Вестник Российского университета дружбы народов.
Серия: АГРОНОМИЯ И ЖИВОТНОВОДСТВО

2019 Том 14 № 3

DOI: 10.22363/2312-797X-2019-14-3

agrojournal.rudn.ru

Научный журнал

Издается с 2006 г.

Издание зарегистрировано Федеральной службой по надзору в сфере связи, информационных технологий и массовых коммуникаций (Роскомнадзор)
Свидетельство о регистрации ПИ № ФС 77-61171 от 30.03.2015 г.
Учредитель: Федеральное государственное автономное образовательное учреждение высшего образования «Российский университет дружбы народов»

Вестник Российского университета дружбы народов. Серия: АГРОНОМИЯ И ЖИВОТНОВОДСТВО

ISSN 2312-7988 (online); 2312-797X (print)
4 выпуска в год.
http://agrojournal.rudn.ru e-mail: agroj@rudn.ru
Языки: русский, английский.
Индексируется в РИНЦ (НЭБ), Cyberleninka, DOAJ, CABI, Ulrich's Periodicals Directory, EBSCOhost.

Цели и тематика. Журнал *Вестник Российского университета дружбы народов. Серия: Агрономия и животноводство* (*Вестник РУДН. Серия: Агрономия и животноводство*) — периодическое рецензируемое научное издание в области сельского хозяйства. Журнал является международным как по составу авторов и тематике публикаций, отражающей проблематику научных исследования в различных регионах мира, так и по составу редакционной коллегии и экспертного совета (рецензентов). Журнал предназначен для публикаций результатов фундаментальных и прикладных научных исследований российских и зарубежных ученых в виде оригинальных научных статей, обзорных научных материалов, научных сообщений, библиографических обзоров по определенным темам научных исследований. Также журнал публикует и распространяет результаты фундаментальных и прикладных исследований, проводимых в коллаборации отечественных и зарубежных ученых по приоритетным проблемам сельскохозяйственной отрасли. В журнале могут быть опубликованы материалы, научная ценность которых и пригодность для публикации оценена рецензентами и редакционной коллегией журнала. Во всех материалах должны соблюдаться этические нормы научных публикаций.

Редакционная коллегия принимает к рассмотрению материалы по направлениям: агрономия, животноводство, ветеринария, зоотехния, ветеринарно-санитарная экспертиза, техносферная безопасность, землеустройство и кадастры, ландшафтная архитектура — для подготовки тематических выпусков с участием приглашенных редакторов.

Журнал рекомендован диссертационными советами РУДН; входит в перечень изданий, публикации которых учитываются Высшей аттестационной комиссией России (ВАК РФ) при защите диссертаций на соискание ученых степеней кандидата и доктора наук по специальностям: 03.02.01 Ботаника, 03.02.13 Почвоведение, 06.01.01 Общее земледелие растениеводство, 06.01.02 Мелиорация, рекультивация и охрана земель, 06.01.04 Агрохимия, 06.01.05 Селекция и семеноводство сельскохозяйственных растений, 06.01.06 Луговодство и лекарственные эфирно-масличные культуры, 06.01.07 Защита растений, 06.01.09 Овощеводство, 06.02.01 Диагностика болезней и терапия животных, патология, онкология и морфология животных (ветеринарные науки), 06.02.02 Ветеринарная микробиология, вирусология, эпизоотология, микология с микотоксикологией и иммунология (ветеринарные науки), 06.02.04 Ветеринарная хирургия (ветеринарные науки), 06.02.07 Разведение селекция и генетика сельскохозяйственных животных (сельскохозяйственные науки), 06.02.10 Частная зоотехния, технология производства продуктов животноводства (сельскохозяйственные науки).

Требования к статьям и правила рецензирования, электронный архив в открытом доступе и иная дополнительная информация размещены на сайте журнала: http://agrojournal.rudn.ru.

Редакторы: *О.В. Горячева, М.И. Яблонская*
Компьютерная верстка: *Ю.Н. Ефремова*

Адрес редакции:
115419, Москва, Россия, ул. Орджоникидзе, д. 3
Тел.: (495) 955-07-16; e-mail: ipk@rudn.ru

Почтовый адрес редакции
117198, Москва, Россия, ул. Миклухо-Маклая, д. 8/2
Тел.: (495) 434-70-07; e-mail: agroj@rudn.ru

Подписано в печать 27.09.2019. Выход в свет 30.09.2019. Формат 70×100/16.
Бумага офсетная. Печать офсетная. Гарнитура «Times New Roman».
Усл. печ. л. 9,1. Тираж 500 экз. Заказ № 1102. Цена свободная.

Федеральное государственное автономное образовательное учреждение высшего образования
«Российский университет дружбы народов» (РУДН)
117198, Москва, Россия, ул. Миклухо-Маклая, д. 6

Отпечатано в типографии ИПК РУДН
115419, Москва, Россия, ул. Орджоникидзе, д. 3,
тел. (495) 952-04-41; ipk@rudn.ru

RUDN JOURNAL OF AGRONOMY AND ANIMAL INDUSTRIES

2019 VOLUME 14 No. 3
DOI: 10.22363/2312-797X-2019-14-3
agrojournal.rudn.ru

Founded in 2006

Founder: PEOPLES' FRIENDSHIP UNIVERSITY OF RUSSIA

RUDN JOURNAL OF AGRONOMY AND ANIMAL INDUSTRIES
Published by the RUDN University (Peoples' Friendship University of Russia), Moscow, Russian Federation

ISSN 2312-7988 (online); 2312-797X (print)
Publication frequency: 4 issues per year
http://agrojournal.rudn.ru
e-mail: agroj@rudn.ru
Languages: Russian, English
Indexed/abstracted by Russian Science Citation Index (elibrary.ru), Cyberleninka, DOAJ, CABI, Ulrich's Periodicals Directory, EBSCOhost.

Aims and Scope

RUDN Journal of Agronomy and Animal Industries is an peer-reviewed periodical covering the latest research in the field of Agricultural Sciences. The journal is international with regard to its editorial board, contributing authors and thematic foci of the publications reflecting problems of various regions in the world.

The journal publishes original results of Russian and foreign scientific researchers and welcomes research articles, review articles, scientific reports, and bibliographic researches. The journal also publishes and disseminates the results of fundamental and applied research conducted by international collaborations of scientists on the priority problems of the agricultural sector.

The most common topics include Agronomy, Animal industries, Veterinary, Veterinary-sanitary expertise, Land use planning and cadaster, Landscape architecture.

The editors are open to thematic issue initiatives with guest editors.

Submitted papers are evaluated by independent reviewers and the Editorial Board members specialized in the article field. All materials must comply with the ethical standards of scientific publications.

In order to expand our readership, we present our journal at scientific conferences, including the annual international conference "Innovation Processess in Agriculture", which is traditionally held at the base of the Agrarian Technological Institute of RUDN University. Each year the conference attracts many agrarian specialists from different parts of the world and continents: Europe, Asia, Africa, North and South America.

Full information for authors, reviewers, and readers (open access to electronic versions and subscription to print editions) can be found at http://agrojournal.rudn.ru.

Editors *O.V. Goryacheva, M.I. Yablonskaya*
Computer design *Yu.N. Efremova*

Address of the Editorial Board:
3 Ordzhonikidze str., 115419 Moscow, Russian Federation
Ph. +7 (495) 952-04-41
e-mail: ipk@rudn.ru

Postal Address of the Editorial Board:
8/2 Miklukho-Maklaya str., 117198 Moscow, Russian Federation
Ph. +7 (495) 434-70-07; e-mail: agroj@rudn.ru

Printing run 500 copies. Open price

Peoples' Friendship University of Russia (RUDN University)
6 Miklukho-Maklaya str., 117198 Moscow, Russian Federation

Printed at RUDN Publishing House:
3 Ordzhonikidze str., 115419 Moscow, Russian Federation,
Ph. +7 (495) 952-04-41; e-mail: ipk@rudn.ru

СОДЕРЖАНИЕ

CONTENTS

RUDN Journal of Agronomy and Animal Industries
Вестник РУДН. Серия: АГРОНОМИЯ И ЖИВОТНОВОДСТВО
2019; 14(3):185—195
http://agrojournal.rudn.ru

БОТАНИКА
BOTANY

Научная статья / Research article

DOI 10.22363/2312-797X-2019-14-3-185-195
УДК 581.192:581.522.4:635.92

Физико-химический анализ и биохимический состав амаранта, интродуцированного в Дагестане

Р.Г. Магомедмирзоева[1], М.С. Гинс[2*], М.Н. Дадашев[3]

[1]Федеральный аграрный научный центр Республики Дагестан,
Махачкала, Российская Федерация

[2]Федеральный научный центр овощеводства,
п. ВНИИССОК, Российская Федерация

[3]РГУ нефти и газа (НИУ) имени И.М. Губкина,
Москва, Российская Федерация

** e-mail: anirr@bk.ru*

Аннотация. Рассмотрены результаты физико-химического анализа состава интродуцированного на юге Дагестана растения амарант. Приведены результаты определения биохимического состава: витаминов, органических кислот, антиоксидантов, бетацианина — амарантина, хлорофилла, каротиноидов и хлорогеновой кислоты в листьях интродуцированного амаранта. Показано, что культура амарант может служить важным источником витаминов и ценных биологически активных веществ как для человека, так и животных. На основании полученных результатов по интродукции амаранта, можно отметить, что его выращивание как промышленной культуры имеет большие перспективы на юге Дагестане.

Ключевые слова: интродукция, Дагестан, амарант, фенольные соединения, фотосинтетические пигменты, гидроксикоричные кислоты, органические кислоты, антиоксиданты, амарантин, витамины

История статьи:
Поступила в редакцию: 6 августа 2019 г. Принята к публикации: 27 августа 2019 г.

Для цитирования:
Магомедмирзоева Р.Г., Гинс М.С., Дадашев М.Н. Физико-химический анализ и биохимический состав амаранта, интродуцированного в Дагестане // Вестник Российского университета дружбы народов. Серия: Агрономия и животноводство. 2019. Т. 14. № 3. С. 185—195. doi: 10.22363/2312-797X-2019-14-3-185-195

Physical and chemical analysis and biochemical composition of amaranth introduced in Dagestan

Ramida G. Magomedmirzoeva[1], Murat S. Gins[2]*, Mirali N. Dadashev[3]

[1]Federal Agrarian Scientific Center of the Republic of Dagestan,
Makhachkala, Russian Federation

[2]Federal Research Vegetable Center,
Moscow region, Russian Federation

[3]Gubkina Russian State University of Oil and Gas (NRU),
Moscow, Russian Federation

** Correspondent author: e-mail: anirr@bk.ru*

Abstract. Amaranth leaves are of high nutritional value, containing various metabolites, mono- and disaccharides, photosynthetic pigments, unsaturated acids, phenolcarboxylic acids with high antioxidant activity. Vegetable amaranth is grown in different soil and climatic conditions all over the world. The article describes the results of physicochemical analysis of composition of amaranth plant introduced in southern Dagestan. The results of determining biochemical composition of vitamins, organic acids, antioxidants, betacyanin — amaranthin, chlorophyll, carotenoids and chlorogenic acid in the leaves of the introduced amaranth are presented. It has been shown that amaranth culture can be an important source of vitamins and valuable biologically active substances for both humans and animals. Based on the results obtained on amaranth introduction, it can be noted that cultivation of amaranth has great prospects in Southern Dagestan, as a mass crop.

Keywords: Dagestan, amaranth, introduction, phenolic compounds, antioxidants, chlorophylls, carotenoids, hydroxycinnamic acids, organic acids, anthocyanins, vitamins

Article history:
Received: 6 August 2019. Accepted: 27 August 2019

For citation:
Magomedmirzoeva RG, Gins MS, Dadashev MN. Physical and chemical analysis and biochemical composition of amaranth introduced in Dagestan. *RUDN Journal of Agronomy and Animal Industries*, 2019; 14 (3):185—195. doi: 10.22363/2312-797X-2019-14-3-185-195

Введение

Амарант относится к семейству Amaranthacea, род *Amaranthus*, многоцелевая культура: семенного, овощного, кормового, декоративного, технического и лекарственного назначения. Листья и семена пищевых видов амаранта богаты водорастворимым белком, не содержащим глютена, и разнообразными эссенциальными и минорными компонентами, необходимыми для функционирования организма человека [1].

Амарант овощного назначения выращивают в разных почвенно-климатических условиях земного шара. Листья амаранта отличаются высокой пищевой ценностью, содержат огромный набор метаболитов, моно- и дисахара, фотосинтетические пигменты, ненасыщенные кислоты, фенолкарбоновые кислоты с высокой антиокислительной активностью [2, 3]. При недостаточном содержании биологически ценных веществ и полноценного белка в корме для животных амарант может применяться как высокобелковая кормовая культура с широким

набором витаминов и антиоксидантов. Семена амаранта могут служить источником биологически активного вещества сквалена, содержание которого в масле составляет 7…11 %, содержат легко усваиваемый крахмал. Однако, не все сорта вызревают на территории России, поэтому перспективно выращивать их на юге России, например, в южной части Дагестана [4].

Цель настоящей работы — исследование особенностей накопления биологически активных веществ и антиоксидантов в листьях растений амаранта овощного и зернового направления, интродуцированных на юге Дагестана.

Материалы и методы

Исследовали растения амаранта вида *Amaranthus tricolor* L. овощных сортов Валентина и Дон Педро и вида *Amaranthus hipochondriacus* L. сорта Крепыш селекции ФГБНУ ФНЦО (ВНИИССОК) (авторы — проф. П.Ф. Кононков, В.К. Гинс и М.С. Гинс). Материалом исследования являлись листья амаранта, которые изучали в процессе вегетации. Растения выращивали на опытном участке в Сулейман-Стальском районе (юг Дагестана) с использованием технологии выращивания для получения листовой биомассы [5]. Определение витаминов B_2, B_6, C, B_3, никотиновой кислоты и B_C проводили с помощью капиллярного электрофореза [6]. Для проведения анализов свежие листья растений измельчали на блендере. Среднюю пробу в количестве 3 г экстрагировали дистиллированной водой до объема 50 мл и центрифугировали в течение 5 мин при скорости вращения 8 тыс. оборотов в минуту. Надосадочную жидкость вводили в анализатор Капель 105 и снимали электрофореграмму при температуре 30 °C при длинах волн 200 и 267 нм. Перенастройка длин волн производится самим прибором автоматически. Исследуемый раствор получается цветным, окраска зависит от цвета листьев.

Содержание восстановленной формы аскорбиновой кислоты определяли йодометрическим методом, основанным на титровании аскорбиновой кислоты в окрашенных экстрактах йодатом калия в кислой среде в присутствии йодистого калия и крахмала [7].

Для определения органических кислот свежие листья растения измельчали на блендере, отбирали среднюю пробу в количестве 1 г, которую разбавляли дистиллированной водой до объема 50 мл и тщательно перемешивали. Полученный раствор центрифугировали в течение 5 мин при 8 тыс. оборотов в минуту. Надосадочную жидкость вводили в анализатор Капель 105 и снимали электрофореграмму при температуре 20 °C, длине волны 254 нм и напряжении 20 кВ.

Содержание кислот в миллиграммах на 1 кг листьев рассчитывали по соответствующей формуле [8]. Было определено содержание муравьиной, уксусной, яблочной и щавелевой кислот, а также фосфорной кислоты.

Для определения содержания амарантина использовали спектрофотометрический метод, который отличается быстротой выполнения и высокой точностью. Содержание амарантина в водных экстрактах определяли с использованием молярного коэффициента экстинкции $5{,}66 \cdot 10^4$ л·моль$^{-1}$·см$^{-1}$ и молярного веса 726,6 г·моль$^{-1}$ [9, 10]. Определение суммарного содержания антиоксидантов проводили амперометрически на приборе Яуза 01-АА. Для этого использовали сырье, измельченное до размеров частиц, проходящих сквозь сито с отверстиями

диаметром 1 мм. Навеску сырья 0,5 г заливали 25 мл 70%-го этанола, перемешивали в колбе в течение 1 ч, фильтровали в мерную колбу на 50 мл и доводили до метки. Градуировку анализатора проводили по галловой кислоте [11].

Фенольные соединения определяли спектрофотометрически. Свежие листья растений измельчали на блендере и отбирали среднюю пробу в количестве 10 г. Навеску помещали в колбу емкостью 100 мл, добавляли дистиллированную воду и перемешивали, доводили до метки. От полученного раствора отбирали 1 мл и добавляли 2 мл специально приготовленного реактива Фолина — Чокальтеу и 10 мл 20%-го раствора пищевой соды. В колбу добавляли дистиллированную воду до 100 мл, перемешивали в течение 30 мин. Оптическую плотность полученного раствора определяли на волне $\lambda \approx 630$ нм в кювете с толщиной слоя 10 мм с помощью прибора СФ 46 [12].

Определение содержания хлорофилла, каротиноидов и гидроксикоричных кислот (хлорогеновой кислоты) также проводили спектрофотометрически на приборе СФ-46 [13]. Способ определения количества хлорофилла, каротиноидов и гидроксикоричных кислот при их совместном присутствии в листьях амаранта включает последовательное экстрагирование каждой пробы в течение 1 ч. При этом предварительно сырье измельчали до размера частиц 1,0 мм. Экстракцию проводили 70%-м этанолом с использованием водяной бани при температуре 100 °С в соотношении сырья и экстрагента 1 : 100, с последующим доведением объема раствора (растворителем) до 100 мл и последующим его разведением 96%-м этанолом в соотношении 2 : 25. Затем измеряли оптическую плотность раствора относительно 96%-го этанола в области максимумов поглощения 328±1, 442±1 и 667±1 нм. Вычисление содержания суммы: гидроксикоричных кислот (в пересчете на хлорогеновую кислоту), каротиноидов (в пересчете на виолоксантин) и хлорофилла в процентах (в пересчете на абсолютно сухую массу сырья) производили по соответствующим формулам [14]. Способ обеспечивает доступность, простоту выполнения и необходимую точность. Следует отметить, что определение содержания биохимических соединений позволяет разработать эффективные способы и технологии использования амаранта в практических целях [15—16].

Результаты исследований

Количественное определение витаминов B_2, B_3, B_5(PP), B_6, B_C, С и Р (биофлавоноидов) и состав фенольных соединений в свежих листьях амаранта приведены в табл. 1.

