Soil nematode *Caenorhabditis elegans* Maupas as a convenient model organism to study anthelmintic activity of plant extracts

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Abstract. The article deals with the study of the possibility to use free-living soil nematode *C. elegans* in researches of anthelmintic activity of plant raw material. *A. sativum* juice and aqueous extract of *T. vulgare* flowers were used in experiments. It is shown that both *A. sativum* juice and tansy flowers extract caused dose-dependent disturbances of *C. elegans* swimming motor program. These disturbances are similar with disturbances caused by agonist of cholinoreceptors levamisole. Therefore, the target of *A. sativum* juice and *T. vulgare* extract action is *C. elegans* cholinergic system. The mechanism of toxic action of *A. sativum* and *T. vulgare* on *C. elegans* organism consists in hyperactivation of nicotinic cholinoreceptors. The possibility of quick adaptation of *C. elegans* nicotinic cholinoreceptors to active components of tansy flowers extract was revealed. Soil nematode *C. elegans* may be used for investigation of anthelmintic activity of *A. sativum* and *T. vulgare* for the purpose of identification of secondary metabolites responsible for the toxic action on helminths.

1 Introduction

One of the most common diseases of farm and domestic animals are helminthiases. Animals infected by helminths decline their producing capacity. Helminths infections make animals more vulnerable to other diseases owing to immunity impairment. Helminthiases may cause lesion of eyes, gastrointestinal tract, cardiovascular and respiratory systems of humans. From the middle of XX century synthetic drugs of different chemical groups are generally used for treatment helminthiases of human and animals. These drugs are agonists of nicotinic acetylcholine receptors (levamisole, pyrantel), macrocyclic lactones (ivermectin), and benzimidazoles (albendazole, mebendazole) [1]. One of consequences of routine usage of synthetic anthelmintic drugs is appearance of drug resistance in parasites. The first reports about the decrease in effectiveness of many anthelmintic drugs appeared in 1960s, and in

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1980s the problem of drug resistance of parasites was recognized as really existent [2]. At the same time, we should not forget about possible by-effects of these drugs on organisms of homoiotherms. At present the so-called organic agriculture is intensively developed in many countries, and one of its principles is animal breeding without usage of synthetic drugs. On organic farms grazings are not treated by nematicides, therefore, they are favorable environment for distribution of helminthiases among farm animals. An alternative for regular application of synthetic anthelmintic drugs may be usage of plant juices and plant extracts. In folk medicine and ethnoveterinary such plants as tansy flowers, wormwood herb, garlic bulbs, pumpkin seeds, caraway seeds et al. are used for treatment and prevention of helminthiases. Plants with immunomodulatory and laxative action are often added to multicomponent anthelmintic mixtures [3].

One of the most popular plants using in folk medicine and ethnoveterinary for treatment of helminthiases is tansy *Tanacetum vulgare* L. Tansy flowers contain essential oils, flavonoids and other biologically active substances. The mechanism of anthelmintic action of tansy consists in damage of nervous system, which leads to decrease in locomotion activity followed by paralysis and death of helminths. In some cases, morphological changes in tegument of parasites are observed [3].

Garlic *Allium sativum* L. is often used for treatment and prevention of helminthiases on organic farms in many countries. Garlic contains more than 30 sulfur compounds, about 20 amino acids, enzymes and inorganic substances, specifically selenium [4]. The question about mechanisms of anthelmintic action of *A. sativum* is still open. Secondary metabolites of garlic, determining its negative action on parasites are not identified by now. The most biological activity possesses allicin, which is synthesized from alliin when garlic bulbs are mechanically crushed [4]. There is lack of direct evidences of allicin anthelmintic activity. Moreover, inefficiency of allicin for treatment ascariasis in chickens was shown [5].

Mechanism of antiparasitic action of juices and extracts of plants are studied not enough because of difficulties of carrying out experiments with parasitic nematodes in vitro. Several last decades for studying mechanisms of action of synthetic nematicides free-living soil nematode *Caenorhabditis elegans* is successfully used as model organism. *C. elegans* is one of the most popular model organisms in molecular genetics, molecular and cell biology from the middle of 1960s. From 1990s this nematode is intensively used in toxicological researches in order to study mechanisms of neurotoxic action of pesticides, heavy metals, et al. Attractivity of *C. elegans* as model organism is determined by simplicity and low cost of its cultivation in laboratory. Short lifespan (nematode becomes adulthood for three days), high breeding power (up to 300 offspring from one worm) and compact size of laboratory culture (nematodes are grown in Petri dishes) allows to maintain in laboratory large number of *C. elegans* strains and use in experiments practically unrestricted amount of animals of the same age [6]. Well-studied functions of single cells along with great amount of mutant strains makes it possible to study mechanisms of action of drugs and toxicants. The anatomy, physiology and neurochemistry of *C. elegans* organism are similar with such of parasitic nematodes, and so it is successfully used to study mechanisms of action of anthelmintic drugs [7]. In addition, experiments with *C. elegans* are quite harmless for researcher.

