedly smaller1.4-fold and 1.8-fold compared tocontrol, respectively. Developmental rate of tadpoles in 0.5 and 1.5 mg/Ltreatments was delayed by 5 and 8 stages, respectively(Figure 4c).

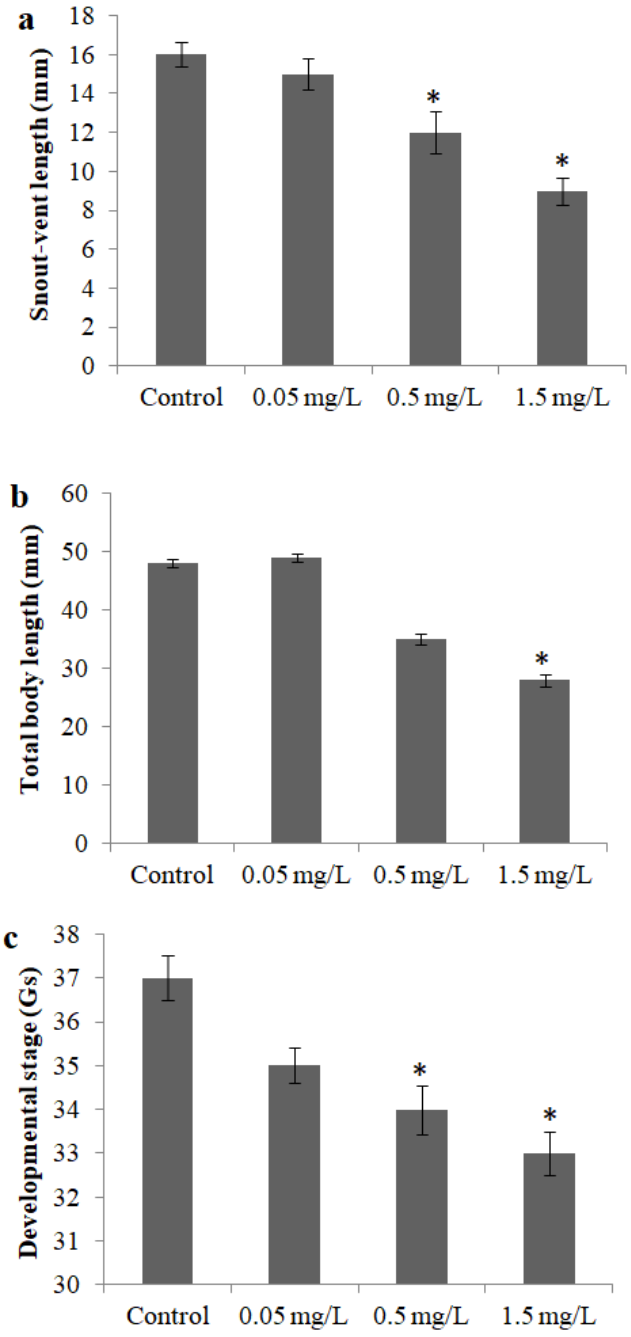

FIGURE 4 – **SVL (a), total body length (b) and developmental stage (c) of *B. viridis* tadpoles chronically (60 days) exposed to WSFO. Stars indicate $p < 0.01$**

Moreover, several tadpoles with axial curvature and visceral edema were observed (Figure 5).