Таблица 1

Содержание витаминов в зеленых листьях амаранта сортов Валентина, Крепыш и Дон Педро

Сорта амаранта	Содержание витаминов, мг%						
	С	B_2	B_3	B_5	B_6	B_C	Фенольные соединения
Валентина	15, 67	–	1,87	0,21	0,41	2,01	17,3
Крепыш	8,97	0, 26	1,04	0,04	0, 09	0,15	24,2
Дон Педро	11,27	–	1,80	0,17	0,23	1,57	25,2

Table 1

Vitamin content in green leaves of Valentina, Krepysh and Don Pedro amaranth varieties

Amaranth varieties	Vitamin content, mg%						
	C	B_2	B_3	B_5	B_6	B_C	Phenolic compounds
Valentina	15.67	–	1.87	0.21	0.41	2.01	17.3
Krepysh	8.97	0. 26	1.04	0.04	0. 09	0.15	24.2
Don Pedro	11.27	–	1.80	0.17	0.23	1.57	25.2

Эти данные показывают, что свежие листья амаранта содержат указанные витамины в неодинаковом количестве. Для лучшего понимания сравнительного содержания витаминов в растениях относительно среднесуточной потребности человека приведем данные, показывающие, какая часть такой потребности удовлетворяется потреблением в пищу 100 г свежих листьев амаранта по сортам (табл. 2) [16].

Таблица 2

Доля витаминов, содержащаяся в 100 г зеленых листьев амаранта относительно среднесуточной потребности человека

Сорта амаранта	Доля витаминов, %					
	C	B_2	B_3	B_5	B_6	B_C
Валентина	16	–	15	1	19	>100
Крепыш	9	10	8	0,2	4	>100
Дон Педро	11	–	14	1	11	>100
Среднесуточная потребность человека, мг	100	2,6	12,5	20,5	2,1	0,15

Table 2

Percentage of vitamins contained in 100 g of amaranth green leaves in relation to the average daily human need

Amaranth variety	Percentage of vitamins, %					
	C	B_2	B_3	B_5	B_6	B_C
Valentina	16	–	15	1	19	>100
Krepysh	9	10	8	0.2	4	>100
Don Pedro	11	–	14	1	11	>100
Daily human need, mg	100	2.6	12.5	20.5	2.1	0.15

Сравнение данных табл. 2 показывает, что по большинству витаминов в 100 г зеленых листьев амаранта содержится примерно 10…20 % суточной потребности человека. По витамину B_5 это около 1 %, а по витамину B_C — даже больше, чем среднесуточная доза. Следовательно, амаранты всех трех сортов: Валентина, Крепыш и Дон Педро — могут служить серьезным источником витаминов для человека, если использовать в пищу их свежие зеленые листья.

Из органических кислот было определено содержание в фотосинтезирующих листьях щавелевой, муравьиной, яблочной и уксусной кислот, а также фосфорной кислоты. Органические кислоты достаточно активно участвуют в обмене веществ и энергетических реакциях [17]. Образование органических кислот у растений амаранта связано, в т. ч., с процессом дыхания и диссимиляции углеводов. Сахара служат источником для синтеза органических кислот, которые

подвергаются окислительной диссимиляции и являются продуктом неполного окисления сахаров [18]. Они являются промежуточным продуктом цикла Кребса, включаются в реакцию конденсации ацетильного радикала и образуют лимонную кислоту. Лимонная кислота затем включается в реакции цикла лимонной кислоты, в результате которых образуются восстановленные формы коферментов НАД в виде НАД · Н и ФАД в виде ФАД H_2, а также некоторое количество молекулярных энергоносителей в виде АТФ. В реакциях цикла лимонной кислоты помимо щавелевой и уксусной также участвует яблочная кислота. Щавелевоуксусная кислота играет важную роль в биосинтезе аспарагиновой кислоты, которая, являясь промежуточным продуктом цикла Кребса, связывает собой взаимопревращения углеводов и аминокислот.

Муравьиная кислота служит донором формильных групп в обмене веществ, а также участвует в ряде биосинтетических реакций в организме.

Очень большую роль в формировании структуры, состава и функции биохимических и биоорганических соединений играет ортофосфорная кислота. Она входит в состав жизненно важных групп липидов, молекулярных энергоносителей АТФ, ГТФ, АДФ, ГДФ, других макроэргических соединений и фосфорных эфиров.

Таким образом, органические кислоты: щавелевая, уксусная, яблочная и муравьиная, а также фосфорная кислота — являются необходимыми участниками обмена веществ в целом и энергетического обмена живого организма в частности.

Полученные данные по количественному содержанию этих кислот в зеленых листьях амаранта приведены в табл. 3.

Таблица 3

Содержание органических кислот и фосфорной кислоты в листьях амаранта

Амарант	Содержание кислот, мг/кг				
	Щавелевая	Уксусная	Яблочная	Муравьиная	Фосфорная
Валентина	348,9	166,8	2476,6	476,2	8,0
Крепыш	1340	998,6	107,3	–	40,0
Дон Педро	601,3	105,7	1275,5	255,8	3,0

Table 3

Content of organic acids and phosphoric acid in amaranth leaves

Amaranth variety	Acid content, mg/kg				
	Oxalic acid	Acetic acid	Malic acid	Formic acid	Phosphoric acid
Valentina	348.9	166.8	2476.6	476.2	8.0
Krepysh	1340	998.6	107.3	–	40.0
Don Pedro	601.3	105.7	1275.5	255.8	3.0

Из приведенных данных (табл. 3) видно, что максимальное количество муравьиной кислоты содержат листья амаранта Валентина, фосфорной, щавелевой, уксусной кислот сравнительно много обнаружено у амаранта Крепыш, а яблочной — в листьях сорта Дон Педро.

Антиоксиданты выполняют в организме протекторную функцию [19]. Они защищают биологические мембраны, другие клеточные структуры и молекулы живого организма от перекисного окисления. В растениях образуется достаточно много антиоксидантов. Среди них более изученными являются токоферолы,

и в частности, витамин Е. Витамин Е совместно с витамином С предохраняют субклеточные и молекулярные структуры, липиды от повреждающего действия свободных радикалов и активных форм кислорода, обеспечивая целостность этих систем.

Эффективность токоферолов в существенной мере определяется их взаимодействием с другими антиоксидантами, например, витамином С и фенольными соединениями [20]. Суммарное содержание антиоксидантов в листьях изученных сортов амаранта приведено в табл. 4.

Таблица 4

Суммарное содержание антиоксидантов, мг/г, в надземной части амаранта сортов Валентина, Крепыш, Дон Педро в разные фазы развития растений

Фаза развития	Сорта амаранта		
	Валентина	Крепыш	Дон Педро
Вегетативная	0,82	1,62	1,32
Бутонизация	0,94	1,14	1,35
Цветение	1,32	1,84	2,35
Плодоношение	1,44	1,17	1,24

Table 4

The total antioxidant content, mg/g, in amaranth shoots at different growth stages

Growth stages	Amaranth variety		
	Valentina	Krepysh	Don Pedro
Vegetative phase	0,82	1,62	1,32
Flower-bud formation	0,94	1,14	1,35
Blooming	1,32	1,84	2,35
Fruiting	1,44	1,17	1,24

В краснокрашенных листьях амаранта сорта Валентина в большом количестве накапливается красно-фиолетовый пигмент амарантин. Он является мощным антиоксидантом: обезвреживает супероксидный радикал кислорода, свободные радикалы, хелатирует двухвалентные ионы переходных металлов: железа, меди и др. Предшественником амарантина является аминокислота тирозин — антистрессовый антиоксидант. Амарантин относится к вторичным соединениям — водорастворимым азотсодержащим пигментам — бетацианинам, которые входят в состав группы беталаинов [21].

Как следует из табл. 5, в краснокрашенных листьях сортов Валентина и Дон Педро наблюдали варьирование содержания красного пигмента амарантина в процессе вегетации растения. Максимальное количество амарантина образуется в листьях сорта Валентина в фазу бутонизации, а у сорта Дон Педро — в фазу цветения.

Таблица 5

Содержание бетацианинов, %, в листьях амаранта сортов Валентина и Дон Педро в разные фазы вегетации

Фазы развития	Сорта амаранта	
	Валентина	Дон Педро
Вегетативная	0,50	0,50
Бутонизация	0,69	0,49
Цветение	0,63	0,60
Плодоношение	0,52	0,46

Table 5

Betacyanin content, %, in amaranth leaves at different growth stages

Growth stages	Amaranth variety	
	Valentina	Don Pedro
Vegetative phase	0,50	0,50
Flower-bud formation	0,69	0,49
Blooming	0,63	0,60
Fruiting	0,52	0,46

Данные по содержанию хлорофилла, каротиноидов и хлорогеновой кислоты в свежих листьях исследованных сортов амаранта приведены в табл. 6.

Таблица 6

Содержание хлорофилла, каротиноидов и хлорогеновой кислоты в листьях амаранта сортов Валентина, Крепыш и Дон Педро в фазу бутонизации

Сорт амаранта	Хлорофилл, %	Каротиноиды, мг%	Хлорогеновая кислота, %
Валентина	0,11	55,4	0,67
Крепыш	0,11	35,6	0,81
Дон Педро	0,13	59,7	1,35

Table 6

Chlorophyll, carotenoids and chlorogenic acid levels in amaranth leaves at budding stage

Amaranth variety	Chlorophyll, %	Carotenoids, mg%	Chlorogenic acid, %
Valentina	0.11	55.4	0.67
Krepysh	0.11	35.6	0.81
Don Pedro	0.13	59.7	1.35

Как видно из этих данных, в растениях содержится довольно много хлорогеновой кислоты, при этом ее максимальное количество обнаружено в листьях сорта Дон Педро.

Заключение

Приведенный физико-химический анализ биохимического состава листьев интродуцированных сортов амаранта в южном Дагестане показал, что эта культура богата полезными биологически активными соединениями, которые необходимы как пищевой и кормовой материал, и имеет большие перспективы как в Дагестане, так и в других регионах России, являясь ценным сырьем для создания функциональных продуктов.

Библиографический список

1. *Das S.* Amaranths: the crop of great prospect // Amaranthus: A Promising Crop of Future. Singapore : Springer. 2016. P. 13—48. doi: 10.1007/978-981-10-1469-7_3
2. *Repo-Carrasco-Valencia R., Hellstrom J.K., Pihlava J.M., Mattila P.H.* Flavonoids and other phenolic compounds in Andean indigenous grains: Quinoa (Chenopodium quinoa), kaniwa (Chenopodium pallidicaule) and kiwicha (Amaranthus caudatus) // Food Chemistry. 2010. Vol. 120. № 1. P. 128—133. doi: 10.1016/j.foodchem.2009.09.087

3. *Steffensen S.K., Rinnan A., Mortensen A.G., Laursen B., Troiani R.M., Noellemeyer E.J. и др.* Variations in the polyphenol content of seeds of field grown Amaranthus genotypes // Food Chemistry. 2011. Vol. 129. № 1. P. 131—138. doi: 10.1016/j.foodchem.2011.04.044
4. *Султанов Ю.М.А., Магомедмирзоева Р.Г., Алимирзоева З.М., Рабаданов Г.А.* Исследование возможности интродукции амаранта в условиях Южного Дагестана // Актуальные проблемы развития овощеводства и картофелеводства : сб. науч. тр. Региональной научно-практической конференции. 2017. С. 185—188.
5. *Гинс М.С., Гинс В.К., Кононков П.Ф., Пивоваров В.Ф., Романова Е.В., Осокин И.Е., Гинс Е.М.* Технология выращивания овощного (листового) амаранта. М.: РУДН, 2017. 49 с.
6. ГОСТ 7047—55. Витамины A, C, D, B1, B2, и PP. Отбор проб, методы определения витаминов и испытания качества витаминных препаратов.
7. *Сапожникова Е.В., Дорофеева Л.С.* Определение содержания аскорбиновой кислоты в окрашенных растительных экстрактах йодометрическим методом // Консервная и овощесушильная промышленность. 1966. № 5. С. 29—31.
8. *Коломиец Н.Э. Калинкина Г.И., Сапронова Н.Н.* Стандартизация листьев крапивы двудомной // Фармация. 2011. № 6. С. 22—24.
9. *Biswas M., Dey S., Sen R.* Betalains from Amaranthus tricolor L. Journal of Pharmacognosy and Phytochemistry. 2013. Vol. 1. № 5. P. 87—95.
10. *Gengatharan A., Dykes G.A., Choo W.S.* Betalains: natural plant pigments with potential application in functional foods // LWT Food Sci Technol. 2015. Vol. 64. № 2. P. 645—649. doi: 10.1016/j.lwt.2015.06.052
11. *Пахомов В.П., Яшин Я.И., Яшин А.Я., Багирова В.Л., Арзамасцев А.П., Кукес В.Г., Ших Е.В.* Способ определения суммарной антиоксидантной активности биологически активных веществ : патент на изобретение RUS 2238554, 2003.
12. *Гинс М.С., Гинс В.К., Колесников М.П., Кононков П.Ф., Чекмарев П.А., Каган М.Ю.* Методика анализа фенольных соединений в овощных культурах. М.: Мин-во сельского хоз-ва Рос. Федерации, 2010.
13. *Lichtenthaler H.K.* Chlorophylls and carotenoids — pigments of photosynthetic biomembranes // Methods in enzymology. 1987. Vol. 148. P. 350—382. doi: 10.1016/0076-6879(87)48036-1
14. *Тринеева О.В., Сливкин А. И., Сафонова Е.Ф.* Определение гидроксикоричных кислот, каротиноидов и хлорофилла в листьях крапивы двудомной (Urtica Dioica L.) // Химия растительного сырья. 2015. № 3. С. 105—110. DOI: https://doi.org/10.14258/jcprm.201503522
15. *Высочина Г.И.* Амарант (Amaranthus L.): химический состав и перспективы использования (обзор) // Химия растительного сырья. 2013. № 2. С. 5—14. doi: 10.14258/jcprm.1302005
16. *Железнов А.В., Железнова Н.Б., Бурмакина Н.В., Юдина Р.С.* Амарант: научные основы интродукции. Новосибирск : Гео, 2009. 236 с.
17. *Осмоловская Н.Г., Кучаева Л.Н., Новак В.А.* Роль органических кислот при формировании ионного состава листьев гликофитов в онтогенезе // Физиология растений. 2007. Т. 54. № 3. С. 381—388.
18. *Любимов В.Ю.* Механизм фотодыхания в листьях С4-растений и его регуляция : автореф. дис. … д-ра биол. наук. Пущино, 1994.
19. *Владимиров Ю.А.* Глава 1. Биологические мембраны — первичные источники и мишени свободных радикалов // Источники и мишени свободных радикалов в крови человека : монография / под ред. Ю.А. Владимирова. М., 2017. С. 5—84.
20. *Коденцова В.М., Рисник Д.В.* Токоферолы: биологическая роль, критерии витаминной обеспеченности, физиологическая потребность организма и рекомендуемые нормы потребления // Вопросы диетологии. 2018. Т. 8. № 2. С. 22—31.
21. *Slimen I.B., Najar T., Abderrabba M.* Chemical and antioxidant properties of betalains. J Agric Food Chem. 2017. Vol. 65. № 4. P. 675—689. doi: 10.1021/acs.jafc.6b04208

References

1. Das S. Amaranths: the crop of great prospect. In: *Amaranthus: A Promising Crop of Future*. Singapore: Springer; 2016. p. 13—48. doi: 10.1007/978-981-10-1469-7_3
2. Repo-Carrasco-Valencia R, Hellstrom JK, Pihlava JM, Mattila PH. Flavonoids and other phenolic compounds in Andean indigenous grains: Quinoa (Chenopodium quinoa), kaniwa (Chenopodium pallidicaule) and kiwicha (Amaranthus caudatus). *Food Chemistry*. 2010; 120(1):128—133. doi: 10.1016/j.foodchem.2009.09.087
3. Steffensen SK, Rinnan A, Mortensen AG, Laursen B, Troiani RM, Noellemeyer EJ, et al. Variations in the polyphenol content of seeds of field grown Amaranthus genotypes. *Food Chemistry*. 2011; 129(1):131—138. doi: 10.1016/j.foodchem.2011.04.044
4. Sultanov YMA, Magomedmirzoeva RG, Alimirzoeva ZM, Rabadanov GA. Investigation of the possibility of introducing amaranth in conditions of Southern Dagestan. In: *Actual problems of development of vegetable growing and potato growing*. 2017. p. 185—188. (In Russ).
5. Gins MS, Gins VK, Kononkov PF, Pivovarov VF, Romanova EV, Osokin IE, Gins EM. *Tekhnologiya vyrashchivaniya ovoshchnogo (listovogo) amaranta* [Technology for growing vegetable (leaf) amaranth]. Moscow: RUDN Publ.; 2017. (In Russ).
6. State Standard 7047–55. Vitaminy A, S, D, V1, V2, i RR. *Otbor prob, metody opredeleniya vitaminov i ispytaniya kachestva* [Vitamins A, C, D, B1, B2, and PP. Sampling, methods for determining vitamins and quality tests].
7. Sapozhnikova EV, Dorofeeva LS. Determination of ascorbic acid content in colored plant extracts by iodometric method. *Food industry*. 1966; (5):29—31. (In Russ).
8. Kolomiyets NE, Kalinkina GI, Sapronova NN. Standardization of stinging nettle (Urtica dioica) leaves. Farmatsiya (Pharmacy). 2011; (6):22-24. (In Russ).
9. Biswas M, Dey S, Sen R. Betalains from Amaranthus tricolor L. *Journal of Pharmacognosy and Phytochemistry*. 2013; 1(5):87—95.
10. Gengatharan A, Dykes GA, Choo WS. Betalains: natural plant pigments with potential application in functional foods. *LWT Food Sci Technol*. 2015; 64(2):645—649. doi: 10.1016/j.lwt.2015.06.052
11. Pakhomov VP, Yashin YI, Yashin AY, Bagirova VL, Arzamastsev AP, Kukes VG, Shikh EV. *Sposob opredeleniya summarnoi antioksidantnoi aktivnosti biologicheski aktivnykh veshchestv* [Method for determining the total antioxidant activity of biologically active substances]. Patent RUS, no. 2238554, 2004. (In Russ).
12. Gins MS, Gins VK, Kolesnikov MP, Kononkov PF, Chekmarev PA, Kagan MY. *Metodika analiza fenol'nykh soedinenii v ovoshchnykh kul'turakh* [Methodology for analysis of phenolic compounds in vegetable crops]. Moscow: Ministry of Agriculture of the Russian Federation Publ; 2010. (In Russ).
13. Lichtenthaler HK. Chlorophylls and carotenoids - pigments of photosynthetic biomembranes. *Methods in enzymology*. 1987; 148:350—382. doi: 10.1016/0076-6879(87)48036-1
14. Trineeva OV, Slivkin AI, Safonova EF. Determination of hydroxycinnamic acids, carotenoids and chlorophyll in the leaves of stinging nettle (Urtica Dioica L.). *Khimiia rastitel'nogo syr'ia* (*Chemistry of plant raw material*). 2015; (3): 105-110. DOI: https://doi.org/10.14258/jcprm.201503522 (In Russ).
15. Vysochina GI. Amaranthus (Amaranthus L.): chemical composition and prospects of use (review). *Khimija rastitel'nogo syr'ja (Chemistry of plant raw material)*. 2013; (2):5—14. doi: 10.14258/jcprm.1302005 (In Russ).
16. Zheleznov AV, Zheleznova NB, Burmakina NV, Yudina RS. *Amaranth: Scientific Basis of Introduction* [Amaranth: Scientific Basis of Introduction]. Novosibirsk: Geo Publ.; 2009. (In Russ).
17. Osmolovskaya NG, Kuchaeva LN, Novak VA. Role of organic acids in formation of the ionic composition of glycophyte leaves in ontogenesis. *Russian Journal of Plant Physiology*. 2007; 54(3):381—388. (In Russ).

