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STUDY OF PHYTOTOXICITY OF BLACK TEA WITH HERBAL AND FRUIT SUPPLEMENTS BY GROWTH TEST WITH *LEPIDIUM SATIVUM* L.

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ДОСЛІДЖЕННЯ ФІТОТОКСИЧНОСТІ ЧОРНОГО ЧАЮ З ТРАВ'ЯНИМИ ТА ФРУКТОВИМИ ДОБАВКАМИ ЗА РОСТОВИМ ТЕСТОМ З *LEPIDIUM SATIVUM* L.

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ABSTRACT

Tea can contain biologically active compounds which phytotoxicity deserves attention.

The aim of the work was to study the phytotoxicity of black tea with herbal and fruit additives according to the phytotest with *Lepidium sativum* L.

Methodology. Phytotoxicity was evaluated in a growth test with the test plant *L. sativum*, the seeds of which were germinated on filter paper with the tested tea solutions. We studied 6 types of packaged black Ceylon tea without additives (control) and with various herbal and/or fruit additives and natural flavorings, freely available in the retail network of Ukraine. The test indicators were the germination energy of *L. sativum* seeds (3rd day of the study), seed germination (5th day of the study), the length of the roots and the aerial part of the seedlings (the 5th day of the study). Phytotoxic indexes were calculated – seed germination index and root length index. The results were processed statistically.

Scientific novelty. The phytotoxicity of black tea with herbal and fruit supplements was investigated using a growth test with *L. sativum*. The absence of phytotoxicity of the investigated tea variants was shown by the root length index of *L. sativum* seedlings.

Conclusions. The study of phytotoxicity of black tea with herbal and fruit supplements according to the growth test with *L. sativum* showed moderate toxicity based on the plant seed germination index in case of the mixture of black and green teas with supplements of apple, cornflowers, pot marigold, safflower, rose and natural flavoring «Grapes». However, according to the root length index of the seedlings, this tea did not show phytotoxicity, as did other tested variants of black tea with herbal and fruit additives. Further detailed phytotoxicity studies of all constituent teas with herbal and fruit supplements are needed. The risks of cornflower and safflower consumption by women should be indicated on the packaging of products that contain them.

Key words: fruit supplements, herbal supplements, *Lepidium sativum*, phytotesting, tea

АНОТАЦІЯ

Чай може містити біологічно активні сполуки, фітотоксичність яких заслуговує на увагу.

Метою роботи було дослідження фітотоксичності чорного чаю з трав'яними та фруктовими добавками за фітотестом з *Lepidium sativum* L.

Методологія. Фітотоксичність оцінювали у ростовому тесті з тест-рослиною *L. sativum,* насіння якої пророщували на фільтрувальному папері з досліджуваними розчинами чаю. Досліджували 6 видів пакетованого чорного цейлонського чаю без добавок (контроль) та з різними трав'яними та/або фруктовими добавками та природними ароматизаторами, вільно доступні у торговельній мережі України. Тест-показниками було обрано енергію проростання насіння *L. sativum* (3-я доба дослідження), схожість насіння (5-а доба дослідження), довжину коріння та надземної частини проростків (5-а доба дослідження). Розраховували фітотоксичні індекси – індекс схожості насіння та індекс довжини коріння. Результати оброблено статистично.

Наукова новизна. Досліджено фітотоксичність чорного чаю з трав'яними та фруктовими добавками за ростовим тестом з *L. sativum*. Показано відсутність фітотоксичності досліджуваних варіантів чаю за індексом довжини коріння проростків *L. sativum*.

Висновки. Дослідження фітотоксичності чорного чаю з трав'яними та фруктовими добавками за ростовим тестом з *L. sativum* показало помірну токсичність за індексом схожості насіння тест-рослини для чаю, що є сумішшю чорного та зеленого чаїв з добавками яблука, волошок, нагідок, сафлору, троянди та натурального ароматизатора «Виноград». Проте за індексом довжини коріння проростків зазначений чай не проявив фітотоксичності, як й інші досліджувані варіанти чорного чаю з трав'яними та фруктовими добавками. Потрібні подальші детальні дослідження фітотоксичності всіх складових чаїв з трав'яними та фруктовими добавками. Ризики вживання волошки та сафлору жінками повинні бути вказані на упаковці продуктів, які їх містять.