Figure 5 – **Tadpole with axial curvature**

Discussion. Our data revealed that environmentally relevant concentrations of WSFO lead to a dose-dependent increase in mortality rate in *B. viridis* embryos. It was shown that PAHs, such as fluoranthene induce mortality in *X. laevis*, *L. pipiens* and *A. maculatum* tadpoles [19]. Low concentrations of commercial naphthenic acids also caused decrease in survival rate in *L. sylvaticus* tadpoles [24]. Different pollutants demonstrate simliar effects in other aquatic animals, such as amphipods [4]; mollusks [25], crustaceans [26], and fish [27, 28]. The sensitivity of *B. viridis* embryos petroleum products impact may be due to the fact that this specieslay eggs in small ponds, and embryos and tadpoles are not able to escape from a water body with unfavorable conditions [29]. The morphological deformities of *B. viridis* larvae detected in the present study are similar to those that found in other species of amphibians. In all species the most common malformations are developmental delay, axial curvature, gut deformities and edema [30-33]. There were several tadpoles in chronic experiment with axial curvature and edemas. Those tadpoles could not swim normally, mostly floated at the surface, and in case of fear they could only swim in circles. In nature tadpoles with this kind of abnormalities would become prey much earlier than healthy ones.Furthermore, it is notable that negative effects became more pronounced with growth of treatment concentrations. Moreover, it should be marked that tadpoles from chronic experiments were more resistible to WSFO contamination than those from subchronic experiments which can be seen from the results of survival rate measurement. It was shown that embryos and larvae of fish are more sensitive to toxic action of pollutants than juvenile and adult individuals [34]. Our data indicate similarity in amphibians.

The dose-dependent delay of *B. viridis* in growth was observed in chronic experiment where tadpoles demonstrated decrease in weight and linear parameters (SVL, total body length) between 0.05 and 1.5 mg/L WSFO.Furthermore, tadpoles in groups 0.5 and 1.5 mg/L showed developmental delay by 5-8 stages. Growth inhibitionis one of the first response to contamination in aquatic organisms as a need to compensate increased stress. This is not a specific indicator for petroleum products effects, but it is believed to be one of the most sensitive markers [33, 35, 36]. Moreover, some species may be more sensitive, and the composition of particular oil may play a significant role. Hydrophobicity is the main factor for availability of oil components for hydrobions [29].According to Erickson et al. [35],highly soluble hydrocarbons with low hydrophobicity are most accessible to aquatic organisms, while hydrocarbons with higher hydrophobicity exhibit a greater affinity for organic substances contained in sediments, which results in secondary water contamination.

The results of this work show that disruption of growth and development of amphibians tadpoles is an important indicator of environmental pollution. The increased mortality frequency is a general response of embryos and larvae to contamination with petroleum products, which indicates an adverse effect attributed to contaminants.

Conclusions. Data obtained in this work showed embryotoxic and teratogenic effects of WSFO from the Zhanazhol oil field (Aktobe region, Kazakhstan) on green toad (*B. viridis*). Subchronic experiments (7 days) demonstrated that *B. viridis* embryos and larvae are highly sensitive to petroleum contamination which is proved with high mortality and morphological deformities rate. Chronic exposure to WSFO (60 days) resulted in growth inhibition and developmental delay.

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Морфологические нарушения головастиков зеленой жабы (*Bufo viridis*), вызванные нефтепродуктами

Аннотация: Загрязнение окружающей среды нефтью и нефтепродуктами приводит к снижению биоразнообразия животных и заболеваниям человека. В связи с интенсивным загрязнением водоемов Казахстана, расположенных на территории нефтедобывающих регионов, целью данного исследования было изучение влияния различных концентраций водорастворимой фракции нефти (ВРФН) на рост и развитие зеленой жабы (*Bufo viridis*). Этот вид бесхвостых земноводных широко распространен в Казахстане, что особенно важно с учетом засушливости земель нефтедобывающих районов. Было проведено исследование по субхроническому и хроническому воздействию трех концентраций ВРФН на

головастиков зеленой жабы (*Bufo viridis*). Результаты исследования выявили подавление роста (размер и вес) и задержку развития у головастиков из экспериментальных групп в 1,6-1,8 раза. Кроме того, наблюдались пороки развития, такие как искривление позвоночника, отек, нарушение развития хвоста, головы, изменение пигментации. Таким образом, воздействие ВРФН подавляет рост и развитие зеленой жабы (*Bufo viridis*).

Ключевые слова: водорастворимая фракция нефти, *Bufo viridis*, рост, развитие, морфологические деформации.