18. Lyubimov VY. *Mekhanizm fotodykhaniya v list'yakh s4-rastenii i ego regulyatsiya* [Mechanism of photorespiration in leaves of c4 plants and its regulation] [Dissertation] Pushchino; 1994. (In Russ).
19. Vladimirov YA. Biological membranes - primary sources and targets of free radicals. In: *Istochniki i misheni svobodnykh radikalov v krovi cheloveka* [Sources and targets of free radicals in human blood]. Moscow; 2017. p. 5—84. (In Russ).
20. Kodentsova VM, Risnik DV. Tocopherols: biological role, criteria for vitamin supply, physiological need of the body and recommended consumption norms. *Nutrition*. 2018; 8(2):22—31. (In Russ).
21. Slimen IB, Najar T, Abderrabba M. Chemical and antioxidant properties of betalains. *J Agric Food Chem*. 2017; 65(4):675—689. doi: 10.1021/acs.jafc.6b04208

Об авторах:

Магомедмирзоева Рамида Гусеновна — старший научный сотрудник, Федеральный аграрный научный центр Республики Дагестан, Российская Федерация, 367014, г. Махачкала, ул. Шахбанова, д. 18а, e-mail: ramida_nii@mail.ru

Гинс Мурат Сабирович — доктор биологических наук, профессор, член-корреспондент РАН, заведующий лабораторией физиологии и биохимии растений и интродукции, Федеральный научный центр овощеводства, Российская Федерация, 143072, Московская обл., Одинцовский р-н, п. ВНИИССОК, ул. Селекционная, д. 14, e-mail: anirr@bk.ru; ORCID: 0000-0001-5995-2696; Scopus Author ID: 6603575024

Дадашев Мирали Нуралиевич — доктор технических наук, профессор кафедры физической и коллоидной химии, Российский государственный университет нефти и газа (НИУ) имени И.М. Губкина, Российская Федерация, 119991, Москва, Ленинский проспект, д. 65, e-mail: elderismailov@mail.ru

About authors:

Magomedmirzoeva Ramida Gusenovna — Senior Researcher, Federal Agrarian Scientific Center of the Republic of Dagestan, 18 st Shakhbanova, Makhachkala, Russian Federation, 367014; e-mail: ramida_nii@mail.ru

Gins Murat Sabirovich — Doctor of Sciences in Biology, Professor, Corresponding Member of the Russian Academy of Sciences, Head of the Laboratory of Plant Physiology and Biochemistry and Introduction, Federal Scientific Center for Vegetable Production, 14 st. Selektsionnaya, VNIISSOK village, Odintsovo urban district, Moscow Region, Russian Federation, 143072; e-mail: anirr@bk.ru; ORCID: 0000-0001-5995-2696; Scopus Author ID: 6603575024

Dadashev Mirali Nuralievich — Doctor of Sciences in Technical Sciences, Professor, Department of Physical and Colloid Chemistry, Gubkin Russian State University of Oil and Gas (NRU), 65 Leninskii avenue, Moscow, Russian Federation, 119991; e-mail: elderismailov@mail.ru

RUDN Journal of Agronomy and Animal Industries
Вестник РУДН. Серия: АГРОНОМИЯ И ЖИВОТНОВОДСТВО
2019; 14(3):196—208
http://agrojournal.rudn.ru

ГЕНЕТИКА И СЕЛЕКЦИЯ
GENETICS AND BREEDING

Research article

DOI 10.22363/2312-797X-2019-14-3-196-208

Detection of genes associated with qualitative characteristics of gluten

Nagham M. Al-Azawi*[1], Ziyad A. Abed[1], Mohammed Al-Issawi[2]

[1]University of Baghdad, *Baghdad, Iraq*

[2]University of Al-Anbar, *Al-Ramadi, Iraq*

**Corresponding author: naghamalazawi@yahoo.com*

Abstract. The research was aimed at analyzing allelic variants of protein in wheat varieties used in Iraqi bakery and evaluating these varieties via genetic source using grain quality selection. Variety tests were carried out at field experimental station of Russian State Agrarian University — Moscow Timiryazev Agricultural Academy. The analysis of wheat grain quality was made after harvesting in mid August. Allele state of genes controlling the quality of gluten in wheat grain was determined using the PCR method. Samples of Iraqi wheat varieties 12 (soft wheat) and One (durum wheat) are characterized by considerable variation of gluten content and quality. The five varieties whose genotype include an allelic variant of high molecular weight glutenins Glu-D1 5+10 and subunit Glu-A1-2* (Fateh, Tamuz-3, Abighreb-3, Iraq and Maxibak) were also studied. The highest gluten content was from 31.5 % in Iraq to 35.3 % in Fateh variety, while the gluten quality was not below the second group. Five varieties — Farah, Al-Murug, Sham-6, Tahadi and Sabirbeg — had unusual combination of the allelic state of Glu-D1 2+12 (which is usually associated with low gluten quality) with a 2* subunit for the Glu-A1 locus, which determines the possibility of improving gluten quality to the wheat varieties.

Key words: soft wheat, grain quality, protein content, gluten content, allelic, glutenins condition, baking qualities

Article history:
Received: 1 August 2019. Accepted: 29 August 2019

For citation:
Al-Azawi NM, Abed ZA, Al-Issawi M. Detection of genes associated with qualitative characteristics of gluten. *RUDN Journal of Agronomy and Animal Industries*, 2019; 14(3):196—208. doi: 10.22363/2312-797X-2019-14-3-196-208

Introduction

Republic of Iraq has long been known among wheat producers, since the time of Hammurabi and its Babylonian Empire. “Mesopotamia” (the land lies between two Rivers — the Tigris and the Euphrates) was the most productive part of the world in terms of wheat yields. However, The Republic of Iraq was compelled to import wheat grains from other countries in quantity of half its need (41—50 %). Over the last thirty years, wheat grain demand in Iraq is approximately 4.6 million tons per year according to FAO (2014). Wheat production in Iraq declined yearly compared with the other countries. As a result, Iraq ranked 38th place among producing countries with an annual wheat production 2.8 millon tones according to FAO (2014). Therefore, issue of finding genetic originals with high productivity depends on selection of varieties by determinate gluten parameter in food industry.

Protein content is one of the most significant indices of wheat grain quality. The protein problem is directly related to the grain quality issue. The main aspects of the protein problem are protein status and quality as the structural basis for gluten. The most significant technological features of flour and the fact that proteins are the primary nutrient elements of bread, bakery and pasta. Bread is foundation of human nutrition, nutritional value of which mainly relies on flour type and raw dough structure. Content of protein, minerals, and vitamins declines with a reduction in the output of flour in it. Baking properties of flour depend on complexes of protein-proteinase and amylase-carbohydrate.

The protein-protein complex involves protein flour, proteolytic enzymes, as well as proteolysis activators and inhibitors. The complex's state is the primary factor determining the flour's "strength". The quantity and quality of gluten as well as structural and chemical characteristics of the experiment were assessed. Proteins were divided into fractions according to their capacity to dissolve in different solvents: soluble globulins (9.4 %), water-soluble-albumins (16.2 %), alcohol-soluble-prolamin (wheat-gliadins) (34.2 %) and alkali-soluble-glutelins (in wheat-glutenins) (37.6 %). Number on the fractional composition was given for baking flour of the highest grade. Albumins and globulins play an important role in the process of plant growth [1]. DNA marking methods are particularly important in selection of grain quality, making it possible to speed up process of selecting genotypes with outstanding grain technological features, which is mainly due to protein quality. Water insoluble protein fractions, so-called spare proteins-gliadins and glutenins play the primary technological role in bakery manufacturing during dough kneading. Glutenin is the basis, and gliadin is its gluing origin. Analysis of the regularities of genetically determined variability of these proteins is of great importance for the creation of varieties with high grain quality [2—5].

Glutenins have a significant effect on wheat's baking features as they determine gluten elasticity, the most significant being the elevated molecular weight proteins. Thus, the glutenin composition, in particular of the high molecular weight fraction, determines strength of gluten, and its elasticity.

The grain endosperm texture is the most significant wheat quality feature. High quality soft wheat has a tough grain structure with endosperm. In the 1990s protein (friabiline) used as a softness marker was discovered to consist of puroindolines and a family of softness proteins (GSP-1) [6]. As surface-active proteins, puroindolins

interact with lipids of starch grain membranes, forming a layer between them and grain's protein matrix, thus, protecting starch grains from destruction during grinding [7]. Temirbekova et al. found that seed protein content of Moskovskaya 39 was high (15.84 %) in hot and 16.60 % in dry conditions [8]. The ordinary cultivar contained grain protein of 14.1—17.0 %, gluten content of 25.0—38.2 %. The puroindolin content in wheat seeds was 0.07—0.10 % of dry matter. It belongs to the albumin class but is easily dissolved in water only after starch granules are released from the membranes' long-lasting lipid-protein complex [9].

The major determinants of wheat quality are endosperm texture and protein content. Endosperm texture has a profound effect on milling, baking and end-use quality. A varietal character, endosperm hardness, is also influenced by environment. It is controlled by the hardness (Ha) locus on the short arm of 5D chromosome. Grain hardness is mainly influenced by various physical and chemical factors like protein, virtuousness, kernel size, water-soluble pentagons, moisture content and lipids [10]. SDS electrophoresis separation revealed two isoforms of this protein-puroindolin a (PINA) and puroindolin b (PINB) very close to electrophoretic mobility. The genes encoding each of these proteins were fully linked together in chromosome 5DS [11]. PINA and PINB gene collaboration guarantees that soft or hard endosperm texture is formed [7, 12]. Variations of PINA or PINB can modify grain hardness significantly due to tryptophan-rich domain related to PINB (allele Pin b-D1b). The amino acid glycine is changed.

Materials and methods

12 Iraqi varieties of soft wheat *(Triticum aestivum L.*) — Fateh, Almurug, Alrasheed, Ibaa-99, Sham-6, Tamuz-3, Abighreb-3, Iraq, Ibaa-95, Tahadi, Maxibak, Sabirbeg and one durum wheat Farah were studied at field experimental station of Russian State Agrarian University — Moscow Timiryazev Agricultural Academy in 2016—2017. After harvesting wheat grain quality was analyzed in the laboratory of grain technology in Nemchinovka Federal Research Center. Wet gluten was estimated with a device INDEX GLUTEN GLUTOMATIC. Dry gluten was calculated by placing wet clot in an oven at 100 °C for 24 hours.

The allelic state of Glu-D locus high-molecular gluten controlling gluten quality in wheat grain was determined by using the PCR technique of polymerase chain reaction. The presence of high-molecular gluten in locus with a combination of 5+10 or 2+12 subunits was determined using the primers: Dx5F and DxF at the Center of Molecular Biotechnology in Russian State Agrarian University — Moscow Timiryazev Agricultural Academy.

DNA was isolated by the CTAB method as follows:

1. Place young seedlings in eppendorfs;
2. Add 200 μl CTAB, preheated to 65 °C;
3. Grind the contents with a pestle;
4. Add 250 μl 2 × CTAB and 450 μl H_2O;
5. Mix by turning 30 times;
6. Put in a water bath at 65 °C with a rocking chair for 80 for 1.5-2 hours with periodic manual tube turning;
7. Cool to room temperature;

8. Add 600 μl of chloroform-isoamyl (24:1) (to the cap);
9. Stir by turning for 30 minutes until the aqueous fraction becomes milky white;
10. Unscrew 10 minutes at 3,000 rpm;
11. Select the supernatant in a clean;
12. Unscrew 10 minutes at 3,000 rpm;
13. Transfer the aqueous fraction to a clean tube, leaving debris at the bottom;
14. Add 700 μl of isopropanol (2/3 volume);
15. Mix by turning 30 times;
16. Unscrew 15 minutes at 13,400 rpm;
17. Drain the isopropanol;
18. Add 70 % ethanol 100 μl;
19. Unscrew 10 minutes at 13,400 rpm;
20. Ethanol drain;
21. Repeat p/n. 18-20;
22. Dry;
23. Add 150 μl of water;
24. Leave overnight in the refrigerator + 4 °C.

Carrying out a polymerase chain reaction (PCR)

The presence of high molecular weight glutenin locus with a combination of 5 + 10 or 2 + 12 subunits was determined using primers: Dx5F, DxF, DxR.

The composition of the reaction mixture during PCR (final concentration of reagents is given) is 25 μl:

Buffer	1x
dNTP	0.2 mM
$MgCl_2$	1.5 mM
Dx_F primer	0.3 μM
Dx5_F primer	0.1 μM
Dx_R primer	0.4 μM
DNA	50—100 ng
Tag polymerase	1 unit

Program for amplification:

Initial denaturation: 95 °C, 5 min.
32 cycles: Denaturation: 95 °C, 30 sec
Primer annealing: 65 °C, 30 sec
Elongation: 72 °C, 2 min
Final elongation: 72 °C, 7 min
Storage: 4 °C
The results were detected on 2 % agarose gel in 1xTBE buffer.

The main criteria for the quantity and quality of gluten

The determination of crude gluten content in soft wheat flour was carried out mechanized according to GOST R 52189—2003 (Russian State Standard), used in the analysis of grain (Wheat flour. General specifications) — Quality indicators of wheat baking flour.

Gluten gradation (mass fraction of raw gluten from flour, %).

Variety of baking flour:

– Extra — not less than 28.0 %;

– Higher — not less than 28.0 %;

– Grit — not less than 30 %;

– The first — not less than 30 %;

– The second — not less than 25.0 %;

– Large flour (Wallpaper) — not less than 20 %.

At the same time, quality of SDS gluten should not be lower than the second group (Classification standards used by the Central Laboratory of the State Commission for Variety Testing of Agricultural Crops to characterize wheat varieties by baking quality).

Strong wheat:

– excellent improver — not less than 34.0 %;

– good improver — not less than 32.0 %;

– satisfactory improver — not less than 30.0 %.

Moreover, quality of gluten (SDS) 45—75 units;

Valuable wheat — not less than 27.0 %, SDS 45—85 units.

Wheat Fillers:

– Good — not less than 25.0 %, SDS 35—90 units;

– Satisfactory — not less than 23.0 %, SDS 20—100 units.

Weak wheat — not less than 18.0 %, SDS 0—120 units.

Evaluation of the "strength" of flour on the basis of the swelling index (0.5 g flour sample) was carried out by evaluating the sedimentation (ml) at a certain grinding size (silk sieve no. 43) [12].

– Very strong — more than 60 ml;

– Strong 60—40 ml;

– Average 40—20 ml;

– Weak — less than 20 ml.

The indicator of sedimentation of flour was determined according to the methodology of the laboratory of grain technology in Agricultural Research Institute of the Central Regions of Non-Chernozem Zone.

Results and discussion

The percentage of high-molecular glutenins is known to exert the biggest impact on the grain's baking characteristics. We used a score of these characteristics in our research determined by the Glu alleles [13]. Researchers have previously discovered a correlation between the existence of certain subunits of elevated molecular weight glutenins and strength, measured by the sedimentation of sample quantity by SDS [14]. Based on this, a score was developed for each allelic state of high molecular weight glutenins [15].

The higher the Mark was assigned to one or another allele, the more significant influence it had on baking qualities (Table 1). Therefore, the best quality of baking corresponds led to the greatest value (4 Marks — in the presence of subunits HMW 5+10).

Thus, it is feasible to assess baking characteristics of wheat variety with the assistance of this classification by adding three alleles expressed in its genotype. However, this

evaluation shows only the prospective characteristics of the variety, as bakery characteristics are mainly dependent on setting, agrotechnology, and a number of other variables.

Table 1

Mark of baking qualities determining Glu-1 alleles [16]

Mark	Chromosome, allele			Mark	Chromosome, allele			Mark	Chromosome, allele		
	1A	1B	1D		1A	1B	1D		1A	1B	1D
4	—	—	5+10	3	—	7+8	—	1	*zero*	—	—
3	1	—	—	3	—	13+16	—	1	—	7	—
3	2*	—	—	2	—	7+9	—	1	—	6+8	—
3	—	17+18	—	2	—	—	2+12	1	—	20	—

As can be seen, the highest mark 4 corresponds to an allele that expresses the subunits of 5+10. Therefore, the main protein of the marker for the baking qualities of wheat is a pair of high-molecular glutenins -Dx-5+Dy-10 in the Glu-D1 locus, while the alternative combination Dx2+Dy12 is usually associated with low gluten quality [17].

The enhancement in gluten quality associated with the existence of a mixture of elevated molecular weight glutenin 5 + 10 subunits is primarily due to the existence in the Dx-5 subunit of an extra cysteine residue compared to the Dx-2 subunit like Cysteine, and in comparison with other amino acids, which is contributed in the formation of a greater number of disulfide bonds as well as formatted of polymers with many branches and number of relationships. Eight of the 13 wheat variety samples had an allelic state of GluD1 5+10. Varieties Al-Murug, Sham-6, Tahadi and Sabirbeg were characterized by genotype 2+12.