There is lack of information about toxicity of plant juices and plant extracts for *C. elegans* organism in scientific literature. Therefore, the aim of this work was to check the possibility to use *C. elegans* organism for investigation of anthelmintic activity of plant raw material.

2 Materials and methods

Experiments were performed with *C. elegans* wild type strain N2 received from Caenorhabditis Genetics Center. Nematodes were grown on standard nematode growth medium (3 g/L NaCl, 17 g/L Bactoagar, 2.5 g/L Bactopeptone, 5 mg/L cholesterol, 1 mM

CaCl₂, 1mM MgSO₄, 25 mM potassium phosphate buffer (pH=6.0)) in Petri dishes 100 mm in diameter [6]. *E. coli* OP50 were used for nematodes feeding [6]. All experiments were performed in NG buffer (3 g/L NaCl, 1 mM CaCl₂, 1 mM MgSO₄, and 25 mM potassium phosphate buffer (pH=7.0)) [6] at 22°C with nematodes incubated individually in glass tubes with flat bottom (one nematode in 1 mL of NG buffer). For each experiment nematodes were three times rinsed of growth medium, bacteria and exometabolites in glass centrifuge tubes 10 mL in volume. The indexes of toxic effects of *A. sativum* juice and *T. vulgare* extract on *C. elegans* organism were disturbances of motor program of nematodes swimming, as induced by mechanical stimulus, and nematodes paralysis (full loss of swimming ability). Behavior disturbances were recorded after 30, 60 and 90 minutes using stereomicroscope SMZ-05.

A. sativum juice was pressed out from crushed bulbs through filter made from grinding gauze. After that juice was repeatedly filtered through grinding gauze with pores 35 µm in diameter. Juice was stored at temperature -18°C. Juice was defrosted just before the experiment.

To prepare aqueous extract *T. vulgare* 15 g of dried tansy flowers embedded in 200 mL of hot distilled water were kept in boiling water bath for 30 minutes. Then filtrate was cooled down at room temperature and filtered through grinding gauze with pores 35 µm in diameter. After filtration through paper filter filtrate was evaporated in water bath with temperature 60°C till end volume of 10 mL. Ready extract was stored at temperature -18°C and defrosted just before the experiment.

Fig 1. Toxic action of *A. sativum* juice and levamisole on *C. elegans* behavior.

Ordinate shows the percentage of nematodes with disturbances of swimming.

Levamisole hydrochloride was obtained from Sigma Aldrich. Statistical analysis was performed using the ϕ^* criterion of Fisher's angle-transformation.

3 Results and discussion

Nervous system is the target for action of most toxicants on animals' organisms. Synthetic anthelmintic drugs cause paralysis of pharynx and body of nematodes interacting with glutamate-gated chloride channels (ivermectin and its derivatives), disrupt carbohydrate metabolism (albendazole and mebendazole), and affect nematodes cholinergic system by change of the effectiveness of cholinergic synaptic transmission (levamisole, pyrantel et al.) [1]. The most abundant group of synthetic nematicides consists in substances affecting cholinergic system. These substances may influence either on acetylcholine secretion or on sensitivity of receptors to acetylcholine [1, 7–8]. The nematodes nervous system may be a target for toxic action of *A. sativum* juice and *T. vulgare* aqueous extract. To check this assumption, experiments were performed in which the influence of *A. sativum* juice and *T. vulgare* aqueous extract on nematodes locomotion was studied.

Fig. 2. Sensitization of *C. elegans* behavior to toxic action of levamisole by *A. sativum* juice. Ordinate shows the percentage of paralyzed nematodes after 60 and 90 minutes of incubation with toxicants.

A. sativum juice in concentrations 2.5–10.0% caused disturbances of *C. elegans* swimming. Primarily these disturbances revealed in disorders of coordination of muscle contraction and relaxation necessary for sinusoidal movements during swimming. In this case all nematodes sustained the ability to swimming as induced by mechanical stimulus. These disturbances of locomotion were revealed in 15 and 20% nematodes after 30-minutes incubation in NG buffer with 5 and 10% of *A. sativum* juice respectively and after 90-minutes in 21% nematodes in NG buffer with 2.5% *A. sativum* juice (fig. 1). Similar with *A. sativum* juice influence on *C. elegans* locomotion had agonist of nicotinic acetylcholine receptors (nAChRs) levamisole, which is used for treatment helminthiasis of human and animals. Levamisole in concentrations 15, 30 and 60 μ M after 30-minutes exposition caused disturbances of motor program of swimming as induced by mechanical stimulus, in 28, 41 and 62% nematodes respectively. Further increase of time course of exposure to levamisole caused disturbances of swimming in 100% nematodes after 90 minutes at levamisole concentration 60 μ M and after 120 minutes at concentration 30 μ M (fig. 1). The increase of levamisole concentration up to 120 μ M and exposure time up to 90 minutes resulted in paralysis of nematodes (full inability to sustain swimming) (fig. 2).