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Мұнай өнімдерімен қоздырылған бақабастардың (*Bufo viridis*) морфологиялық бұзушылықтары

Аңдатпа: Қоршаған ортаның мұнай және мұнай өнімдерімен ластануы жануарлар биоалуантүрліліктің төмендеуі мен адамдардың ауруға ұшырауына акеледі. Осы зерттеудің мақсаты Қазақстанның мұнай шығаратын аймақтардың су айдындары күрт ластануына байланысты, әртүрлі концентрацияларда мұнайдың су ерігіш фракциялардың (МСЕФ) жасыл бақаның (*Bufo viridis*) өсуі мен дамуына әсерін зерттеу. Бұл аса маңызды мұнай шығаратың аудандардың құрғақшылығын ескерте отырып, құйрықсыз қос мекенділер түрі Қазақстанда кең таралған. Зерттеу жасыл құрбақалардың (*Bufo viridis*) итшабақтарына МСЕФ үш концентрация субхроникалы және хроникалы әсері бойынша жүргізілді. Зерттеудің нәтижелері бақабастардың эксперименталдық топтар өсімінің және дамуының (мөлшері және салмағы) 1,6-1,8 есеге дейін кешігуі анықталды. Бұған қоса, жұлынның қисаюы, ісіну, құйрық, бас дамуның бұзылуы және пигментациядағы өзгерістер байқалды. Осылайша, МСЕФ әсері жасыл бақабастардың (*Bufo viridis*) өсуіне және дамуына кедергі келтіреді .

Түйін сөздер: мұнайдың суда еритін фракциясы, Bufoviridis, өсу, даму, морфологиялық деформациялар.

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«Л.Н. Гумилев атындағы Еуразия ұлттық университетінің Хабаршысы. Биологиялық ғылымдар сериясы» журналында мақала жариялау ережесі

1. Журнал мақсаты. Биохимия, молекулалық биология, биотехнология, биоинформатика, вирусология, биофизика, биоинженерия, физиология, ботаника, зоология, эволюциялық биология, генетика, микробиология, биомедицина салалары бойынша мұқият тексеруден өткен ғылыми құндылығы бар мақалалар жариялау.

2. Журналда мақала жариялаушы автор мақаланың қол қойылған 1 дана қағаз нұсқасын Ғылыми басылымдар бөліміне (редакцияға, мекенжайы: 010008, Қазақстан Республикасы, Нұр-Сұлтан қаласы, Қ. Сәтбаев көшесі, 2, Л.Н. Гумилев атындағы Еуразия ұлттық университеті, Бас ғимарат, 409 кабинет) және *eurjourbio@enu.kz* электрондық поштасына PDF, Tex форматтарындағы нұсқаларын жіберу қажет. Мақаланұң мәтінінің қағаз нұсқасы мен электронды нұсқасұмен бірдей болуы қажет. Мақалалар қазақ, орыс, ағылшын тілдерінде қабылданады. Мақаланың тех фарматындағы үлгісі bulbio.enu.kz журнал сайытында берілген. Сонымен қатар, автор(лар) ілеспе хат ұсынуы керек.

3. Автордың қолжазбаны редакцияға жіберуі мақаланың Л.Н. Гумилев атындағы Еуразия ұлттық университеті Хабаршысында басуға және, шетел тіліне аударылып қайта басылуына келісімін білдіреді. Автор мақаланы редакцияға жіберу арқылы автор туралы мәліметтің дұрыстығына, мақала көшірілмегендігіне (плагиаттың жоқтығына) және басқа да заңсыз көшірмелердің жоқтығына кепілдеме береді.

4. Мақаланың көлемі 18 беттен аспауға тиіс (6 беттен бастап).

5. Мақаланың құрылымы

ҒТАМРК http://grnti.ru/

Автор(лар)дың аты-жөні

Мекеменің толық атауы, қаласы, мемлекеті (егер авторлар әртүрлі мекемеде жұмыс жасайтын болса, онда әр автор мен оның жұмыс мекемесі қасында бірдей белгі қойылу керек)

Автор(лар)дың E-mail-ы

Мақала атауы

Аңдатпа (100-200 сөз; формуласыз, мақаланың атауын мейлінше қайталамауы қажет; әдебиеттерге сілтемелер болмауы қажет; мақаланың құрылысын (кіріспе /мақаланың мақсаты/ міндеттері /қарастырылып отырған сұрақтың тарихы, зерттеу әдістері, нәтижелер/талқылау, қорытынды) сақтай отырып, мақаланың қысқаша мазмұны берілуі қажет).