The 5 + 10 group has a major impact on the sample kneading moment, strength and SDS sedimentation value relative to the 2 + 12 subunit [18, 19]. Different genotypes of wheat differentiate between 3 and 5 subunits of elevated molecular gluten. Allelic variants GluA1a and GluA1b encoding subunits 1 and 2* have a beneficial impact on cooking quality (3 points), the null allele has a score of 1 point [13, 20].

The variety of samples from Iraq were divided into three groups depending on the allele state of genes influencing baking characteristic (Tables 2, 3 and 4). In particular, the first group included varieties (Table 2), whose genotype contained an allelic variant of elevated molecular weight glutenins 5+10-Dx-5+Dy-10 at the Glu-D1 locus, as well as the subunit 2* at the Glu-A1 locus.

Table 2

Content of glutenin in grain of wheat samples from Iraq with allelic state Glu-D1 5+10 and subunit, 2016 (First group)

Indicators of quantity and gluten quality, harvest, 2016		Chromosome, allele		
		A1	B1	D1
Varieties	Fateh	2*	—	5+10
	Tamuz-3	2*	—	5+10
	Abighreb-3	2*	—	5+10
	Iraq	2*	—	5+10
	Maxibak	2*	—	5+10

Varieties in Figure 1 (first group) are characterized by high gluten content (from 31.5 to 35.3 %) characteristic of strong wheat varieties and gluten quality in all five varieties (according to SDS consequences) is only the second group.

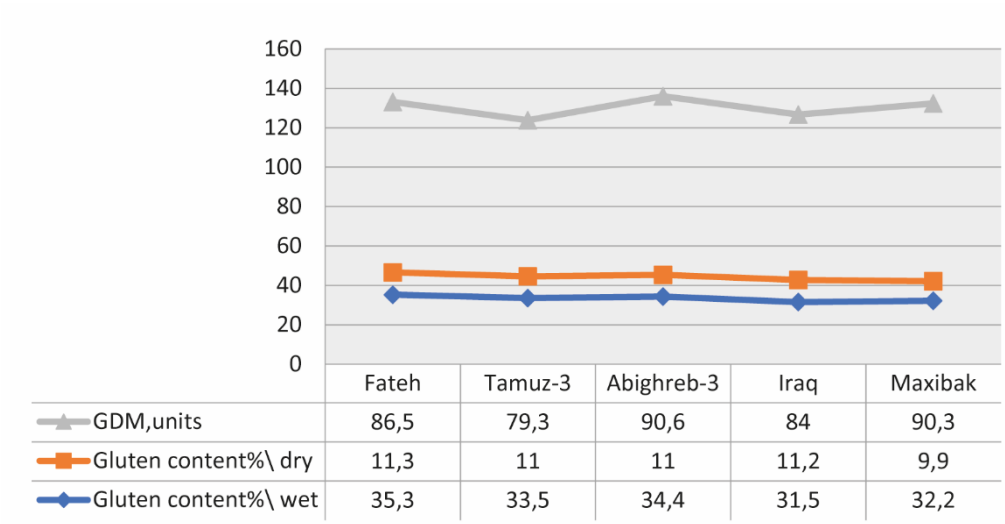


Fig. 1. Content of glutenin in grain of wheat from Iraq, 2016 (First group)

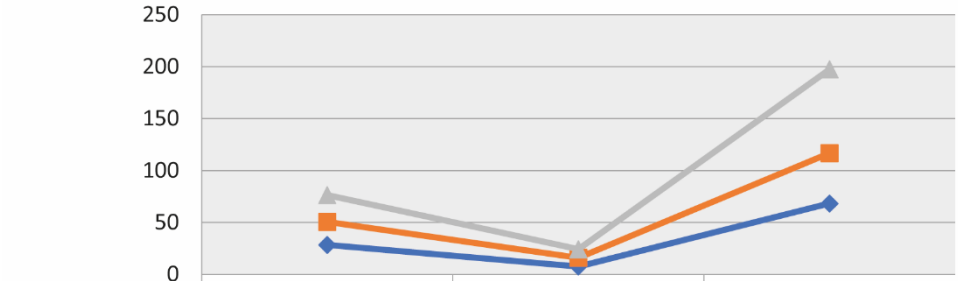
Two types of excellent fillers-two varieties Fateh and Maxibak and one variety Abighreb-3 are categorized as precious varieties Tamuz-3 and Iraq. The second group (Table 3) includes varieties combining the variant Glu-D1 5+10 and subunit 1 at the Glu-A1 locus.

Table 3

Content of gluten in grain of wheat samples from Iraq with allelic state Glu-D1 5 + 10 and subunit Glu-A1-1 (Second group)

Indicators of quantity and gluten quality, harvest, 2016		Chromosome, allele		
		A1	B1	D1
Varieties	Alrasheed	1	—	5+10
	Ibaa-99	1	—	5+10
	Ibaa-95	1	—	5+10

The qualitative feature of gluten (in accordance with SDS) in the second group of samples is as follows: two varieties Alrasheed and Ibaa-99 are allocated to the second group of quality varieties, while the third group is Ibaa-95 (Fig. 2). The general assessment of the varieties of this group by the quantity and quality of gluten: Alrasheed variety is a valuable variety; Ibaa-95 is a good filler, and the third grade of this group Ibaa-99 has the characteristics of weak wheat variety. The third group involves varieties with an alternative mixture of Dx2 + Dy-12 alleles, generally associated with low quality gluten.



	Gluten content%\ wet	Gluten content%\ dry	GDM,units
Ibaa-95	25,6	8,3	80,6
Ibaa-99	22,1	8,4	48,4
Alrasheed	28,6	7,6	68,4

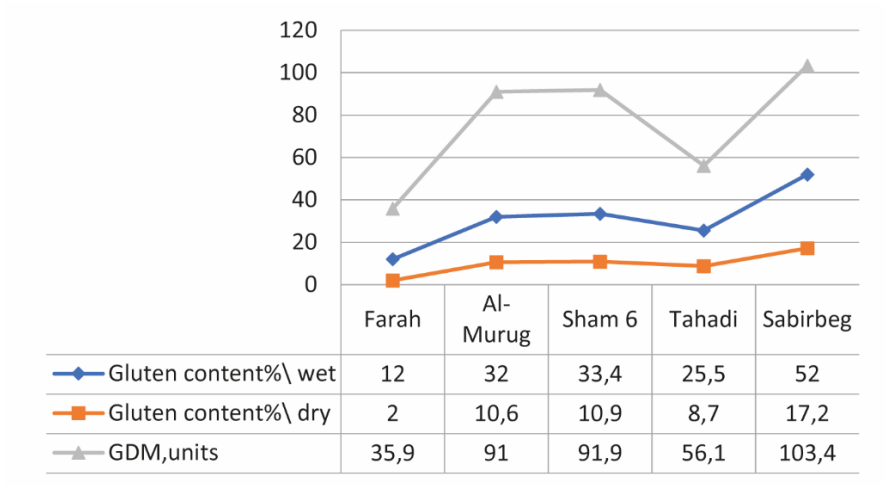
Fig. 2. Content of glutenin in grain of wheat from Iraq, 2016 (Second group)

According to the characteristic of the allelic state of Glu-D1 2+12, two varieties are assigned to the third group. Two varieties of this group — Tahadi and Sabirbeg — are an example of the contrast ratio of gluten content and its quality. The content of gluten in the second grade (Tahadi) is half that (25.5 %), but the qualitative characteristics of gluten are classified as strong varieties, based on the screening of allelic composition of genes related to gluten quality, considering the results of the analysis of content and quality of gluten in the grain of varietal samples from Iraq (Table 4, Figure 3).

Table 4

Content of gluten in grain of varietal wheat samples from Iraq with allelic state Glu-D1 2 + 12

Indicators of quantity and gluten quality, harvest, 2016		Chromosome, allele		
		A1	B1	D1
Varieties	Farah	1	—	2+12
	Al-Murug	Segregation	—	2+12
	Sham 6	Segregation	—	2+12
	Tahadi	2*	—	2+12
	Sabirbeg	2*	—	2+12



	Farah	Al-Murug	Sham 6	Tahadi	Sabirbeg
Gluten content%\ wet	12	32	33,4	25,5	52
Gluten content%\ dry	2	10,6	10,9	8,7	17,2
GDM,units	35,9	91	91,9	56,1	103,4

Fig. 3. Content of glutenin in grain of Iraqi wheat 2016 (Third group)

In our studies, using the dominant PCR marker for the allelic state of the PinaD1 gene, amplification is observed only for the wild-type allele PinaD1a identified in Sham 6 and Sabirbeg varieties. Amplification was not observed in the Nine cultivars having over the null allele (PinaD1b) associated with hardness. The Ibaa-99 sample was heterogeneous on the basis of soft grain/hardness (Figure 4).

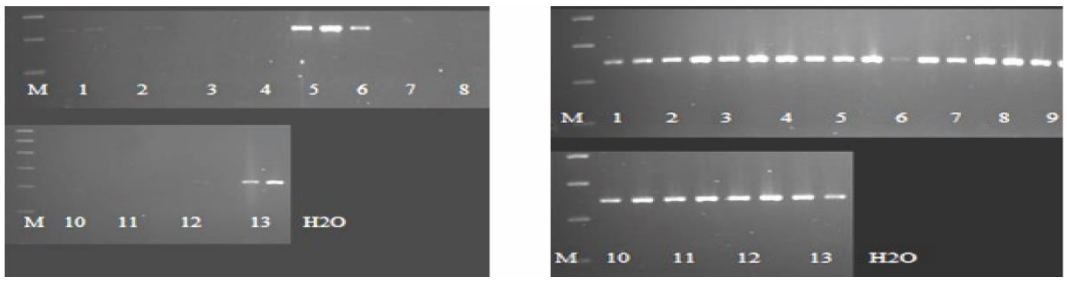


Fig. 4. Electrophoregrams of puroindolins A and in variety samples
(1 — Farah, 2 — Al-Murug, 3 — Fateh, 4 — Alrasheed, 5 — Sham 6, 6 — Ibaa-99, 7—Tamuz 3, 8 — Abighreb-3, 9 — Iraq, 10 — Ibaa-99, 11 — Tahadi, 12 — Maxibak, 13 — Sabirbeg)

In accordance with the Classification Standards used by the State Commission for Variety Testing of Agricultural Crops' Central Laboratory for characterizing wheat varieties by baking traits, the "strong" wheat category involves varieties whose gluten quality in grain and flour in normal SDS units ranges from 45 to 75 units: Tahadi, Alrasheed, Ibaa-99. The Saberbeg sample belongs to the third group. The remaining Iraqi genotypes are allocated to the second quality group for this indicator. The sedimentation index is an indirect technique of evaluating the baking characteristics of flour.

It is the consequence of determining the level of flour swelling and rainfall on a unique device in a soft acetic acid solution (2 %) and flour swelling is determined by the milliliter size of the precipitate. The use of indirect techniques to determine the baking characteristics of wheat is prompted by the need for quality assessment of the source material in the early generation of selection, when the breeder has several grams of grain obtained from one plant. For analysis by this method, 2—5 g of grain is sufficient, which is ground in a micro mill.

This technique is commonly used for a preliminary evaluation of grain quality not only in Russia, but also in other countries. The appropriate classification of soft wheat according to sedimentation indicators has been developed: strong — 40 ml and higher; valuable — 20—40 ml; weak — less than 30 ml. An analysis of our collection showed that 8 variety samples can be classified as valuable wheat by sedimentation level. Studies of wheat grain quality and flour also revealed variations in raw and dry gluten content. Fateh and Abighreb-3 had the highest raw gluten content — 35.3 % and 34.4 %, respectively. Farah variation had the smallest proportion (12 %). The same grade had the lowest proportion (2 %) of dry gluten. GOST R 52189-2003 (Russian State Standard) was not met by the amount and quality of gluten varieties Farah and Sabirbeg.

The remaining 11 samples studied accepted the standard's particular criteria.

Conclusions

Thus, soft wheat collection surveys showed the heterogeneity of grain and flour quality variety samples and revealed interesting samples as sources of economically precious selection characteristics, as well as introduction of Middle Eastern wheat in Russia. The Varietal samples Abighreb-3, Tamuz-3, Fateh, Iraq showed elevated outcomes in terms of a set of gluten quality indices and other economically important features connected with the wheat gene allegiance. Short-range forms separated from the collection samples can be used directly for short-range reproduction of wheat and, indirectly, for lodging resistance. Marked samples may be included in the selection method for the quality of soft wheat grain and flour.

References

1. Wiesner H, Seilmeier W, Belitz HD. Vergleichende untersuchungen über partielle aminosäuresequenzen von prolaminen und glutelinen verschriedener getreidearten. *Z Lebensm Unters Forsch*. 1980; 170(1):17—26. doi: 10.1007/BF01113461
2. Romanova EV, Martynov OL, Tumanyan AF. 2003. Biochemical polymorphism of proteins in crops. *Vestnik of the Russian agricultural science*. 2003; (6):39—40. (In Russ).
3. Konarev AV. Development of biochemical and molecular-genetic studies of the global plant genetic diversity conserved at the Vavilov institute (VIR). *Agrarnaya Rossiya (Agrarian Russia)*. 2006; (6):2—3. (In Russ). doi: 10.30906/1999-5636-2006-6-2-3
4. Romanova EV, Al Labban AA. Influence of alleles state of protein loci on qualities of wheat meal. *Agrarian science*. 2011; (7):18—19. (In Russ).
5. Torbica A, Antov M, Mastilović J, Knežević D. The influence of changes in gluten complex structure on technological quality of wheat (Triticum aestivum L.). *Food Research International*. 2007; 40(8):1038—1045. doi: 10.1016/j.foodres.2007.05.009
6. Jolly CJ, Rahman S, Kortt AA, Higgins TJV. Characterization of the wheat Mr 15,000 "grain-softness protein" and analysis of the relationship between its accumulation in the whole seed and grain softness. *Theoretical and Applied Genetics*. 1993; 86(5):589—597. doi: 10.1007/BF00838714
7. Gautier MF, Aleman ME, Guirao A, Marion D, Joudrier P. Triticum aestivum puroindolines, two basic cystine-rich seed proteins: cDNA sequence analysis and developmental gene expression. *Plant Molecular Biology*. 1994; 25(1):43—57. doi: 1007/BF00024197
8. Temirbekova SK, Ovsyankina AV, Ionova NE, Cheremisova TD, Afanasyeva YV, Mitrofanova OP, Al-Azawi Nagham MH. Enzymatic activity in the resistance stress of winter wheat from different sources in the Non-black land of the center of Russian Federation. *Plant Archives*. 2019; 19 (1):1653—1658.
9. Douliez JP, Michon T, Elmorjani K, Marion D. Structure, biological and technological functions of lipid transfer proteins and indolines, the major lipid binding proteins from cereal kernels. J Cereal Sci. 2000; 32(1):1—20. doi: 10.1006/jcrs.2000.0315
10. Pasha I, Anjum FM, Morris CF. Grain hardness: a major determinant of wheat quality. *Food Science and Technology International*. 2012; 16(6):511—522. doi: 10.1177/1082013210379691
11. Blochet JE, Chevalier C, Forest E, Pebay-Peyroula E, Gautier MF, Joudrier P et al. Complete amino acid sequence of puroindoline, a new basic cystine-rich protein with unique tryptophan-rich domain, isolated from wheat endosperm by Triton X-114 phase partitioning. *FEBS Letters*. 1993; 329(3): 336—340. doi: 10.1016/0014-5793(93)80249-T
12. Morris CF. Puroindolines: the molecular genetic basis of wheat grain hardness. *Plant Molecular Biology*. 2002; 48(5—6):633—647. doi: 10.1023/A:1014837431178
13. Payne PI, Nigtingale MA, Krattiger AF, Holt LM. The relationship between HMW glutenin subunit composition and the breadmaking quality of British-grown wheat varieties. *J. Sci. Food and Agriculture*. 1987; 40(1):51—65. doi: 10.1002/jsfa.2740400108

14. Nettevich ED. Grain quality of spring wheat in relation to resistance to preharvest sprouting. Russian Agricultural Sciences. 1999; (6):6—8. (In Russ).
15. Obukhova LV, Laikova LI, Shumny VK. Analysis of storage proteins (prolamines, puroindolines and Waxy) in common wheat lines Triticum aestivum L.× (Triticum timopheevii Zhuk.× Triticum tauschii) with complex resistance to fungal infections. *Russian Journal of Genetics.* 2010; 46(6):672—676. (In Russ).
16. Semenov OG. Study of fertility reducing agents of Triticum aestivum L. on the cytoplasm T. timopheevi Zhuk. *Conference proceeding: Collected scientific works: Cytoplasmic male sterility and plant breeding*. Kiev: Naukova dumka Publ; 1979. p. 207—209. (In Russ).
17. Murashev VA, Kuperman FM. Morphological and physiological analysis of potential and real productivity of Einkorn wheat and soft wheat. *Biologicheskie nauki*. 1976; (3):86—89. (In Russ).
18. Luo C, Griffin WB, Branlard G, McNeil DL. Comparison of low- and high molecular-weight wheat glutenin allele effects on flour quality. *Theor Appl Genet*. 2001; 102(6—7):1088—1098. doi: 10.1007/s001220000433
19. Liang D, Tang J, Pena RJ, Singh R, He X, Shen X, et al. Characterization of CIMMYT bread wheats for high-and low-molecular weight gluteninsubunits and other quality-related genes with SDS-PAGE, RP-HPLC and molecular markers. *Euphytica.* 2010; 172(2):235—250. doi.org/10.1007/s10681-009-0054-x
20. Rogers WJ, Payne PI, Harinder K. The HMW glutenin subunit and gliadin compositions of German-grown wheat varieties and their relationship with bread making quality. *Plant Breeding*. 1989; 103(2):89—100. doi: 10.1111/j.1439-0523.1989.tb00356.x

About authors:
Al-Azawi Nagham Majeed — University of Baghdad, College of Agricultural Engineering Sciences, Baghdad, Jadiriya, Iraq, 17635; e-mail: naghamalazawi@yahoo.com

Abed Ziyad Asmail — PhD, Department of Field Crop Sciences, University of Baghdad, College of Agricultural Engineering Sciences, Baghdad, Jadiriya, Iraq, 17635

Al-Issawi Mohammed — PhD, Department of Field Crops, University of Al-Anbar, College of Agricultural Sciences Al-Ramadi, 11 Ramadi, Anbar, Iraq, 55431, Scopus ID: 38360983000; ORCID ID: 0000-0002-2551-8768

Научная статья

DOI 10.22363/2312-797X-2019-14-3-196-208

Определение генов, контролирующих качественные характеристики глютена

Н.М.Х. Аль-Азауи[*1], З.А. Абед[1], М. Аль-Иссави[2]

[1]Багдадский университет, *Багдад, Ирак*

[2]Университет Анбара, *Рамади, Ирак*

* *naghamalazawi@yahoo.com*

Аннотация. Исследование посвящено анализу аллельных вариантов белка, подходящих для использования в хлебобулочных изделиях, изготавливаемых из сортов иракской пшеницы, а также оценке этих сортов с помощью генетического источника с использованием методики качественного отбора зерна. Испытания сортов проводились на полевой опытной станции Российского аграрного университета им. Тимирязева. Анализ качества зерна пшеницы проведен после сбора урожая в середине августа, с помощью метода полимеразной цепной реакции определено

аллельное состояние генов, контролирующих качество клейковины зерна пшеницы. Объектом исследования являлись 12 иракских сортов мягкой пшеницы и 1 сорт твердой пшеницы, характеризующиеся значительными колебаниями содержания глютена и его качества. Пять сортов пшеницы содержат в своем генотипе аллельный вариант высокомолекулярных глютенинов Glu-D1 5 + 10 и субъединицы Glu-A1-2* (Fateh, Tamuz-3, Abighreb-3, Iraq и Maxibak). Наибольшее содержание глютена в зернах этих сортов составляет от 31,5 (Iraq) до 35,3 % (Fateh), при этом качество глютена не опускается ниже второй группы. У сортов Farah, Al-Murug, Sham-6, Tahadi и Sabirbeg встречается интересная комбинация аллельного состояния гена Glu-D1 2 + 12, обычно ассоциирующегося с низким качеством глютена, и субъединицей 2* для локуса Glu-A1, которая позволяет повысить качественные показатели глютена до уровня изучаемых сортов пшеницы.