Ключові слова: *Lepidium sativum*, трав'яні добавки, фітотестування, фруктові добавки, чай

Introduction

Tea made from the dried leaves of *Camellia sinensis* L. is the second most consumed beverage in the world after water (McKay & Blumberg, 2002). Tea from China, India and Sri Lanka, countries where current regulations of pesticide use, worker protection and environmental pollution often fall short of international standards, consistently show pesticide residues (Naik, 2015). Pesticide residues, including chlorpyrifos, were recently detected in Ukraine (Chernivtsi region) in jasmine (originating from China) used in various tea blends exported from the Federal Republic of Germany (Shevchenko, 2024). The Food and Drug Administration (in the USA) constantly detects a high level of prohibited compound residues in imported tea that reaches the American consumer. In particular, they detect permethrin (a synthetic pyrethroid linked to cancer and endocrine disruption), DDE (a metabolite of DDT, banned in the US in 1972), heptachlor epoxide (a derivative of the pesticide heptachlor, which was banned in the US for use in agriculture as itself as a termiticide, due to its carcinogenicity and persistence in the environment), as well as acetamiprid (a neonicotinoid, toxic to bees). Given these concerns, consumers should choose certified and labeled organic products that prohibits residual pesticides and is verified for producers' compliance with organic management systems plans and contains only permitted substances (Naik, 2015). Recently, it has been found that heavy metals are a serious problem because they are highly absorbed by tea plants, causing significant risk of tea contamination (Han et al., 2005; Shi et al., 2008; Schwalfenberg et al., 2013).

The toxicity and safety of a number of teas and herbal supplements were tested using a bioassay method (Dosumu et al., 2010; Lee et al., 2019; Li et al., 2019; Rojo et al., 2020; Sotiropoulou et al., 2020; Sunandar & Alfarabi, 2021). Thus, using *Vibrio fischeri* as a test organism, toxicity was shown for aqueous extracts of chamomile (*Matricaria chamomilla* L., syn: *Matricaria recutita*) and sage (*Salvia officinalis* L.), which are popular in herbal teas (Sotiropoulou et al., 2020). When studying the toxicity of different types of tea using shrimp (*Artemia salina* L.) as a test object, they were found to be safe (Dosumu et al., 2010). The highest toxicity was noted for high concentrations of caffeinated Tetley tea, decaffeinated Tetley tea, and raw lemongrass, and the authors emphasize the need for pre-dilution before consumption (Dosumu et al., 2010). In a shrimp (*Artemia salina* L.) bioassay, it was shown that taxus herbal teas brewed at 75°C had a greater toxic effect (290.58 ppm) compared to teas brewed at 95°C (536.38 ppm) (Sunandar & Alfarabi, 2021). The nontoxic safety of fermented green tea with *Aquilariae Lignum* was shown in a toxicological experiment with mice or rats (Lee et al., 2019). It has also been shown on rats that huanghuali (*Dalbergia odorifera* T. Chen) tea shows no obvious toxicity (Li et al., 2019). Oral administration of a concentrated mixture of green tea polyphenols to mice potentiated the toxic effects caused by intraperitoneal administration of ebulin f. The obtained conclusions raise questions about the abuse of concentrated extracts of green tea as food antioxidant supplements, in particular, when using medical drugs affecting the gastrointestinal tract or taking toxic compounds (Rojo et al., 2020). It is known that a sensitive plant for toxicological experiments is *Lepidium sativum* L. (Khan et al., 2018; Bożym et al., 2021; Tkachuk et al., 2022; Tkachuk & Zelena, 2023). Therefore, the aim of this study was to investigate the phytotoxicity of black tea with herbal and fruit supplements by phytotesting with *L. sativum*.