Түйін сөздер (6-8 сөз не сөз тіркесі. Түйін сөздер мақала мазмұнын көрсетіп, мейлінше мақала атауы мен аннотациядағы сөздерді қайталамай, мақала мазмұнындағы сөздерді қолдану қажет. Сонымен қатар, ақпараттық-іздестіру жүйелерінде мақаланы жеңіл табуға мүмкіндік беретін ғылым салаларының терминдерін қолдану қажет).

Негізгі мәтін мақаланың мақсаты/ міндеттері/ қарастырылып отырған сұрақтың тарихы, зерттеу әдістері, нәтижелер/талқылау, қорытынды бөлімдерін қамтуы қажет.

Таблица, суреттер – аталғаннан кейін орналастырылады. Әр таблица, сурет қасында оның аталуы болуы қажет. Сурет айқын, сканерден өтпеген болуы керек.

Мақаладағы ***формулалар*** тек мәтінде оларға сілтеме берілсе ғана нөмірленеді.

Жалпы қолданыста бар ***аббревиатуралар*** мен ***қысқартулардан*** басқалары міндетті түрде алғаш қолданғанда түсіндірілуі берілуі қажет. ***Қаржылай көмек туралы*** ақпарат бірінші бетте көрсетіледі.

Әдебиеттер тізімі

Мәтінде әдібиеттерге сілтемелер тікжақшаға алынады. Мәтіндегі әдебиеттер тізіміне сілтемелердің нөмерленуі мәтінде қолданылуына қатысты жүргізіледі: мәтінде кездескен әдебиетке алғашқы сілтеме [1] арқылы, екінші сілтеме [2] арқылы т.с.с. жүргізіледі. Кітапқа жасалатын сілтемелерде қолданылған беттер де көрсетілуі керек (мысалы, [1, 45 бет]). Жарияланбаған еңбектерге сілтемелер жасалмайды. Сонымен қатар, рецензиядан өтпейтін басылымдарға да сілтемелер жасалмайды (әдебиеттер тізімінін әзірлеу үлгілерін төмендегі мақаланы рәсімдеу үлгісінен қараңыз).

Мақала соңындағы әдебиеттер тізімінен кейін ***библиографиялық мәліметтер*** орыс және ағылшын тілінде (егер мақала қазақ тілінде жазылса), қазақ және ағылшын тілінде (егер мақала орыс тілінде жазылса), орыс және қазақ тілінде (егер мақала ағылшын тілінде жазылған болса) беріледі.

Авторлар туралы мәлімет: автордың аты-жөні, ғылыми атағы, қызметі, жұмыс орны, жұмыс орнының мекенжайы, телефон, e-mail – қазақ, орыс және ағылшын тілдерінде толтырылады.

6. Қолжазба мұқият тексерілген болуы қажет. Техникалық талаптарға сай келмеген қолжазбалар қайта өңдеуге қайтарылады. Қолжазбаның қайтарылуы оның журналда басылуына жіберілуін білдірмейді.

7. Редакцияға түскен мақала жабық (анонимді) тексеруге жіберіледі. Барлық рецензиялар авторларға жіберіледі. Автор (рецензент мақаланы түзетуге ұсыныс берген жағдайда) үш күн аралығында қайта қарап, қолжазбаның түзетілген нұсқасын редакцияға қайта жіберуі керек. Рецензент жарамсыз деп танығаң мақала қайтара қарастырылмайды. Мақаланың түзетілген нұсқасы мен автордың рецензентке жауабы редакцияға жіберіледі.

8. Төлемақы. Басылымға рұқсат етілген мақала авторларына төлем жасау туралы ескертіледі. Төлем көлемі – ЕҰУ қызметкерлері үшін 4500 тенге және 5500 тенге басқа ұйым қызметкерлеріне.