Ключевые слова: мягкая пшеница, качество зерна, содержание белка, содержание глютена, аллель, глютенины, хлебопекарные качества

История статьи:
Поступила в редакцию: 1 августа 2019 г. Принята к публикации: 29 августа 2019 г.

Для цитирования:
Al-Azawi N.M., Abed Z.A., Al-Issawi M. Detection of genes associated with qualitative characteristics of gluten // Вестник Российского университета дружбы народов. Серия: Агрономия и животноводство. 2019. Т. 14. № 3. С. 196—208. doi: 10.22363/2312-797X-2019-14-3-196-208

Библиографический список

1. *Wiesner H., Seilmeier W., Belitz H.D.* Vergleichende untersuchungen über partielle aminosäuresequenzen von prolaminen und glutelinen verschriedener getreidearten // Z Lebensm Unters Forsch. 1980. Vol. 170. № 1. P. 17—26. doi: 10.1007/BF01113461
2. *Романова Е.В., Мартынов О.Л., Туманян А.Ф.* О биохимическом полиморфизме белков сельскохозяйственных растений // Вестник РАСХН. 2003. № 6. С. 39—40.
3. *Конарев А.В.* Развитие биохимических и молекулярно-биологических исследований мирового генофонда растений в ВИР им. НИ Вавилова // Аграрная Россия. 2006. № 6. С. 2—3.
4. *Романова Е.В., Аль Л.А.А.* Влияние аллельного состояния белковых локусов на показатели качества пшеничной муки // Аграрная наука. 2011. № 7. С. 18—19.
5. *Torbica A., Antov M., Mastilović J., Knežević D*. The influence of changes in gluten complex structure on technological quality of wheat (Triticum aestivum L.) // Food Research International. 2007. Vol. 40 № 8. P. 1038—1045. doi: 10.1016/j.foodres.2007.05.009
6. *Jolly C.J., Rahman S., Kortt A.A., Higgins T.J.V.* Characterization of the wheat Mr 15,000 “grain-softness protein” and analysis of the relationship between its accumulation in the whole seed and grain softness // Theoretical and Applied Genetics. 1993. Vol. 86. № 5. P. 589—597. doi: 10.1007/BF00838714
7. *Gautier M.F., Aleman M.E., Guirao A., Marion D., Joudrier P.* Triticum aestivum puroindolines, two basic cystine-rich seed proteins: cDNA sequence analysis and developmental gene expression // Plant Molecular Biology. 1994. Vol. 25. № 1. P. 43—57. doi: 1007/BF00024197
8. *Temirbekova S.K., Ovsyankina A.V., Ionova N.E., Cheremisova T.D., Afanasyeva Y.V., Mitrofanova O.P., Al-Azawi Nagham M.H.* Enzymatic activity in the resistance stress of winter wheat from different sources in the Non-black land of the center of Russian Federation // Plant Archives. 2019. Vol. 19. № 1. P. 1653—1658.
9. *Douliez J.P., Michon T., Elmorjani K., Marion D.* Structure, biological and technological functions of lipid transfer proteins and indolines, the major lipid binding proteins from cereal kernels // J Cereal Sci. 2000. Т. 32. № 1. С. 1—20. doi: 10.1006/jcrs.2000.0315

10. *Pasha I., Anjum F.M., Morris C.F.* Grain hardness: a major determinant of wheat quality // Food Science and Technology International. 2012. Vol. 16. № 6. P. 511—522. doi: 10.1177/1082013210379691
11. *Blochet J.E., Chevalier C., Forest E., Pebay-Peyroula E., Gautier M.F., Joudrier P. et all.* Complete amino acid sequence of puroindoline, a new basic cystine-rich protein with unique tryptophan-rich domain, isolated from wheat endosperm by Triton X-114 phase partitioning // FEBS Letters. 1993. Vol. 329. № 3. P. 336—340. doi: 10.1016/0014-5793(93)80249-T
12. *Morris C.F.* Puroindolines: the molecular genetic basis of wheat grain hardness // Plant Molecular Biology. 2002. Vol. 48. № 5—6. P. 633—647. doi: 10.1023/A:1014837431178
13. *Payne P.I., Nigtingale M.A., Krattiger A.F., Holt L.M.* The relationship between HMW glutenin subunit composition and the breadmaking quality of British-grown wheat varieties // J. Sci. Food and Agriculture. 1987. Vol. 40. № 1. P. 51—65. doi: 10.1002/jsfa.2740400108
14. *Нettевич Э.Д.* Качество зерна яровой мягкой пшеницы в связи с устойчивостью к прорастанию на корню // Доклады РАСХН. 1999. № 6. С. 6—8.
15. *Обухова Л.В., Лайкова Л.И., Шумный В.К.* Анализ запасных белков (проламинов, пуроиндолинов и waxy) у линий мягкой пшеницы Triticum aestivum L. × (Triticum timopheevii Zhuk. × Triticum tauschii) с комплексной устойчивостью к грибным инфекциям // Генетика. 2010. Т. 46. № 6. С. 764—768.
16. *Семенов О.Г.* Изучение восстановителей фертильности Triticum aestivum L. на цитоплазме T. timopheevi Zhuk // Цитоплазматическая мужская стерильность и селекция растений : сб. науч. трудов. Киев : Наукова думка, 1979. С. 207—209.
17. *Мурашев В.В., Куперман Ф.М.* Морфофизиологический анализ потенциальной и реальной продуктивности однозернянки культурной и мягкой пшеницы // Биологические науки. 1976. № 3. С. 86—89.
18. *Luo C., Griffin W.B., Branlard G., McNeil D.L.* Comparison of low- and high molecular-weight wheat glutenin allele effects on flour quality // Theor Appl Genet. 2001. Vol. 102. № 6—7. P. 1088—1098. doi: 10.1007/s001220000433
19. *Liang D., Tang J., Pena R.J., Singh R., He X., Shen X. et all.* Characterization of CIMMYT bread wheats for high- and low-molecular weight gluteninsubunits and other quality-related genes with SDS-PAGE, RP-HPLC and molecular markers // Euphytica. 2010. Vol. 172. № 2. P. 235—250. doi.org/10.1007/s10681-009-0054-x
20. *Rogers W.J., Payne P.I., Harinder K.* The HMW glutenin subunit and gliadin compositions of German-grown wheat varieties and their relationship with bread making quality // Plant Breeding. 1989. Vol. 103. № 2. P. 89—100. doi: 10.1111/j.1439-0523.1989.tb00356.x

Об авторах:

Аль-Азауи Нагам Маджид Хамид — Багдадский университет, Колледж сельскохозяйственных инженерных наук, Ирак, 17635, Джадирия, Багдад; e-mail: naghamalazawi@yahoo.com; SCOPUS ID – 38360983000; ORCID ID – 0000-0002-2551-8768

Абед Зияд Асмаил — Багдадский университет, Колледж сельскохозяйственных инженерных наук, Ирак, 17635, Джадирия, Багдад

Аль-Иссави Мохаммед — PhD, Университет Анбара, Колледж сельскохозяйственных наук Аль-Рамади, Ирак, 55431, Анбар, Рамади, 11

RUDN Journal of Agronomy and Animal Industries
Вестник РУДН. Серия: АГРОНОМИЯ И ЖИВОТНОВОДСТВО
2019; 14(3):209—224
http://agrojournal.rudn.ru

РАСТЕНИЕВОДСТВО
CROP PRODUCTION

Research article

DOI 10.22363/2312-797X-2019-14-3-209-224

Effect of combined use of fertilizer and plant growth stimulating bacteria *Rhizobium*, *Azospirillum*, *Azotobacter* and *Pseudomonas* on the quality and components of corn forage in Iran

Yousef Naserzadeh*[1], Abdorreza M. Nafchi[2], Niloufar Mahmoudi[1], Davoud K. Nejad[3], Anvar Sh. Gadzhikurbanov[1]

[1]Peoples' Friendship University of Russia (RUDN University), *Moscow, Russian Federation*

[2]Universiti Sains Malaysia, *Penang, Malaysia*

[3]Semnan University, *Semnan, Iran*

**Corresponding author: unaserzadeh@gmail.com*

Abstract. *Zea mays* variety 704 (single cross) was studied to investigate effect of chemical fertilizers and growth-promoting bacteria on yield and yield components of corn (*Zea mays*). A factorial experiment was conducted in a completely randomized block design with three replications at Tehran-Varamin Research Farm (Iran) in 2017. The treatments were as follows: inoculation of the seeds with growth promoters in four levels: *Rhizobium*, *Azospirillum*, *Azotobacter* and *Pseudomonas*; *Rhizobium*, *Azospirillum* and *Pseudomonas*; *Rhizobium*, *Azotobacter* and *Pseudomonas*; *Azospirillum*, *Azotobacter* and *Pseudomonas* and use of nitrogen (N) and phosphorus (P) fertilizers at four levels: no use, 1/3, 2/3, and 100 % recommended were applied. The results showed that the use of fertilizer was significant on the traits such as several leaves per plant, number of seeds per row, number of seeds per ear, plant height and forage yield at 1 % level. The results indicated that the highest forage yield of 33.78 t ha^{-1} was obtained from the interaction between the use of fertilizers and biological fertilizers, *Rhizobium*, *Azospirillum*, *Azotobacter* and *Pseudomonas*, which was 42 % higher than control.

Key words: growth promoting bacteria, forage corn, fertilizer, Varamin Plain

ACKNOWLEDGMENTS
Ministry of Education and Science of the Russian Federation on the program to improve the competitiveness of RUDN University among the world's leading research and education centers during 2017—2021 financially supported this research.

Article history:
Received: 10 July 2019. Accepted: 7 August 2019

For citation:
Naserzadeh Y, Nafchi AM, Mahmoudi N, Nejad DK, Gadzhikurbanov ASh. Effect of combined use of fertilizer and plant growth stimulating bacteria *Rhizobium*, *Azospirillum*, *Azotobacter* and *Pseudomonas* on the quality and components of corn forage in Iran. *RUDN Journal of Agronomy and Animal Industries*, 2019; 14(3):209—224. doi: 10.22363/2312-797X-2019-14-3-209-224

Introduction

Application of chemical fertilizers increases plant yields, however, promotes quickly availability of nutrients to plants [1, 2]. A huge amount of mineral nutrients accumulates the soil due to use of synthetic fertilizers and in the long run, causes environmental hazards such as leaching, resulting in groundwater contamination [3, 4]. To meet global demands for crops, farming systems in industrialized countries have undergone profound transformations. On the one hand, high application rates of synthetic fertilizers and manure together with the use of pesticides, irrigation, and short crop rotations have increased yields and have helped to reduce hunger in these countries [5]. Sorghum is one of the most important crop plants whose seeds are used for feeding poultry and its aerial parts after harvest are used for production of silage forage. Highest absorption of nitrogen in corn occurs at the stages of male and female organ formation. Corn requires urgent N uptake during one to two weeks before flowering, and 3-4 weeks of flowering [6, 7]. These soil bacterial species burgeoning in plant rhizosphere, which grow in, on or around the plant stimulate plant growth by a plethora of mechanisms that are collectively known as plant growth-promoting rhizobacteria (PGPR) [8]. Today, due to the untapped use of chemical fertilizers, organic matter of agricultural land has declined in the world and soil composition has become hard and undesirable [9, 10]. Researchers have reported that use of growth promoters, while reducing their intake and increasing efficiency of chemical fertilizers, increases plant growth by increasing N and P absorption [9]. In sustainable agricultural systems, the use of biological fertilizers is important in increasing product production and maintaining sustainable soil fertility. Today, bio-fertilizers are considered as an alternative to chemical fertilizers to increase soil fertility and production of products in sustainable agriculture [11, 12]. Biological fertilizers increase the effects of organic and chemical fertilizers on agricultural production by increasing the activity of growth-promoting bacteria [13]. *Azotobacter*, *Azospirillum*, *Pseudomonas*, and *Rhizobium* bacteria are some of the most important plants` growth promoters. In addition to nitrogen biomass and phosphorus solubilization, the production of significant amounts of growth-stimulating hormones, especially auxin, gibberellin and cytokines during growth and development of plants affects the crop [14]. Several reports on the ability to produce phytohormones by *D-isotropy* PGPR bacteria, including *Azotobacter* bacteria [9], *Azospirillum* [15], as well as *Rhizobium* bacteria [16]. In some cases, it has been observed that levels of nitrogen fertilizers inoculant plants with di-isotropy bacteria have increased growth and development of plants, in which case there are other mechanisms other than nitrogen fixation, including the production of regulating agents such as indoleacetic acid, the reason for the increase in plant growth for this particular study. Many *Rhizobia* species have shown the ability to produce indoleacetic acid (IAA). Increasing concentration of IAA in rhizosphere also leads to an increase in growth and development of plant root system. This, in turn, increases the number of radionuclides, including signals (IAA),

and ultimately, as an expanding ring or loop, generates more amounts of indoleacetic acid and increases growth and yield of the product [17]. The research on sunflower plant showed that simultaneous use of *Azotobacter*, *Azospirillum*, *Pseudomonas* and U.S. cadmium increased the grain yield [18]. A study [19] stated that indigenous *Rhizobium* bacteria can produce the auxin hormone and that this ability is not the same among different species of rhizobia. The most important mechanism of stimulating plant growth by *Rhizobium* strains is the production of Indo-phytonutrient, which results in better root growth, followed by increased water absorption and nutrient uptake, resulting in increased plant growth [20]. In a laboratory study, the researchers stated that inoculation of sorghum seeds with *Rhizobium* bacteria did not fix the nitrogen in the roots, but the bacterium could naturally increase growth hormones such as auxin, cytokine and riboflavin molecules, oligosaccharides and vitamins, which increased root development and increased adsorption of phosphorus [21]. Plant height, dry weight and dry leaves of corn plants increased by inoculation with *Azospirillum* bacteria [20], fresh weight of the aerial part of the plant, leaf number and corn plant height increased by the inoculation of its seeds with the bacteria of the genus *Pseudomonas* [22]. The dry weight of corn (biomass) was increased, with the seeds inoculated with bacteria *A. chroococcum* and *A. brasilense* [23]. The beneficial and plant growth-enhancing effects of PGPR are well reported and explained. PGPR inoculation has increased different crop yields in normal and stress conditions. From the recent literature, PGPR inoculation increased the stress resistance and production of the crops, including tomato [24], lettuce [25], wheat [26]. The authors of [11] reported in a corn study that the use of phosphorus-soluble mycorrhiza and microorganisms reduced consumption of fertilizers by at least 50 %. The purpose of this study was to investigate the effect of combination of chemical fertilizers and plant growth-stimulating bacteria on yield and its components. The yield of corn fodder was recorded in Iran (Tehran, Varamin City) [11].

Materials and methods

In order to investigate the effect of chemical fertilizers and growth-enhancing bacteria on growth stages of corn, forage hybrids of a single cross 704 cultivar were tested at the Tehran-Varamin Research Farm University of Varamin located in the southern region of Tehran City in 2017, using a factorial design in the form of completely randomized blocks and was replicated three times. Geographically, this training farm is located at 51 degrees and 38 minutes north latitude and 35 degrees and 19 minutes east longitude with a height of 920 meters above sea level. The area has warm summers and semi-cold winters. The treatments were as follows: inoculation of the seeds with growth promoters in four levels: B1 = *Rhizobium*, *Azospirillum*, *Azotobacter* and *Pseudomonas*, B2 = *Rhizobium*, *Azospirillum* and *Pseudomonas*, B3 = *Rhizobium*, *Azotobacter* and *Pseudomonas*, B4 = *Azospirillum*, *Azotobacter* and *Pseudomonas.* Use of nitrogen and phosphorus fertilizers was at four levels: A1 = no use, A2 = 1/3 recommended, A3 = 2/3 recommended, A4 = 100 % recommended. Before the beginning of the experiment and applying the seedlings, soil samples were taken from the soil and, based on the results of the soil test, chemical component treatments were based on 100 % fertilizer recommendation of 230.4 kg N, 69 kg of P and 100 kg of potassium (K) as a pure element, was applied per hectare (Table 1). The cultivar used in this study was a single-

grain hybrid single-grain hybrid 704 (forage) from PueblaSeed and Plant Research Institute (Iran). After creating a groove on the stack manually, non-fungicidal seeds after inoculation with *Azotobacter chroococcum* (strain 5), *Azospirillum lipoferum* (Strain OF) and *Rhizobium leguminosarum* bv. phaseoli in a value of 1 liter per 25 kg of seed and phosphate solubilizing bacterium, *Pseudomonas fluorescens* (Snain P21) in a quantity of 100 g per 25 kg of seed per hectare, based on experimental data of approximately 108 live and active bacteria per ml. All of these bacteria were natural and native to Iranian soils and were isolated and purified by inoculation by the Department of Biological Research of the Iranian Institute of Soil and Water Research, in collaboration with the Agrarian and Technological Institute of RUDN, Moscow, Russia, and inoculated to corn seed (single cross) 704 cultivar.