Similarities between negative action of levamisole and *A. sativum* juice on nematodes behavior may be a consequence of similar mechanism of their toxic action on *C. elegans* organism. To check this assumption, we performed experiments in which joint action of

levamisole and *A. sativum* juice on *C. elegans* swimming was studied. *A. sativum* juice in concentration 5% after 15-minutes exposure did not cause any disturbances of nematodes swimming. When nematodes were incubated for 15 minutes in the medium containing 30 μ M of levamisole, addition of 5% of *A. sativum* juice increased the percentage of nematodes with disturbances of swimming as induced by mechanical stimulus from 13 up to 46%. The addition of 5% of *A. sativum* juice into the medium containing 120 μ M of levamisole increased from 52 up to 90% the percentage of nematodes with disturbances of locomotion after 15-minutes incubation with toxicants (data not shown). *A. sativum* juice in concentration 5 and 10% increased the percentage of paralyzed nematodes after incubation with 30–120 μ M levamisole during 60 or 90 minutes (fig. 2).

It is known that levamisole disturbs nematodes swimming due to binding with nAChRs in neural circuit regulating locomotion or in body muscles. In both cases the hypercontraction of locomotory muscles occurs leading to failure in coordination of movements during swimming, and under more continuous impact – to nematodes paralysis [9–10]. Results of our experiments give the evidence that *A. sativum* juice contains components which increase nAChRs sensitivity to levamisole action. The existence of lag-phase in *A. sativum* juice action on *C. elegans* organism allows to suppose the presence in it of some components with opposite action upon nAChRs.

Table 1. Action of aqueous extract of *T. vulgare* on *C. elegans* behavior.

Experimental conditions	The percentage of nematodes with disturbances of motor program of swimming, %			
	Time course of nematodes exposition to <i>T. vulgare</i> extract, minutes			
	15	30	60	90
5% extract of <i>T. vulgare</i>	30.0±3.7	40±4.0	50.0±4.1	56.5±4.0
10% extract of <i>T. vulgare</i>	43.5±4.0	50.0±4.1	56.5±4.0	60.0±4.0

Aqueous extract of tansy flowers in concentrations 5 and 10% caused disturbances of motor program of swimming in 30 and 43.5% nematodes respectively after 15-minutes exposure. When exposure time was increased up to 90 minutes, the percentage of nematodes with disturbances of motor program of swimming rose along with reduction of differences between toxicant concentrations (table 1). Aqueous extract of *T. vulgare* as *A. sativum* juice increased the negative levamisole action on *C. elegans* behavior (fig. 3). Therefore, it is possible to suppose the presence in tansy flowers extract of some components, which activate *C. elegans* nAChRs. Equalizing of differences between effects of different concentration of *T. vulgaris* extract on nematodes locomotion may be explained by the presence in extract of active components with opposite effects on *C. elegans* behavior. The second possible explanation of this phenomenon may be quick adaptation of *C. elegans* nAChRs to active components containing in tansy flowers extract. In favor of this explanation is the absence of nematodes paralysis after long-term (4-hours) incubation of *C. elegans* with tansy flowers extract (data not shown).

Fig. 3. Sensitization of *C. elegans* behavior to toxic action of levamisole by *T. vulgare* extract. Ordinate shows the percentage of paralyzed nematodes after 16 and 90 minutes of incubation with toxicants.

4 Conclusions

1. Soil nematode *C. elegans* may be used for studying anthelmintic activity of *A. sativum* and *T. vulgare* in order to identify secondary metabolites, determining toxic action on helminths.

2. In experiments with soil nematode *C. elegans* it was shown that *A. sativum* juice and aqueous extract of *T. vulgare* flowers cause dose-dependent disturbances of motor program of nematodes swimming.

3. The target of toxic action of *A. sativum* juice and aqueous extract of *T. vulgare* flowers on *C. elegans* organism is cholinergic system of nematodes.

4. The mechanism of anthelmintic action of *A. sativum* juice and aqueous extract of *T. vulgare* flowers consists in activation of nicotinic cholinoreceptors.

5. In experiments with *C. elegans* the possibility of quick adaptation of *C. elegans* nicotinic cholinoreceptors to active components of aqueous extract of tansy flowers was revealed.

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