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1.Purpose of the journal. Publication of carefully selected original scientific works in the fields of Biochemistry, Molecular Biology, Biotechnology, Bioinformatics, Virology, Biophysics, Bioengineering, Physiology, Botany, Zoology, Evolutionary Biology, Genetics, Microbiology, Biomedicine.

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4. The volume of the article should not exceed 18 pages (from 6 pages).

5. Structure of the article

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Initials and Surname of the author (s)

Full name of the organization, city, country (if the authors work in different organizations, you need to put the same icon next to the name of the author and the corresponding organization)

Author's e-mail (s)

Article title

Abstract (100-200 words, it should not contain a formula, the article title should not repeat in the content, it should not contain bibliographic references, it should reflect the summary of the article, preserving the structure of the article - introduction/ problem statement /goals/ history, research methods, results /discussion, conclusion).

Keywords (6-8 words/word combination. Keywords should reflect the main content of the article, use terms from the article, as well as terms that define the subject area and include other important concepts that make it easier and more convenient to find the article using the information retrieval system).

The main text of the article should contain an introduction/ problem statement/ goals/ history, research methods, results / discussion, conclusion. Tables, figures should be placed after the mention. Each illustration should be followed by an inscription. Figures should be clear, clean, not scanned.

In the article, only those ***formulas*** are numbered, to which the text has references.

All ***abbreviations***, with the exception of those known to be generally known, must be deciphered when first used in the text.

Information on ***the financial support*** of the article is indicated on the first page in the form of a footnote.

References

In the text references are indicated in square brackets. References should be numbered strictly in the order of the mention in the text. The first reference in the text to the literature should have the number [1], the second - [2], etc. The reference to the book in the main text of the article should be accompanied by an indication of the pages used (for example, [1, 45 p.]). References to unpublished works are not allowed. Unreasonable references to unreviewed publications (examples of the description of the list of literature, descriptions of the list of literature in English, see below in the sample of article design).

At the end of the article, after the list of references, it is necessary to indicate bibliographic data in Russian and English (if the article is in Kazakh), in Kazakh and English (if the article is in Russian) and in Russian and Kazakh languages (if the article is English language).

Information about authors: surname, name, patronymic, scientific degree, position, place of work, full work address, telephone, e-mail - in Kazakh, Russian and English.

6. The article must be **carefully verified**. Articles that do not meet technical requirements will be returned for revision. Returning for revision does not mean that the article has been accepted for publication.

7. Work with electronic proofreading. Articles received by the Department of Scientific Publications (editorial office) are sent to anonymous review. All reviews of the article are sent to the author. The authors must send the proof of the article within three days. Articles that receive a negative review for a second review are not accepted. Corrected versions of articles and the author's response to the reviewer are sent to the editorial office. Articles that have positive reviews are submitted to the editorial boards of the journal for discussion and approval for publication.

Periodicity of the journal: 4 times a year.

8. Payment. Authors who have received a positive conclusion for publication should make payment (for ENU employees - 4,500 tenge, for outside organizations - 5,500 tenge).

Положение о рукописях, представляемых в журнал «Вестник Евразийского национального университета имени Л.Н.Гумилева. Серия Биологические науки»

1. Цель журнала. Публикация тщательно отобранных оригинальных научных работ по следующим направлениям: биохимия, молекулярная биология, биотехнология, биоинформатика, вирусология, биофизика, биоинженерия, физиология, ботаника, зоология, эволюционная биология, генетика, микробиология, биомедицина.

2. Автору, желающему опубликовать статью в журнале необходимо представить рукопись в твердой копии (распечатанном варианте) в одном экземпляре, подписанном автором в Отдел научных изданий (по адресу: 010008, Казахстан, г.Нур-Султан, ул. Сатпаева, 2, Евразийский национальный университет им. Л.Н.Гумилева, Учебно-административный корпус, каб. 349) и по e-mail *eurjourbio@enu.kz* в формате Tex и PDF . При этом должно быть строго выдержано соответствие между Tex-файлом, PDF-файлом и твердой копией. Шаблон статьи в формате tex приведен на сайте журнала bulbio.enu.kz. Также автору(ам) необходимо предоставить сопроводительное письмо.