Materials and methods

Test plant and research samples

Lepidium sativum was chosen as the test plant, the seeds of which were placed in a quantity of 10 pieces in a Petri dish on filter

paper, which was moistened with the investigated tea solution. The experiment was repeated three times. We studied 6 types of packaged black Ceylon tea without additives (control) and with various herbal and/or fruit additives and natural flavorings, freely available in the retail network of Ukraine. The investigated tea solutions were prepared as specified by the manufacturer: 1 tea bag (2 g of tea) was poured with 200 ml of boiled water and infused for 3-5 minutes; then the solution was cooled to room temperature and used in further research. The names of the researched teas and their manufacturers are withheld to prevent accusations of advertising/anti-advertising. The studied teas are conventionally marked from T1 (control) to T6. The composition of the studied teas (according to the manufacturer) is as follows:

T1 – small Ceylon black tea;

T2 – small Ceylon black tea, small Chinese green tea (10 %), chopped apple (1.5 %), flower petals (cornflowers, pot marigold, safflower, roses) (1.5 %), natural flavoring «Grapes»;

T3 – small Ceylon black tea, crushed raspberry berries (5 %), safflower flower petals (1 %), natural flavoring «Raspberry», natural flavoring «Vanilla»;

T4 – small Ceylon black tea, chopped berries in an equal portion (strawberry, rose hip) (5 %), natural flavoring «Forest Berries»;

T5 – small Ceylon black tea, small Chinese green tea (10 %), crushed strawberries (1.5 %), cornflower petals (0.5 %), natural flavoring «Strawberry»;

T6 – small Ceylon black tea, chopped fruits in equal portions (pineapple and peach) (5 %), orange peel (3 %), safflower petals (1 %), natural flavoring «Peach», natural flavoring «Passion fruit».

Test indicators and phytotoxic indices

The test parameters were the germination energy of *L. sativum* seeds (3rd day of the study), seed germination (5th day of the study), the length of the roots and the aerial part of the seedlings (5th day of the study) (Tkachuk et al., 2022). Phytotoxic indices were calculated – seed germination index (SGI) and root length index (RLI) (Bagur-González et al., 2011; Tkachuk & Zelena, 2022; Tkachuk et al., 2022):

$$SGI = \frac{N_t(i) - N_c}{N_c} \quad (1)$$

$$RLI = \frac{L_t(i) - L_c}{L_c} \quad (2)$$

where $N_t(i)$ and N_c – the number of germinated seeds in experiment (i) and control, respectively; $L_t(i)$ and L_c – the average root length in experiment (i) and control, respectively.

A scale was used to assess phytotoxicity (Bagur-González et al., 2011):

−0.25 ≤ SGI or RLI < 0 – slight toxicity;
−0.5 ≤ SGI or RLI < −0.25 – moderate toxicity;
−0.75 ≤ SGI or RLI < −0.5 – high toxicity'
−1 ≤ SGI or RLI < −0.75 – extreme toxicity.

Statistical data processing

The obtained results were processed statistically (the arithmetic mean, the error of the arithmetic mean, the significance of the differences of the arithmetic means were determined), as previously described (Tkachuk & Zelena, 2023).

Results and Discussion

The results of the study of the phytotoxicity of tea solutions according to the growth test with *L. sativum* are presented in Fig. 1 and in Table 1.

Table 1

Test indicators of *L. sativum* and phytotoxic indices

Research option	The energy of germination, %	Germination, %	Root length, mm	The length of the above-ground part, mm	SGI	RLI
T1	80.0 ± 5.8	80.0 ± 5.8	14.1 ± 1.1	28.1 ± 1.6	–	–
T2	53.3 ± 3.3*	56.7 ± 6.7*	16.2 ± 1.7	25.2 ± 2.5	-0.29	0.15
T3	90.0 ± 10.0	90.0 ± 10.0	18.7 ± 1.5*	27.8 ± 1.5	0.13	0.33
T4	73.3 ± 3.3	73.3 ± 3.3	11.5 ± 1.0	25.9 ± 2.3	-0.08	-0.18
T5	70.0 ± 5.8	70.0 ± 5.8	13.3 ± 1.1	28.9 ± 2.1	-0.13	-0.06
T6	73.3 ± 6.7	73.3 ± 6.7	11.3 ± 1.9	22.0 ± 3.0	-0.08	-0.20

Notes: − - the index is not calculated;