Table 1

Some physical and chemical characteristics of the soil

Depth (sm)	Saturation	Neutralizing agents (%)	Electric conductivity (ds/m)	pH	Organic carbon (%)	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Sand (%)	Mud (%)	Clay (%)
0.30	36.5	18	2.9	7.5	0.30	0.03	8	180	30	42	28

To mix and inoculate seeds, the seed was first applied to a broad and clean plastic, and then gradually spread the appropriate amount of the seed. The seeds were inoculated by a method of stirring. The inoculated seeds were left in the shade and after drying; they were placed at 15 cm intervals in the grooves and covered with dirt. Each experimental plot consisted of 4 cultivars with a length of 6 m. Inter-row spacing was 65 cm and intra-row spacing was 15 cm. The traits such as several leaves per plant, number of seeds per row, number of seeds per ear, plant height and forage yield were evaluated. In the end, two lateral and half-lines from the beginning and the end of each row in each plot were eliminated as marginal effects, and the sampling was performed from two midpoints. For this purpose, in corn grain pulp (R4), 10 berets were harvested from each plot and agronomic traits were measured. Forage harvesting was also taken from two rows in the middle after removing the half-meter marginal effects from the beginning and the end of each plot in one row and the forage yield was calculated. Statistical analysis of data was done by SAS software. For comparing the means, Duncan's multi-domain test was used at 5 % probability level.

Results and discussion

Plant height

Based on the results of the analysis of variance, plant height was affected by the use of chemical and biological fertilizers at 1 % level and their interaction effects were significant at 5 % level (Table 2). The results of the main effects showed that with the increasing use of fertilizer, the plant height also increased so that the highest plant height with an average of 208 cm was related to the recommended level of fertilizer and the lowest of it with an average of 185.08 cm belonged to non-fertilizer treatment. Among the different levels of biofertilizer application, the highest plant height with a mean of 201.25 cm was related to the treatment of *Rhizobium, Azospirillum,*

Azotobacter, and Pseudomonas. The concentration of *Rhizobium*, *Azospirillum*, and *Pseudomonas* was in the range of 194.87 cm (Table 3). The results of comparison of the mean effects of interaction of traits showed that the highest plant height with an average of 201.66 cm belonged to the fertilizer application based on the recommended amount of fertilizers. Bioassay, *Rhizobium, Azospirillum, Azotobacter* and *Pseudomonas*, and the lowest plant height with an average of 180.50 cm, was related to non-fertilization and biodiversity use of *Rhizobium*, *Azospirillum* and *Pseudomonas* (Table 4). It seems that the use of biofertilizers has a positive effect on plant growth (plant height). Zahir and colleagues observed an increase in the height of the 704 maize plant corn that was inoculated with *Azospirillum* bacteria [14]. In addition, an increase of 8.5 % was reported in corn plant height, which seeds were inoculated with *Azospirillum* and *Pseudomonas* [27]. In a study, Radha *et al*. reported an increase in the height of the corn plant inoculated with *Azospirillum lipopherom* [20].

Table 2

Results of variance analysis of traits

Source of changes	Degrees of freedom	Average of square						
		Plant height	Stem diameter	Number of the row in per ear	Number of seeds per row	Number of seeds per ear	Number of active leaves in the bush	Fodder yield
Repetition	2	ns203/0	**5.02	ns65/0	ns25/2	ns06/3607	ns 002/0	**9.00
Chemical fertilizer (A)	3	**1518.26	**46.35	**6.70	**244/63	**126256.73	**7.16	**2580.96
Chemical fertilizer (B)	3	**106.14	**29.71	**0.97	**38.74	ns00/2070	**3.30	**165.25
B × A	9	*4.03	**1.21	**0.71	**20.54	Ns80/3287	*0.02	**7.07
Error	30	1.52	0.10	0.20	5.63	2091.95	0.006	0.56
Coefficient of variation (%)		0.6	5.41	3.01	4.93	6.24	3.61	1.2

ns, * and **: respectively, are meaningless, significant at a probability level of 5 % and 1 % respectively.

These results are also consistent with Tilak et al. (1982), who observed the increase in corn grain yield due to inoculation with E. coli and Azospirillum Brazilian bacteria [23].

Stem diameter

The results of variance analysis of traits showed that stem diameter was affected by chemical and biological fertilizer application as well as their interaction effects and was statistically significant ($p < 0.05$). Based on the results of the comparison of the mean of the main effects of the traits, with the increase in the use of fertilizer, the stem diameter also increased. So that the highest stem diameter with an average of 25.20 mm belonged to chemical fertilizer application based on 100 % and the lowest stem diameter with an average of 21.58 mm for treatment where no fertilizer was used (Table 3). Among the different levels of biofertilizer, the highest stem diameter with

an average of 24.87 mm was related to the treatment of *Rhizobium, Azospirillum, Azotobacter,* and *Pseudomonas* (Table 3).

Based on the results of the comparison table, average interaction effects were observed, with the highest stem diameter with an average of 27 mm belong to chemical fertilizer application based on the recommended dose of 2.3 % with *Rhizobium, Azospirillum, Azotobacter* and *Pseudomonas* and the lowest stem diameter with a mean of 19.83 mm for non-use of fertilizer and the use of bio-fertilizers of *Rhizobium, Azospirillum* and *Pseudomonas* (Table 4).

Number of rows per ear

The results of the table of variance analysis of traits showed that the number of rows per ear was affected by chemical fertilizer, biofertilizer, and their interaction effect, and it was statistically significant at 1 % level (Table 2). Based on the results of comparison of the main effects, the number of rows in the ear increased with increasing the use of chemical fertilizer, so that the highest number of rows in ear with a mean of 15.97 rows belonged to chemical fertilizer application based on 100 %, and the lowest with a mean of 14.24 rows related to non-fertilizer treatment. Among the different levels of biofertilizers, the highest number of rows in the ear with a mean of 15.40 rows was related to *Rhizobium, Azotobacter* and *Pseudomonas* consumption and the lowest number of rows in the ear with a mean of 14.77 rows related to *Azospirillum, Azotobacter* and *Pseudomonas* consumption (Table 3).

Table 3

Comparison of the mean of the main effects

Treatment	Plant height (cm)	Stem diameter (mm)	Number of rows per ear	Number of seed in the row	Number of seed in the ear	Number of leaves per plant	Forage yield (t/ha)
Chemical Fertilizer A							
Control (a1)	c185.08	c21.58	14.24 d	c41.97	c597.24	c12.60	b47.62
1/3 Recommended dose (a2)	b 193.25	b 21.87	c15.01	b47.36	b710.35	b13.20	c51.75
2/3 Recommended dose (a3)	a208.00	a25.04	b15.55	a51.35	a797.66	a14.17	b74.58
100 % Recommended dose1 (a4)	a207.54	a25.20	a15.97	a51.64	a824.88	a14.17	a75.25
Bio-Fertilizer B							
B1=RZ+AS+AZ+PS	a201.25	a24.87	a15.30	b47.23	a723.77	a14.00	a64.95
B2=RZ+AS+PS	c194.87	b21.45	15.30 a	b47.54	a730.81	c12.95	c56.91
B3=RZ+AZ+PS	b197.20	c22.87	a15.40	b46.81	a723.94	b13.23	b62.79
B4=AS+AZ+PS	a200.54	b24.50	b14.77	a50.47	a751.61	a13.95	a64.54

The meanings of at least one letter do not have a significant statistical difference in the Duncan multi-scope test at the 5 % probability level.

The authors of [28] stated that *Rhizobium* bacteria increased root contact in soil by increasing root length and increasing root system in cereals and eventually increasing absorption of nutrients by production of hormones that increased production of photosynthetic material in vegetative stage and its allocation to reproductive organs resulting in an increase in number of rows in the ear. Based on the results of the comparison of the mean interactions effects showed that the highest number of rows per ear

with a mean of 16.8 rows was related to chemical fertilizer application based on 100 % recommended dose plus *Rhizobium*, *Azospirillum* and *Pseudomonas*, and the lowest value was 13.88 rows belonged to the non-fertilization treatment and the use of biological fertilizers *Azospirillum*, *Azotobacter* and *Pseudomonas* (Table 4).

The authors of [29] concluded that combined use of nitrogen fertilizers and inoculum with *Azotobacter*, in addition to increasing soil fertility, improves yield and yield components in plants. Increasing number of ear bean seeds by inoculation of corn seed with *Azospirillum* bacteria also increased airborne dry weight of 42.6 and 67.4 % increase in corn root weight, which seeds were inoculated with growth-enhancing bacteria [19, 30].

Number of seeds per row

Based on the results of the analysis of variance of traits, number of seeds per row was affected by biofertilizer and biological effects as well as their interactions at the level of 1 % (Table 2). The results of the comparison of the mean of the main effects showed that with increase in fertilizer application, the number of seeds per row also increased, so that the highest number of seeds per row with an average of 51.64 belonged to chemical fertilizer application based on 100 % recommended and the lowest with a mean of 41.97 % of seeds belonging to the non-use of chemical fertilizers (Table 3). Among the different levels of biofertilizer use, the highest number of seeds in the row with a mean of 50.74 % belonged to *Azospirillum*, *Azotobacter* and *Pseudomonas*, and the lowest with a mean of 46.81 seeds was related to treatment with *Rhizobium*, *Azotobacter* and *Pseudomonas* (Table 3).

Table 4

Comparison of mean effects of traits

Treatment	Bush height (cm)	Stem diameter (mm)	Number of rows per ear	Number of seeds per row	Number of seeds per ear	Number of active leaves per pant	Forage yield (ton.ha^{-1})
							(A×B)Bio-fertilizer ×Chemical fertilizer
A_1B_1	188.00 E	22.50 d-e	14.70 d-g	40.70 ef	598.30 fg	13.00 f	f 49.83
A_1B_2	f 180.50	19.83 h	14.40 e-g	f 38.16	549.60 g	i11.96	44.66 h
A_1B_3	f 182.50	21.50 F	14.00 fg	42.50 de	595.00 fg	12.30 h	g47.00
A_1B_4	189.33 e	22.50 d-e	g13.86	46.53 b-d	646.07 ef	13.16 e	f49.00
A_2B_1	c196.33	c23.50	15.56 cd	de42.60	d-f663.10	13.76 bc	54.33 d
A_2B_2	189.66 e	20.83 g	d-f14.83	50.46 ab	749.03 bc	g12.60	g47.66
A_2B_3	193.16 d	21.00 fg	de15.13	45.66 cd	690.93 c-e	f 12.93	e01.33
A_2B_4	193.83 d	e22.16	g14.53 e-g	ab50.73	738.33 b-d	13.50 d	d53.66
A_3B_1	a210.66	a27.00	15.46 cd	a53.03	820.20 ab	a14.63	ab77.33
A_3B_2	204.83 b	22.33 d-e	15.16 de	ab50.86	771.00 a-c	13.63 cd	67.83 c
A_3B_3	b206.66	b24.33	ab16.33	48.93 a-c	ab798.60	13.83 b	b76.00
A_3B_4	a209.83	a26.50	15.23 de	a52.56	800.83 ab	14.60 a	ab77.16
A_4B_1	a210.00	a26.50	cd15.46	a52.60	ab813.47	14.63 a	a78.33
A_4B_2	b204.50	22.83 d	16.80 a	ab50.66	853.60 a	cd13.63	c67.50
A_4B_3	b206.50	b206.50	a-b16.16	ab50.16	811.23 ab	b13.86	b76.33
A_4B_4	a209.16	a26.83	cd15.46	a53.13	ab821.20	a14.56	a78.33

The meanings of at least one letter do not have a significant statistical difference in the Duncan multi-scope test at the 5 % probability level.

Based on the results of the comparison of the mean interactions effects, the highest number of seeds per row with an average of 53.13 seeds belonged to chemical fertilizer application 100 % recommended dose with *Azospirillum*, *Azotobacter* and

Pseudomonas, and the lowest number of seeds per row with an average of 38.16 seeds related to non-use of fertilizer and consumption of *Rhizobium*, *Azospirillum* and *Pseudomonas* (Table 4). The increase of 19.8 % of grain yield due to inoculation of maize seeds with *Azotobacter*, and *Pseudomonas* bacteria reported by [27] is consistent with the findings of this research.

Number of seeds per ear

Based on the results of the analysis of variance of traits, number of seeds per ear was affected by the use of fertilizer and was significant at 1 % level, but the use of bio-fertilizer, as well as the effects of biological and chemical fertilizer, had a significant difference in grain number ear did not show up (Table 2). The results of the comparison of the mean of the main effects showed that with an increase in fertilizer, number of seeds per ear also increased so that the highest number of seeds per ear with a mean of 824.88 seeds belonged to the treatment. The use of chemical fertilizer was based on 100 % recommended and the lowest with a mean of 597.24 seeds belonging to non-fertilizer treatment (Table 3). Among the different levels of consumption of biofertilizer also the highest the number of grains per ear with an average of 61.751 grains belonged to *Azosperillum*, *Azotobacter* and *Pseudomonas* treatments and the lowest with 77.732 grains belonged to *Rhizobium*, *Azotobacter* and *Pseudomonas* treatments (Table 3).

According to the results of comparison of mean interaction effects, it was observed that the highest number of kernels per ear with 60.853 seed was related to 100 % recommended fertilizer treatment along with *Rhizobium*, *Azospirillum* and *Pseudomonas* and the lowest with 60.549. Seeds belonged to non-fertilizer treatment and biofertilizer application of *Rhizobium*, *Azospirillum* and *Pseudomonas* (Table 4). This study is consistent with the results of [23], which shows that corn grain yield increased by inoculation with *Azotobacter chrocococcus* and *Azospirillum brasilense*. Also, growth of dry weight of plant in millet stage of corn seeds whose seeds were inoculated with *Azospirillum brasilense* bacteria [28].

Number of leaves per plant

Based on the results of the analysis of variance, the number of leaves in the ear was affected by the use of fertilizer and biofertilizers at 1 % level and the effect of chemical and biological fertilizer interaction at the 5 % statistical level (Table 2). Based on the results of the comparison, the average of the main effects with the increase in the use of chemical fertilizer is the number of active leaves in plants. The highest number of leaves per plant with an average of 14.17 leaves belonged to chemical fertilizer application based on 100 % recommended and the lowest with a mean of 12.60 % belonged to non-fertilizer treatment (Table 3). Among the application of different levels of biofertilizers, the highest number of leaves per plant with an average of 14 leaves belonged to *Rhizobium*, *Azospirillum*, *Azotobacter*, *Pseudomonas* and the lowest value was 12/95 for *Rhizobium*, *Azospirillum* and *Pseudomonas* use (Table 3). Based on the results of the comparison, the average interaction effects, the highest number of leaves in the plant with an average of 14.63 leaves for fertilizer application based on 100 % recommended dose with combined use of *Rhizobium*, *Azospirillum*, *Azotobacter*, *Pseudomonas* and the lowest number of leaves per plant with a mean of 11.96 leaves for

non-fertilizer application (Table 4). Treatment with the bio-fertilizers increases the fresh air mass, the number of leaves and height. The corn plants, which were inoculated with *Pseudomonas* bacteria, as reported authors of [31], had an increase in the number of leaves. The authors of the study [18] reported an increase in fresh weight, height and number of sunflower leaves that were inoculated with *Azotobacter, Azospirillum* and *Pseudomonas* biodiversity, which is consistent with the results of this research.

More fodder yield

The results of the analysis of variance of traits showed that fresh forage yield was affected by the use of fertilizer; bio-fertilizer and their interactions were statistically significant at 1 % (Table 2). Based on the results of the comparison, the average of the main effects of traits increased with increasing fertilizer use of forage yields as well the highest forage yield (75.25 t ha^{-1}) belonged to chemical fertilizer application based on 100 % recommended and minimum forage yield with mean of 64.95 t ha^{-1} was related to non-fertilizer treatment (Table 3). Among the different levels of biofertilizers, the highest forage yield with a mean of 64.95 t ha^{-1} was related to the combined application of bio-fertilizers of *Rhizobium, Azospirillum*, *Pseudomonas* and *Azotobacter*, and the lowest forage yield with an average of 56.91 t ha^{-1} *Rhizobium, Azospirillum* and *Pseudomonas* were used for treatment of biological fertilizers (Table 3). Based on the results of the comparison of the effects of the mean interaction, the highest forage yield with average of 78.33 t ha^{-1} was chemical fertilizer application based on 100 % recommended diet plus biofertilizer, *Rhizobium, Azospirillum, Azotobacter* and *Pseudomonas*, and the lowest forage yield with 44.66 t ha^{-1} mean of non-fertilizer application and use of biological fertilizers of *Rhizobium, Azospirillum* and *Pseudomonas* (Table 4), which was treated with 2/3 fertilizer with 4 types of bacteria and 2/3 of chemical fertilizer along with the *Azotobacter, Azospirillum* and *Pseudomonas* bacteria with a yield of 77.16 t ha^{-1} was considered statistically significant. According to the mean comparison table, the use of 2/3 fertilizer with four species of forage increased the forage yield compared to non-fertilizer treatment, combined with *Rhizobia* bacteria, *Azospirillum*, and ascorbate 42 %. On the other hand, with application of four types of bacteria, use of chemical fertilizers decreased by 25 %, without reducing yields, which could be an effective step towards sustainable agriculture. Inoculation of corn with *Azotobacter* bacteria increased yields. In [18] it is also reported that inoculation with biological fertilizers increased the rate of crop growth. They considered the increase in crop growth rate to improve the absorption of food by the plant. Positive effects of *Azotobacter* on wheat growth and yield have been reported. The authors of [32] have reported the positive effects of this bacterium on corn. Quantitative analysis is a method for justifying and interpreting plant reactions relative to different environmental conditions during its growth stage, through which the transposition and accumulation of the products of photosynthesis in different organs can be determined by measuring the amount of dry matter produced [32]. Besides, some researchers reported an increase of 33 % in fresh weight of corn inoculated with *Pseudomonas spp*. [33]. These results are consistent with the findings of other researchers regarding the application of biological fertilizers [34].