Язык публикаций: казахский, русский, английский.

3. Отправление статей в редакцию означает согласие авторов на право Издателя, Евразийского национального университета имени Л.Н. Гумилева, издания статей в журнале и переиздания их на любом иностранном языке. Представляя текст работы для публикации в журнале, автор гарантирует правильность всех сведений о себе, отсутствие плагиата и других форм неправомерного заимствования в рукописи, надлежащее оформление всех заимствований текста, таблиц, схем, иллюстраций.

4. Объем статьи не должен превышать 18 страниц (от 6 страниц).

5. Схема построения статьи

ГРНТИ http://grnti.ru/

Инициалы и Фамилию автора(ов)

Полное наименование организации, город, страна (если авторы работают в разных организациях, необходимо поставить одинаковый значок около фамилии автора и соответствующей организации)

E-mail автора(ов)

Название статьи

Аннотация (100-200 слов; не должна содержать формулы, не должна повторять по содержанию название статьи; не должна содержать библиографические ссылки; должна отражать краткое содержание статьи, сохраняя структуру статьи – введение/ постановка задачи/ цели/ история, методы исследования, результаты/обсуждения, заключение/выводы).

Ключевые слова (6-8 слов/словосочетаний. Ключевые слова должны отражать основное содержание статьи, использовать термины из текста статьи, а также термины, определяющие предметную область и включающие другие важные понятия, позволяющие облегчить и расширить возможности нахождения статьи средствами информационно-поисковой системы).

Основной текст статьи должен содержать введение/ постановку задачи/ цели/ историю, методы исследования, результаты/обсуждение, заключение/выводы.

Таблицы, рисунки необходимо располагать после упоминания. Каждой иллюстрации должна следовать надпись. Рисунки должны быть четкими, чистыми, несканированными.

В статье нумеруются лишь те ***формулы***, на которые по тексту есть ссылки.

Все ***аббревиатуры и сокращения***, за исключением заведомо общеизвестных, должны быть расшифрованы при первом употреблении в тексте.

Сведения о ***финансовой поддержке*** работы указываются на первой странице в виде сноски.

Список литературы

В тексте ссылки обозначаются в квадратных скобках. Ссылки должны быть пронумерованы строго по порядку упоминания в тексте. Первая ссылка в тексте на литературу должна иметь номер [1], вторая - [2] и т.д. Ссылка на книгу в основном тексте статьи должна сопровождаться указанием использованных страниц (например, [1, 45 стр.]). Ссылки на неопубликованные работы не допускаются. Нежелательны ссылки на нерецензируемые издания (примеры описания списка литературы, описания списка литературы см. ниже в образце оформления статьи).

В конце статьи, после списка литературы, необходимо указать ***библиографические данные*** на русском и английском языках (если статья оформлена на казахском языке), на казахском и английском языках (если статья оформлена на русском языке) и на русском и казахском языках (если статья оформлена на английском языке).

Сведения об авторах: фамилия, имя, отчество, научная степен, должность, место работы, полный служебный адрес, телефон, e-mail – на казахском, русском и английском языках.

6. Рукопись должна быть **тщательно выверена**. Рукописи, не соответствующие техническим требованиям, будут возвращены на доработку. Возвращение на доработку не означает, что рукопись принята к опубликованию.

7. Работа с электронной корректурой. Статьи, поступившие в Отдел научных изданий (редакция), отправляются на анонимное рецензирование. Все рецензии по статье отправляются автору. Авторам в течение трех дней необходимо отправить корректуру статьи. Статьи, получившие отрицательную рецензию, к повторному рассмотрению не принимаются. Исправленные варианты статей и ответ автора рецензенту присылаются в редакцию. Статьи, имеющие положительные рецензии, представляются редколлегии журнала для обсуждения и утверждения для публикации.

Периодичность журнала: 4 раза в год.

8.Оплата. Авторам, получившим положительное заключение к опубликованию необходимо произвести оплату (для сотрудников ЕНУ – 4500 тенге, для сторонних организаций – 5500 тенге).