*** – the difference is statistically significant compared to the T1 option**

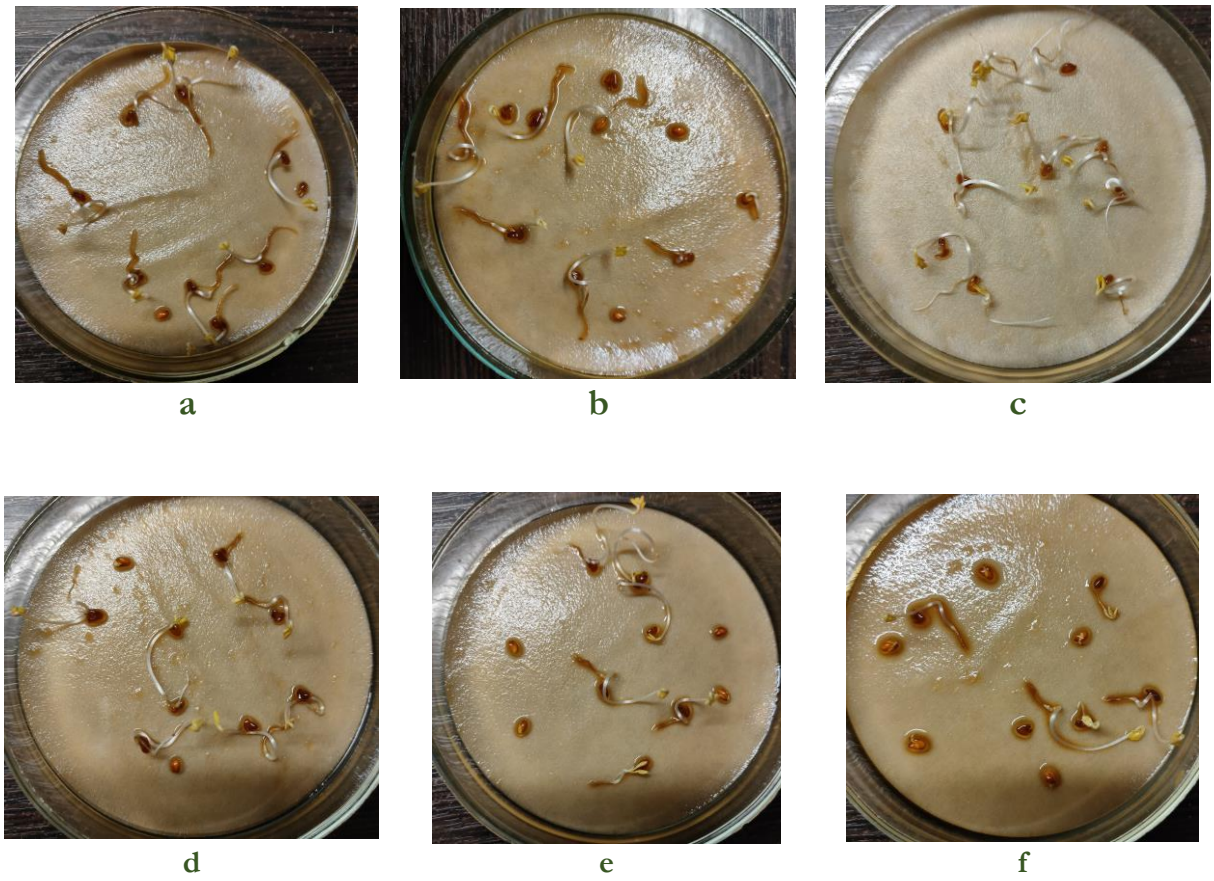

Fig. 1. Seedlings of the test plant (5th day) growing on the tested tea solutions: a – tea T1; b – tea T2; c – tea T3; d – tea T4; e – tea T5; f – tea T6

It was showed that the investigated tea solutions did not affect the germination energy and germination of *L. sativum* seeds, compared to the control (T1), except for the T2 option (Table 1). For variant T2, statistically significant differences compared to the control are shown: a 1.5-fold decrease in seed germination energy and a 1.4-fold decrease in seed germination (Table 1). According to the SGI phytotoxicity index, tea is moderately toxic. At the same time, according to the RLI phytotoxicity index, T2 tea is not phytotoxic (Table 1).