Conclusions

In general, the application of livestock manure and biological fertilizers can increase soil organic matter and, consequently, improve soil structure, increase cation exchange capacity, microorganisms, activity, gas exchange, and water storage capacity. The positive effects of fertilizer combinations with organic and biological fertilizers on growth previously confirmed for other crops are also true for corn. In addition, the results of this study showed that growth-stimulating bacteria have a positive role in absorption and stabilization of essential elements required for plant and can significantly reduce use of synthetic fertilizers, which ultimately maintains plant performance along the lines of agriculture. Sustained when the fertilizer is completely consumed, these bacteria can be a good alternative to reducing the use of chemical fertilizers in the fields and improving the environment.

References

1. López P, Casanova E, Chacon L, Paz P, Guerrero JR. Residual effect of three phosphate rocks from Tachina [Venezuela] in a greenhouse experiment with maize (Zea mays L.) as indicator plant. *Revista Cientifica UNET*. 1990; 4(1—2):29—48.
2. Bostick WM, Bado VB, Bationo A, Soler CT, Hoogenboom G, Jones JW. Soil carbon dynamics and crop residue yields of cropping systems in the Northern Guinea Savanna of Burkina Faso. *Soil and Tillage Research*. 2007; 93(1):138—151. doi: 10.1016/j.still.2006.03.020
3. Sarkar S, Singh SR, Singh RP. The effect of organic and inorganic fertilizers on soil physical condition and the productivity of a rice–lentil cropping sequence in India. *The Journal of Agricultural Science*. 2003; 140(4):419—425. doi: 10.1017/S0021859603003186
4. Tittonell P, Giller KE. When yield gaps are poverty traps: The paradigm of ecological intensification in African smallholder agriculture. *Field Crops Research*. 2013; 143:76—90. Available from: doi: 10.1016/j.fcr.2012.10.007
5. Zimmerer KS. The compatibility of agricultural intensification in a global hotspot of smallholder agrobiodiversity (Bolivia). *Proceedings of the National Academy of Sciences*. 2013; 110(8):2769—2774. doi: 10.1073/pnas.1216294110
6. Kumar A, Patel JS, Bahadur I, Meena VS. The molecular mechanisms of KSMs for enhancement of crop production under organic farming. In: *Potassium solubilizing microorganisms for sustainable agriculture*. New Delhi: Springer; 2016. p.61—75. doi: 10.1007/978-81-322-2776-2_5
7. Naserzadeh Y, Kartoolinejad D, Mahmoudi N, Zargar M, Pakina E, Heydari M, et al. Nine strains of Pseudomonas fluorescens and P. putida: Effects on growth indices, seed and yield production of Carthamus tinctorius L. *Research on Crops*. 2018; 19(4):622—632. doi: 10.31830/2348-7542.2018.0001.39
8. Vessey JK. Plant growth promoting rhizobacteria as biofertilizers. *Plant and soil*. 2003; 255(2):571—586. doi: 10.1023/A:1026037216893
9. Jat LK, Singh YV, Meena SK, Meena SK, Parihar M, Jatav HS, et al. Does integrated nutrient management enhance agricultural productivity. *Pure Appl Microbiol*. 2015; 9(2):1211—1221.
10. Naserzadeh Y, Mahmoudi N. Chelating Effect of Black Tea Extract Compared to Citric Acid in the Process of the Oxidation of Sunflower, Canola, Olive, and Tallow oils. *International Journal of Agricultural and Biosystems Engineering*. 2018; 12(5). DOI: 10.13140/RG.2.2.12552.26887

11. Wu S, Cao Z, Li Z, Cheung K, Wong MH. Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. *Geoderma*. 2005; 125(1—2):155—166. doi: 10.1016/j.geoderma.2004.07.003
12. Parewa HP, Yadav J, Rakshit A. Effect of fertilizer levels, FYM and bioinoculants on soil properties in inceptisol of Varanasi, Uttar Pradesh, India. *International Journal of Agriculture, Environment and Biotechnology*. 2014; 7(3):517. doi: 10.5958/2230-732X.2014.01356.4
13. Shata SM, Mahmoud A, Siam S. Improving calcareous soil productivity by integrated effect of intercropping and fertilizer. *Research Journal of Agriculture and Biological Sciences*. 2007; 3(6):733—739.
14. Zahir ZA, Arshad M, Frankenberger WT. Plant growth promoting rhizobacteria: applications and perspectives in agriculture. *Advances in Agronomy*. 2004; 81(1):98—169.
15. Tien T, Gaskins M, Hubbell D. Plant growth substances produced by Azospirillum brasilense and their effect on the growth of pearl millet (Pennisetum americanum L.). *Appl. Environ. Microbiol*. 1979; 37(5):1016—1024.
16. Lambrecht M, Okon Y, Broek AV, Vanderleyden J. Indole-3-acetic acid: a reciprocal signalling molecule in bacteria–plant interactions. *Trends in microbiology*. 2000; 8(7):298—300. doi: 10.1016/S0966-842X(00)01732-7
17. Ahemad M, Kibret M. Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. *Journal of King Saud University — science*. 2014; 26(1):1—20. doi: 10.1016/j.jksus.2013.05.001
18. Krystofova O, Shestivska V, Galiova M, Novotny K, Kaiser J, Zehnalek J, et al. Sunflower plants as bioindicators of environmental pollution with lead (II) ions. *Sensors*. 2009; 9(7):5040—5058. doi: 10.3390/s90705040
19. Hernández-Rodríguez A, Heydrich-Pérez M, Acebo-Guerrero Y, Velazquez-Del Valle MG, Hernandez-Lauzardo AN. Antagonistic activity of Cuban native rhizobacteria against Fusarium verticillioides (Sacc.) Nirenb. in maize (Zea mays L.). *Applied soil ecology*. 2008; 39(2):180—186. doi: 10.1016/j.apsoil.2007.12.008
20. Radha TK, Rao DLN. Plant growth promoting bacteria from cow dung based biodynamic preparations. *Indian journal of microbiology*. 2014; 54(4):413—418. doi: 10.1007/s12088-014-0468-6
21. Grönemeyer JL, Kulkarni A, Berkelmann D, Hurek T, Reinhold-Hurek B. Rhizobia indigenous to the Okavango region in Sub-Saharan Africa: diversity, adaptations, and host specificity. *Appl. Environ. Microbiol*. 2014; 80(23):7244—7257. doi: 10.1128/AEM.02417-14
22. Hernandez AN, Hernandez A, Heydrich M. Selection of rhizobacteria for use in maize cultivation. *Cultivos Tropicales*. 1995; 6:5—8.
23. Tilak KVBR, Singh CS, Roy NK, Subba Rao NS. Azospirillum brasilense and Azotobacter chroococcum inoculum: effect on yield of maize (Zea mays) and sorghum (Sorghum bicolor). *Soil Biology and Biochemistry*. 1982; 14(4): 417—418. doi: 10.1016/0038-0717(82)90016-5
24. Almaghrabi OA, Massoud SI, Abdelmoneim TS. Influence of inoculation with plant growth promoting rhizobacteria (PGPR) on tomato plant growth and nematode reproduction under greenhouse conditions. *Saudi journal of biological sciences*. 2013; 20(1):57—61. doi: 10.1016/j.sjbs.2012.10.004
25. Kohler J, Hernández JA, Caravaca F, Roldán A. Induction of antioxidant enzymes is involved in the greater effectiveness of a PGPR versus AM fungi with respect to increasing the tolerance of lettuce to severe salt stress. *Environmental and Experimental Botany*. 2009; 65(2—3):245—252. doi: 10.1016/j.envexpbot.2008.09.008
26. Jäderlund L, Arthurson V, Granhall U, Jansson JK. Specific interactions between arbuscular mycorrhizal fungi and plant growth-promoting bacteria: as revealed by different

combinations. *FEMS microbiology letters*. 2008; 287(2):174—180. doi: 10.1111/j.1574-6968.2008.01318.x

27. Zahir A, Arshad M, Khalid A. Improving maize yield by inoculation with plant growth promoting rhizobacteria. *Pakistan Journal of Soil Science*. 1998; 15:7—11.
28. Belimov AA, Safronova VI, Sergeyeva TA, Egorova TN, Matveyeva VA, Tsyganov VE, et al. Characterization of plant growth promoting rhizobacteria isolated from polluted soils and containing 1-aminocyclopropane-1-carboxylate deaminase. *Canadian Journal of Microbiology*. 2001; 47(7):642—652. doi: 10.1139/w01-062
29. Soleimanzadeh H, Habibi D, Ardakani M, Paknejad F, Rejali F. Response of sunflower (Helianthus annuus L.) to inoculation with Azotobacter under different nitrogen levels. *American-Eurasian Journal of Agricultural and Environmental Science*. 2010; 7(3):265—268.
30. Fulchieri M, Frioni L. Azospirillum inoculation on maize (Zea mays): effect on yield in a field experiment in central Argentina. *Soil Biology and Biochemistry*. 1994; 26(7):921—923. doi: 10.1016/0038-0717(94)90308-5
31. Rohitashav-Singh S, Sharma VK, Singh R. Response of forage maize (Zea mays L.) to Azotobacter inoculation and nitrogen. *Indian J. Agronomy*. 1993; 38:555—558.
32. Ferrise R, Triossi A, Stratonovitch P, Bindi M, Martre P. Sowing date and nitrogen fertilisation effects on dry matter and nitrogen dynamics for durum wheat: An experimental and simulation study. *Field Crops Research*. 2010; 117(2—3):245—257. doi: 10.1016/j.fcr.2010.03.010
33. Chabot R, Antoun H, Cescas MP. Stimulation de la croissance du maïs et de la laitue romaine par des microorganismes dissolvant le phosphore inorganique. *Canadian journal of microbiology*. 1993; 39(10):941—947. doi: 10.1139/m93-142
34. Peiffer JA, Ley RE. Exploring the maize rhizosphere microbiome in the field: a glimpse into a highly complex system. *Communicative & integrative biology*. 2013; 6(5):e25177. doi: 10.4161/cib.25177

About authors:

Naserzadeh Yousef — PhD candidate, Department of AgroBiotechnology, RUDN University, 8 Miklukho-Maklaya St., 117198, Moscow, Russian Federation, Researcher in Russian Centre for Plant Quarantine, Moscow, Russian Federation; e-mail: unaserzadeh@gmail.com; ID: 10421751554; ORCID: 0000-0003-2585-5307

Mahmoudi Niloufar — PhD candidate, Department of AgroBiotechnology, RUDN University, 8 Miklukho-Maklaya St., 117198, Moscow, Russian Federation; Researcher in Russian Centre for Plant Quarantine. Moscow, Russian Federation; e-mail: niloofarmahmoodi@ymail.com

Nafchi Abdorreza Mohammadi — Professor of Food Technology School of Industrial Technology, Universiti Sains Malaysia, Chancellory II, Building E42, Level 1, Penang, Malaysia, 11800, e-mail: amohammadi@usm.my; ID: 55886527800; ORCID: 0000-0002-6065-5098

Nejad Davoud Kartuli — Assistant Professor of Forestry, Faculty of Desert Studies, Semnan University, Iran, 35196-45399, Semnan, Motahari Square, Mowlawi Boulevard, e-mail: Kartooli58@gmail.com

Gadzhikurbanov Anvar Shikhragimovich — Department of AgroBiotechnology, RUDN University, 8 Miklukho-Maklaya St., 117198, Moscow, Russian Federation; e-mail: gadcgikurbanow@mail.ru

Научная статья

DOI 10.22363/2312-797X-2019-14-3-209-224

Влияние комбинированного использования удобрений и ростостимулирующих бактерий *Rhizobium*, *Azospirillum*, *Azotobacter* и *Pseudomonas* на качество и состав кукурузного корма в Иране

Ю. Насерзаде*[1], А.М. Нафчи[2], Н. Махмуди[1], Д.К. Нежад[3], А.Ш. Гаджикурбанов[1]

[1] Российский университет дружбы народов, *Москва, Российская Федерация*

[2] Научный университет Малайзии, *Пенанг, Малайзия*

[3] Семнанский университет, *Семнан, Иран*

* *unaserzadeh@gmail.com*

Аннотация. Для исследования влияния химических удобрений и ростостимулирующих бактерий на урожайность и качество зерна кукурузы (*Zea mays*) сорта 704 (одиночный кросс) был проведен факторный рандомизированный блочной эксперимент с тремя повторностями в 2017 г. Исследовательская ферма Варамин находится в Тегеране, Иран. Обработку семян стимулятором роста проводили в четырех комбинациях: *Rhizobium*, *Azospirillum*, *Azotobacter* и *Pseudomonas*; *Rhizobium*, *Azospirillum* и *Pseudomonas*; *Rhizobium*, *Azotobacter* и *Pseudomonas*; *Azospirillum*, *Azotobacter* и *Pseudomonas* — на фоне применения азотных N и фосфорных P удобрений в четырех вариантах: без удобрений, 1/3, 2/3 и 100 % рекомендуемой концентрации. Результаты исследований показали, что использование удобрений оказало значительный эффект на такие параметры, как количество листьев на одно растение, количество семян в ряду, количество семян на колосе, высота растения и урожайность кормов на уровне 1 %. Лучшая кормовая урожайность 33,78 т/га была получена при кобинированном использовании удобрений и биологических ростостимулирующих препаратов на основе *Rhizobium*, *Azospirillum*, *Azotobacter* и *Pseudomonas*, что оказалось на 42 % выше, чем в контроле.

Ключевые слова: ростостимулирующие бактерии, кормовая кукуруза, удобрение, Варамин

БЛАГОДАРНОСТИ

Исследование проводилось в 2017—2021 гг. при финансовой поддержке Министерства образования и науки Российской Федерации по программе повышения конкурентоспособности РУДН среди ведущих мировых научно-образовательных центров.

История статьи:

Поступила в редакцию: 10 июля 2019 г. Принята к публикации: 7 августа 2019 г.

Для цитирования:

Naserzadeh Y., Nafchi A.M., Mahmoudi N., Nejad D.K., Gadzhikurbanov A.Sh. Effect of combined use of fertilizer and plant growth stimulating bacteria *Rhizobium*, *Azospirillum*, *Azotobacter* and *Pseudomonas* on the quality and components of corn forage in Iran // Вестник Российского университета дружбы народов. Серия: Агрономия и животноводство. 2019. Т. 14. № 3. С. 209—224. doi: 10.22363/2312-797X-2019-14-3-209-224

Библиографический список

1. *López P., Casanova E., Chacon L., Paz P., Guerrero J.R.* Residual effect of three phosphate rocks from Tachina [Venezuela] in a greenhouse experiment with maize (Zea mays L.) as indicator plant // Revista Cientifica UNET. 1990. Vol. 4. № 1—2. P. 29—48.
2. *Bostick W.M., Bado V.B., Bationo A., Soler C.T., Hoogenboom G., Jones J.W.* Soil carbon dynamics and crop residue yields of cropping systems in the Northern Guinea Savanna of Burkina Faso // Soil and Tillage Research. 2007. Vol. 93. № 1. P. 138—151. doi: 10.1016/j.still.2006.03.020
3. *Sarkar S., Singh S.R., Singh R.P.* The effect of organic and inorganic fertilizers on soil physical condition and the productivity of a rice–lentil cropping sequence in India // The Journal of Agricultural Science. 2003. Vol. 140. № 4. P. 419—425. doi: 10.1017/S0021859603003186
4. *Tittonell P., Giller K.E.* When yield gaps are poverty traps: The paradigm of ecological intensification in African smallholder agriculture // Field Crops Research. 2013. № 143. P. 76—90. doi: 10.1016/j.fcr.2012.10.007
5. *Zimmerer K.S.* The compatibility of agricultural intensification in a global hotspot of smallholder agrobiodiversity (Bolivia) // Proceedings of the National Academy of Sciences. 2013. Vol. 110. № 8. P. 2769—2774. doi: 10.1073/pnas.1216294110
6. *Kumar A., Patel J.S., Bahadur I., Meena V.S.* The molecular mechanisms of KSMs for enhancement of crop production under organic farming // Potassium solubilizing microorganisms for sustainable agriculture. New Delhi : Springer, 2016. P. 61—75. doi: 10.1007/978-81-322-2776-2_5
7. *Naserzadeh Y., Kartoolinejad D., Mahmoudi N., Zargar M., Pakina E., Heydari M.,* et al. Nine strains of Pseudomonas fluorescens and P. putida: Effects on growth indices, seed and yield production of Carthamus tinctorius L. // Research on Crops. 2018. Vol. 19. № 4. P. 622—632. doi: 10.31830/2348-7542.2018.0001.39
8. *Vessey J.K.* Plant growth promoting rhizobacteria as biofertilizers // Plant and soil. 2003. Vol. 255. № 2. P. 571—586. doi: 10.1023/A:1026037216893
9. J*at L.K., Singh Y.V., Meena S.K., Meena S.K., Parihar M., Jatav H.S.,* et al. Does integrated nutrient management enhance agricultural productivity // Pure Appl Microbiol. 2015. Vol. 9. № 2. P. 1211—1221.
10. *Naserzadeh Y., Mahmoudi N.* Chelating Effect of Black Tea Extract Compared to Citric Acid in the Process of the Oxidation of Sunflower, Canola, Olive, and Tallow oils // International Journal of Agricultural and Biosystems Engineering. 2018. Vol. 12. № 5. DOI: 10.13140/RG.2.2.12552.26887
11. *Wu S., Cao Z., Li Z., Cheung K., Wong M.H.* Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial // Geoderma. 2005. Vol. 125. № 1—2. P. 155—166. doi: 10.1016/j.geoderma.2004.07.003
12. *Parewa H.P., Yadav J., Rakshit A.* Effect of fertilizer levels, FYM and bioinoculants on soil properties in inceptisol of Varanasi, Uttar Pradesh, India // International Journal of Agriculture, Environment and Biotechnology. 2014. Vol. 7. № 3. P. 517. doi: 10.5958/2230-732X.2014.01356.4
13. *Shata S.M., Mahmoud A., Siam S.* Improving calcareous soil productivity by integrated effect of intercropping and fertilizer // Research Journal of Agriculture and Biological Sciences. 2007. Vol. 3. № 6. P. 733—739.
14. *Zahir Z.A., Arshad M., Frankenberger W.T.* Plant growth promoting rhizobacteria: applications and perspectives in agriculture // Advances in Agronomy. 2004. Vol. 81. № 1. P 98—169.
15. *Tien T., Gaskins M., Hubbell D.* Plant growth substances produced by Azospirillum brasilense and their effect on the growth of pearl millet (Pennisetum americanum L.) // Appl. Environ. Microbiol. 1979. Vol. 37. № 5. P. 1016—1024.