Мақаланы рәсімдеу үлгісі

IRSTI 27.25.19

G.S. Mukiyanova [1] , A.Zh. Akbassova [1] , J. Maria Pozo [2] , R.T. Omarov [1]

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Tbsv encoded capcid protein p41 triggers resistance in solanum lycopersicum

Abstract: Efficient infection of Nicotiana benthamiana plants with wild type Tomato bushy stunt virus (TBSV) is influenced by expression of protein P19, which is a potent RNAi suppressor. The capsid protein (CP) P41 is required for virion formation and facilitates long distance movement of the virus. Along with RNAi suppression, P19 protein is involved in the development of severe disease symptoms in N. benthamiana and elicitation of Hypersensitive Response (HR) in tobacco. Our results show that wild type TBSV infection of Solanum lycopersicum (cv. Money maker) triggers resistance to the virus. Despite detectable accumulation levels of P19 protein in leaf and root tissues, the infection was not accompanied with obvious disease symptoms. Contrastingly, inoculation with TBSV mutant, lacking capsid protein P41 demonstrated susceptibility to TBSV. Moreover, Chl-FI analysis of plants infected with virus exhibited significant changes in metabolism. Our data suggests that in response to CP expression tomato plants have evolved defense mechanisms to resist viral infection.

Key words: Tomato bushy stunt virus, capsid protein, virions, resistance, Solanum lycopersicum.

TEXT OF THE ARTICLE

- The main text of the article should be divided into clearly defined and numbered sections (subsections). Subsections must be numbered 1.1, 1.2, etc. Required sections of the article:

1.Introduction should supply the rational of the investigation and its relation to other works in the same scope.

2. Materials and methods should be detailed to enable the experiments to be repeated. Do not include extensive details, unless they present a substantially new modification.

3. Results section may be organized into subheadings. In this section, describe only the results of the experiments. Reserve extensive interpretation for the Discussion section. Avoid combining Results and Discussion sections.

4. Discussion should provide an interpretation of the results in relation to previously published works.

5. Conclusion The main conclusions of the study can be presented in a short section "Conclusions".

6.Author contributions should indicate the individual contribution of authors to the manuscript.

7.Acknowledgments should be brief and should precede the References.

8.Funding the source of any financial support received for the work being published must be indicated.

Ethics approval Manuscripts reporting animals and/or human studies must that relevant Ethics Committee or Institutional Review Board include provided or waived approval.

Tables

Tables must be placed next to the relevant text in the article. Number tables consecutively in accordance with their appearance in the text and place any table notes above the table body.

Таблица 1 – **Title of table**

Prime	Nonprime numbers
2, 3, 5, 7, 11, 13, 17, 19, 23, 29	4, 6, 8, 9, 10, 12, 14

Figures

Figures must be saved individually and separate to text. All figures must be numbered in the order in which they appear in the article (e.g. figure 1, figure 2). In multi-part figures, each part should be labeled (e.g. figure 1(a), figure 1(b)). Figures must be of sufficiently high resolution (minimum 600 dpi). It is preferable to prepare figures in black-and-white or grey color scale. Figures should be clear, clean, not scanned (PS, PDF, TIFF, GIF, JPEG, BMP, PCX).

Рисунок 1 – **Title of figure**

References

1 Alazem M., Lin N. Roles of plant hormones in the regulation of host-virus interactions // Mol Plant Pathol. - 2015. - V. 16, № 5. - P. 529-40. doi: ... (if available) - **Journal article**

2 Abimuldina ST, Sydykova GE, Orazbaeva LA Functioning and development of the infrastructure of sugar production // Innovation in the agricultural sector of Kazakhstan: Mater. Intern. Conf., Vienna, Austria, 2009. - Almaty, 2010. - P. 10-13 - **Proceedings of the conferences**

3 Kurmukov A.A. Angioprotective and lipid-lowering activity of leukomycin. - Almaty: Bastau, 2007. - S. 3-5 - **newspaper articles**

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5 Petushkova G.I. Costume Design: Textbook. for universities / G.I. Petushkova. - Moscow: Academy, 2004. - 416 p. - **the book**