All tested variants of teas did not affect the test indicator of the length of the aerial part – no statistically significant difference with the control was noted. There was also no statistically significant difference compared to the control for the root length indicator, except for the T3 variant (Table 1). It was established that in variant T3 the length of the roots is significantly longer (1.3 times), compared to the control, phytotoxicity was not noted (Table 1). In addition, there is a report that compost tea increased the growth rates of 30-day-old *L. sativum*: relative growth rate, specific leaf area, unit leaf rate and relative leaf growth rate (Farzamisepehr et al., 2014). The authors explain the observed effect of compost tea on the growth of *L. sativum* plants by the better availability of plant growth regulators and humic acid, which is formed as a result of increased microbial activity (Farzamisepehr et al., 2014). Since the tested teas contain herbal and fruit additives, it is important to analyze their phytotoxicity separately.

In particular, among the additives contained in the researched teas, safflower and cornflower petals deserve special attention. Among the chemical compounds of cornflower flowers are: glycoside cyanarin, centaurin and chicorin; anthocyanins pelargonin and cyanidin; flavonoids luteolin, astragalin, kaempferol, apiin, cosmosin, quercetin and rutin; cyanine dye, saponins, resinous and pectin substances, alkaloids, ascorbic acid, carotene and mineral salts (Description and characteristics of the blue cornflower plant, n.d.). Pre-sowing treatment with alcohol extracts of plants of the genus Cornflower (*Centaurea* L.) had an inhibitory effect on the germination of garden cress seeds:

at a concentration of 1 mg/ml, seed germination decreased by 8-42 % compared to the control, at 10 mg/ml – by 30-100 %. Suppression of linear root growth of garden cress seedlings was observed (by 35-52 %) after pre-sowing treatment of seeds with the studied extracts, as well as accumulation of raw biomass of seedlings and roots by 23-89 % (Rakhmaeva et al., 2020). At the same time, there are reports that extracts of cornflower plants did not show phytotoxicity for the germination of garden cress, but inhibited the growth of young plants, especially roots. The greatest phytotoxic effect was found for methyl-tert-butyl ether, where inhibition of growth indicators was 35% or more (Sharonova et al., 2021).

Safflower is considered a valuable source of nutrients. It includes vitamins C, A, E, D, B_{12} and B_6; glycosides; trace elements (sodium, calcium, iron, magnesium, potassium); lignan alcohols; polyunsaturated fatty acids; flavonoids (luteolin, carthamidine and isokarthamidine) (Safflower…, n.d.). Among safflower compounds, sesquiterpene lactones (secondary metabolites of safflower roots) deserve attention, for which phytotoxicity was noted against *Lolium perenne, Lolium rigidum* and *Echinochloa crus-galli* – weeds in agriculture (Rial et al., 2020). According to the authors, the obtained data are the basis for the development of future agrochemicals with allelopathic activity based on natural products without harming the environment (Rial et al., 2020). The remains of safflower shoots also had an inhibitory effect on the test radish plant (Motamedi et al., 2016). At the same time, it was shown that the remains of safflower roots have a higher level of allelochemical substances than the remains of shoots (Motamedi et al., 2016).

Cornflower and safflower have restrictions for use by women. It is stated that cornflower, due to the content of cyan components, is contraindicated during pregnancy and breastfeeding (Description and characteristics of the blue cornflower plant, n.d.). Safflower is contraindicated for use during pregnancy and heavy menstrual discharge (Description and characteristics of the safflower plant, n.d.). However, there is no information on the risks of using a product containing cornflower and safflower on the packaging of the tested teas. In our opinion, manufacturers of teas containing these plants should indicate the possible dangers of their use and risk groups.

Conclusions

The study of phytotoxicity of black tea with herbal and fruit supplements according to the growth test with *L. sativum* showed moderate toxicity based on the plant seed germination index in case of the mixture of black and green teas with supplements of apple, cornflowers, pot marigold, safflower, rose and natural flavoring «Grapes». However, according to the root length index of the seedlings, this tea did not show phytotoxicity, as did other tested variants of black tea with herbal and fruit additives. Further detailed phytotoxicity studies of all constituent teas with herbal and fruit supplements are needed. The risks of cornflower and safflower consumption by women should be indicated on the packaging of products that contain them.

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