6 Кусаинова А.А., Булгакова О.В., Берсимбаев Р.И. Роль miR125b в патогенезе рака легкого // Прикладные информационные аспекты медицины. - 2017. -Т. 20. - №4. -С. 86-92. - **Journal article**

Г.С. Мукиянова [1], А.Ж. Акбасова [1], М.Х. Позо [2], Р.Т. Омаров [1]

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Solanum lycopersicum өсімдігінде резистенттілік жауаптың tomato bushy stunt virus (tbsv) вирусының p41 капсидтік ақуызымен белсендірілуі

Аннотация. Tomato bushy stunt virus (TBSV) вирусымен кодталатын P19 ақуызы РНҚ интерференцияның қуатты супрессоры болып табылады және Nicotiana benthamiana өсімдіктерінің вируспен жұқтырылуында маңызды рөл атқарады. P19 ақуызының экспрессиясы вируспен зақымдануы айқын көрініс береді де, өсімдіктің толық коллапсына әкеліп соқтырады. Сонымен қатар супрессорлық P19 ақуызы Nicotiana tabacum өсімдігінде гиперсезімталдық реакциясын белсендіруге жауапты. Вирустың P41 капсидтік ақуызы вирион құрылымын қалыптастырып, өсімдік бойымен таралауын қамтамасыз етеді. Алынған зерттеу нәтижелері TBSV вирусының жабайы типінің инфекциясы Solanum lycopersicum (Money maker сұрыбы) қызанақ өсімдігінде вирусқа қарсы төзімділік жауабын тудыратынын анықтады. Өсімдіктің тамыр және жапырақ ұлпасында P19 ақуызының жинақталуына қарамастан вируспен зақымдалудың сыртқы көрінісі нашар байқалды. Алайда, Chlorophyll Fluorescence Imaging system (Chl-FI) сараптамасы вируспен зақымдалған өсімдіктерде жасушаішілік

метаболизмінің өзгеруін анықтады. Ал вирустың капсидтік ақуызы экспрессияланбайтын мутантпен инфекция тудырғанда, қызанақ өсімдіктері жоғары сезімталдық көрсетіп, жүйелік некрозға ұшырады. Зерттеу нәтижелері қызанақтың Money maker сұрыбында TBSV вирусына қарсы қорғаныс механизмдері вирустық капсидтік ақуыз P41-ді тану арқылы белсендірілетінін көрсетеді.

Түйін сөздер: Tomato bushy stunt virus (TBSV), вирус, капсидтік ақуыз, вирион, Solanum lycopersicum, резистенттілік, РНҚ-интерференция.

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Капсидный белок p41 вируса tomato bushy stunt virus (tbsv) активирует резистентность у растений вида solanum lycopersicum

Аннотация. Кодируемый вирусом Tomato bushy stunt virus (TBSV), белок P19 является мощным супрессором РНК интерференции и играет важную роль при инфекции растений Nicotiana benthamiana, которая характеризуется ярко выраженными симптомами заболевания и системным коллапсом. Кроме того, белок P19 является элиситором гиперчувствительного ответа у Nicotiana tabacum. Капсидный белок вируса P41 формирует вирионы и способствует развитию системной инфекции. Полученные нами данные показали, что при инфекции диким типом TBSV у растений вида Solanum lycopersicum (сорт Money maker) активируется резистентный ответ. Несмотря на системную аккумуляцию белка супрессора P19 в листьях и корнях, у растений не проявляются видимые симптомы заболевания. Однако анализ Chlorophyll Fluorescence Imaging system (Chl-FI) показал, что в инфицированных вирусом растениях происходят значительные изменения метаболизма. Более того, инфекция растений мутантом TBSV по капсидному белку приводит к системному некрозу гибели растений. Полученные данные указывают на то, что у томатов выработаны защитные механизмы в ответ на экспрессию капсидного белка P41 вируса TBSV.

Ключевые слова: Tomato bushy stunt virus (TBSV), капсидный белок, вирион, Solanum lycopersicum, резистентность, РНК-интерференция.

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