16. *Lambrecht M., Okon Y., Broek A.V., Vanderleyden J.* Indole-3-acetic acid: a reciprocal signalling molecule in bacteria–plant interactions // Trends in microbiology. 2000. Vol. 8. № 7. P. 298—300. doi: 10.1016/S0966-842X(00)01732-7
17. *Ahemad M., Kibret M.* Mechanisms and applications of plant growth promoting rhizobacteria: current perspective // Journal of King Saud University — science. 2014. Vol. 26. № 1. P. 1—20. doi: 10.1016/j.jksus.2013.05.001
18. *Krystofova O., Shestivska V., Galiova M., Novotny K., Kaiser J., Zehnalek J., e*t al. Sunflower plants as bioindicators of environmental pollution with lead (II) ions // Sensors. 2009. Vol. 9. № 7. P. 5040—5058. doi: 10.3390/s90705040
19. *Hernández-Rodríguez A.N., Heydrich-Pérez M., Acebo-Guerrero Y., Velazquez-Del Valle M.G., Hernandez-Lauzardo A.N.* Antagonistic activity of Cuban native rhizobacteria against Fusarium verticillioides (Sacc.) Nirenb. in maize (Zea mays L.) // Applied soil ecology. 2008. Vol. 39. № 2. P. 180—186. doi: 10.1016/j.apsoil.2007.12.008
20. *Radha T.K., Rao D.L.N.* Plant growth promoting bacteria from cow dung based biodynamic preparations // Indian journal of microbiology. 2014. Vol. 54. № 4. P. 413—418. doi: 10.1007/s12088-014-0468-6
21. *Grönemeyer J.L., Kulkarni A., Berkelmann D., Hurek T., Reinhold-Hurek B.* Rhizobia indigenous to the Okavango region in Sub-Saharan Africa: diversity, adaptations, and host specificity // Appl. Environ. Microbiol. 2014. Vol. 80. № 23. P. 7244—7257. doi: 10.1128/AEM.02417-14
22. *Hernandez A., Hernandez A., Heydrich M.* Selection of rhizobacteria for use in maize cultivation // Cultivos Tropicales. 1995. № 6. P. 5—8.
23. *Tilak K.V.B.R., Singh C.S., Roy N.K., Subba Rao N.S.* Azospirillum brasilense and Azotobacter chroococcum inoculum: effect on yield of maize (Zea mays) and sorghum (Sorghum bicolor) // Soil Biology and Biochemistry. 1982. Vol. 14. № 4. P. 417—418. doi: 10.1016/0038-0717(82)90016-5
24. *Almaghrabi O.A., Massoud S.I., Abdelmoneim T.S.* Influence of inoculation with plant growth promoting rhizobacteria (PGPR) on tomato plant growth and nematode reproduction under greenhouse conditions // Saudi journal of biological sciences. 2013. Vol. 20. № 1. P. 57—61. doi: 10.1016/j.sjbs.2012.10.004
25. *Kohler J., Hernández J.A., Caravaca F., Roldán A.* Induction of antioxidant enzymes is involved in the greater effectiveness of a PGPR versus AM fungi with respect to increasing the tolerance of lettuce to severe salt stress // Environmental and Experimental Botany. 2009. Vol. 65. № 2—3. P. 245—252. doi: 10.1016/j.envexpbot.2008.09.008
26. *Jäderlund L., Arthurson V., Granhall U., Jansson J.K.* Specific interactions between arbuscular mycorrhizal fungi and plant growth-promoting bacteria: as revealed by different combinations // FEMS microbiology letters. 2008; 287(2):174—180. doi: 10.1111/j.1574-6968.2008.01318.x
27. *Zahir A., Arshad M., Khalid A.* Improving maize yield by inoculation with plant growth promoting rhizobacteria // Pakistan Journal of Soil Science. 1998. № 15. P. 7—11.
28. *Belimov A.A., Safronova V.I., Sergeyeva T.A., Egorova T.N., Matveyeva V.A., Tsyganov V.E.*, et al. Characterization of plant growth promoting rhizobacteria isolated from polluted soils and containing 1-aminocyclopropane-1-carboxylate deaminase // Canadian Journal of Microbiology. 2001. Vol. 47. № 7. P. 642—652. doi: 10.1139/w01-062
29. *Soleimanzadeh H., Habibi D., Ardakani M., Paknejad F., Rejali F.* Response of sunflower (Helianthus annuus L.) to inoculation with Azotobacter under different nitrogen levels // American-Eurasian Journal of Agricultural and Environmental Science. 2010. Vol. 7. № 3. P. 265—268.
30. *Fulchieri M., Frioni L.* Azospirillum inoculation on maize (Zea mays): effect on yield in a field experiment in central Argentina // Soil Biology and Biochemistry. 1994. Vol. 26. № 7. P. 921—923. doi: 10.1016/0038-0717(94)90308-5
31. *Rohitashav-Singh S., Sharma V.K., Singh R.* Response of forage maize (Zea mays L.) to Azotobacter inoculation and nitrogen // Indian J. Agronomy. 1993. № 38. P. 555—558.

32. *Ferrise R., Triossi A., Stratonovitch P., Bindi M., Martre P.* Sowing date and nitrogen fertilisation effects on dry matter and nitrogen dynamics for durum wheat: An experimental and simulation study // Field Crops Research. 2010. Vol. 117. № 2—3. P. 245—257. doi: 10.1016/j.fcr.2010.03.010
33. *Chabot R., Antoun H., Cescas M.P.* Stimulation de la croissance du maïs et de la laitue romaine par des microorganismes dissolvant le phosphore inorganique // Canadian journal of microbiology. 1993. Vol. 39. № 10. Vol. 941—947. doi: 10.1139/m93-142
34. *Peiffer J.A., Ley R.E.* Exploring the maize rhizosphere microbiome in the field: a glimpse into a highly complex system // Communicative & integrative biology. 2013. Vol. 6. № 5. P. e25177. doi: 10.4161/cib.25177

Об авторах:

Насерзаде Юсеф — аспирант, агробиотехнологический департамент, Аграрно-технологический институт, Российский университет дружбы народов, Российская Федерация, 117198, Москва, ул. Миклухо-Маклая, 8; научный сотрудник Российского центра карантина растений, Москва, Российская Федерация; e-mail: unaserzadeh@gmail.com; ID: 10421751554; ORCID: 0000-0003-2585-5307

Махмуди Нилоуфар — аспирант, агробиотехнологический департамент, Аграрно-технологический институт, Российский университет дружбы народов, Российская Федерация, 117198, Москва, ул. Миклухо-Маклая, 8; научный сотрудник Российского центра карантина растений, Москва, Российская Федерация; e-mail: niloofarmahmoodi@ymail.com

Нафчи Абдорреза Мохаммади — профессор Школы пищевых технологий промышленных технологий, Научный университет Малайзии, Малайзия, 11800, Пенанг, Канцелярия II, Здание E42, Уровень 1, e-mail: amohammadi@usm.my; ID: 55886527800; ORCID: 0000-0002-6065-5098

Неджад Давуд Картули — доцент кафедры лесоводства, факультет исследований пустынь, Университет Семнан, Иран, 35196-45399, Семнан, Motahari Square, Mowlawi Boulevard, e-mail: Kartooli58@gmail.com

Гаджикурбанов Анвар Шихрагимович — агробиотехнологический департамент, Аграрно-технологический институт, Российский университет дружбы народов, Российская Федерация, 117198, Москва, ул. Миклухо-Маклая, 8; e-mail: gadcgikurbanow@mail.ru

RUDN Journal of Agronomy and Animal Industries
Вестник РУДН. Серия: АГРОНОМИЯ И ЖИВОТНОВОДСТВО
2019; 14(3):225—238
http://agrojournal.rudn.ru

Research article / Научная статья

DOI 10.22363/2312-797X-2019-14-3-225-238
УДК 631.529:635.49

Nutritional value of vegetable *Amaranthus tricolor* L. seedlings grown in Moscow region

Iskren G. Sergiev*[1], Desislava A. Todorova[1], Valentina K. Gins[2], Svetlana M. Motyleva[3], Ekaterina M. Gins[4], Evgeny A. Moskalev[4]

[1]Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, *Sofia, Bulgaria*

[2]Federal Scientific Vegetable Center, *VNIISSOK village, Russian Federation*

[3]All-Russian Horticultural Institute for Breeding, Agrotechnology and Nursery, *Moscow, Russian Federation*

[4]Lorch Potato Research Institute, *Kraskovo village, Moscow Region, Russian Federation*

**Corresponding author: iskren@bio21.bas.bg*

Abstract. The use of amperometric express method made it possible to measure quickly and evaluate content of water- and alcohol-soluble antioxidants in extracts from *Amaranthus tricolor* L. plants. Accumulation of low molecular weight antioxidants: ascorbic acid, beta-cyanine (amaranthine) and the total content of antioxidants in various organs of Valentina amaranth seedlings were studied. The maximum amount of low molecular weight antioxidants accumulates in leaves, compared with roots and stems of seedlings grown in open and protected ground. In open ground conditions, amaranth leaves and stems have 1.5-fold and 2-fold increased level of ascorbic acid than seedlings grown in protected ground. But the total content of water-soluble antioxidants in leaves and roots of seedlings is lower compared to seedlings of protected ground. Minimum amount of antioxidants was found in alcohol extracts of stems and roots in open ground, while the total content of antioxidants in stems and roots was 1.6 fold higher in seedlings grown in protected soil. The content of amaranthine is comparable in the studied organs of amaranth seedlings of both cultivation variants. The data obtained allow to recommend use of leaves and stems of amaranth seedlings grown in open and protected ground (early spring and autumn), as a preventive antioxidant dietary product.

Key words: *Amaranthus tricolor* L. seedlings, water and alcohol extracts, low molecular weight antioxidants, amaranthine, ascorbic acid, open ground, protected ground

Article history:
Received: 16 August 2019. Accepted: 30 August 2019

For citation:
Sergiev IG, Todorova DA, Gins VK, Motyleva SM, Gins EM, Moskalev EA. Nutritional value of vegetable *Amaranthus tricolor* L. seedlings grown in Moscow region. *RUDN Journal of Agronomy and Animal Industries*, 2019; 14(3):225—238. doi: 10.22363/2312-797X-2019-14-3-225-238

Пищевая ценность сеянцев овощного вида *Amaranthus tricolor* L., выращенных на зелень рассадным способом в условиях Московской области

И.Г. Сергиев[1], Д.А. Тодорова[1], В.К. Гинс[2], С.М. Мотылева[3], Е.М. Гинс[4], Е.А. Москалев[4]

[1]Институт физиологии и генетики Болгарской академии наук, *София, Болгария*

[2]Федеральный научный центр овощеводства,
п. ВНИИССОК, Московская область, Российская Федерация

[3]Всероссийский селекционно-технологический институт садоводства и питомниководства,
Москва, Российская Федерация

[4]Всероссийский научно-исследовательский институт картофельного хозяйства имени А.Г. Лорха,
п. Красково, Московская область, Российская Федерация

**Автор-корреспондент: iskren@bio21.bas.bg*

Аннотация. Использование амперометрического экспресс-метода позволило оперативно измерить и оценить содержание водо- и спирторастворимых антиоксидантов в экстрактах из растений *Amaranthus tricolor* L. Исследовали накопление низкомолекулярных антиоксидантов: аскорбиновой кислоты, бетацианина — амарантина и суммарное содержание антиоксидантов в различных органах сеянцев амаранта сорта Валентина. Максимальное количество низкомолекулярных антиоксидантов накапливается в листьях, по сравнению с корнями и стеблями сеянцев, выращенных в открытом и защищенном грунте. В условиях открытого грунта уровень аскорбиновой кислоты в листьях в 1,5 и в стеблях амаранта в 2 раза выше, чем в сеянцах в защищенном грунте. В то время как суммарное содержание водорастворимых антиоксидантов в листьях и корнях сеянцев меньше по сравнению с сеянцами защищенного грунта. В спиртовых экстрактах стеблей и корней обнаружено минимальное количество антиоксидантов в открытом грунте, при этом суммарное содержание антиоксидантов в стеблях и корнях в 1,6 раза выше у сеянцев защищенного грунта. Содержание амарантина сравнимо в исследованных органах сеянцев амаранта обоих вариантов выращивания. Полученные данные позволяют рекомендовать использование листьев и стеблей сеянцев амаранта, выращенных в открытом и защищенном грунте (ранней весной и осенью), в качестве профилактического антиоксидантного продукта диетического назначения.

Ключевые слова: сеянцы *Amaranthus tricolor* L., водные и спиртовые экстракты, низкомолекулярные антиоксиданты, амарантин, аскорбиновая кислота, открытый грунт, защищенный грунт

История статьи
Поступила в редакцию: 16 августа 2019 г. Принята к публикации: 30 августа 2019 г.

Для цитирования
Сергиев И.Г., Тодорова Д.А., Гинс В.К., Мотылева С.М., Гинс Е.М., Москалев Е.А. Пищевая ценность сеянцев овощного вида *Amaranthus Tricolor* L., выращенных на зелень рассадным способом в условиях Московской области // Вестник Российского университета дружбы народов. Серия: Агрономия и животноводство. 2019. Т. 14. № 3. С. 225—238. doi: 10.22363/2312-797X-2019-14-3-225-238

Introduction

In hot and humid regions of the world, edible species of leafy amaranths (genus *Amaranthus)* are considered to be popular vegetable crops: *A. tricolor*, *A. blitum*, *A. dubius*, *A. cruentus* and *A. Viridis* [1—3]. In many countries of Africa and Southeast

Asia, India, southern China, amaranth leafy greens are widely used for food purposes, growing it as parsley, leaves and stems of which are used for food purposes. Young plants of vegetable species of amaranth are used in a variety of salads, appetizers, side dishes, soups, fillings for confectionery, drinks, pasta and even traditional medicine [4—7].

Such widespread use of vegetable amaranth as a food product is explained by a number of reasons, including the fact that spicy aromatic plants are practically not grown in these countries, and amaranth makes up for greens in many dishes [8, 9]. The popularity of amaranth vegetables is due to their mild piquant taste and high nutritional value, leaves of which are rich in gluten-free protein, vitamins, minerals, especially calcium, iron, as well as biologically active substances [10]. In addition, in a number of countries there is a shortage of animal protein, and leaves of amaranth vegetable species contain up to 20 % of a complete protein, balanced for essential amino acids. Therefore, population of these regions replenishes the lack of dietary protein by leaves of wild vegetable amaranth, growing seedlings and preparing various diets based on them [11].

Amaranth seedlings (young plants) are a commercial product in some countries. In Indonesia, amaranth vegetables are grown on an area of 2000 hectares. In tropical countries, amaranth is sown year-round. Due to the short development cycle of seedlings (7-8 weeks), they are cut for food several times a year.

Cultivation of vegetable amaranth in non-chernozem zone faces a number of problems. For example, return cold in spring months of April-May does not allow sowing of amaranth seeds in open ground before the end of May or the beginning of June; moisture deficiency in dry years can ruin the crop, since amaranth seedlings require more watering, compared to adult plants. In addition, amaranth seedlings in open ground can suffer from weeds, whose growth rate is much higher than growth of amaranth seedlings, and later snails can harm young seedlings [12]. When growing amaranth seedlings in a protected ground, such problems do not occur. Therefore, it is important to represent change patterns in the main morphological and biochemical parameters that determine productivity and nutritional and pharmacopoeial value of seedlings grown in open and protected ground.

The aim of the work was to study morphological and biochemical parameters of amaranth seedlings when growing them in open and protected ground for food use.

Materials and methods

The object of the study are vegetable amaranth plants (*Amaranthus tricolor* L.) of Valentina cultivar originated in Russian Research Institute of Selection and Seed Production of Vegetable Crops (Moscow Region). Cassettes with pre-moistened peat mixture were used for sowing seeds. Sowing depth was 0.5…1 cm. After 4 weeks, the seedlings were transplanted into soil in protected and open ground [13]. Plants were grown on sod-podzolic soil with a heavy mechanical composition at night temperature of 8…14 °C and day temperature 9…25 °C in open ground, and at night temperature 14…17 °C and day temperature 22…30 °C in protected ground.

Young plants aged 6-7 weeks were cut off and morphometric indicators were studied: plant height, mass of plants, leaves, stem, root, length and width of leaf blades. In the experiment, amaranth plants of Valentina cultivar grown in protected and open ground were compared. From each experimental plot, 15 plants were collected.

Biochemical studies were carried out in the Laboratory of physiology and biochemistry of introduction and functional products of Federal Scientific Technological Center in 2018. Aqueous and alcoholic extracts of leaves, stems and roots of seedlings were used in the experiments.

Extraction of crushed leaves and other plant organs was carried out with distilled water at room temperature (water ratio 1:10), followed by centrifugation at 10,000 rpm. Amount of amaranthine in aqueous extracts was determined spectrophotometrically considering a molar extinction coefficient of $5.66 \cdot 10^4 \cdot M^{-1} \cdot cm^{-1}$ and a molar weight of 726.6 [14]. Content of reduced form of ascorbic acid (AA) was determined by iodometric method based on titration of ascorbic acid in colored extracts with potassium iodate in acidic medium in presence of potassium iodide and starch [15]. The total content of antioxidants was determined by amperometric method, the result was expressed in gallic acid equivalents mEq.GK/g. The measurements were performed on a Tsvet-Yauza 01-AA device in a constant current mode [16]. The samples were crushed on a homogenizer in presence of a certain volume of extracting liquid (double-distilled water, 96% ethanol) at 20…25 °C. Then, the homogenizer was centrifuged at 10,000 g for 15 minutes at 4 °C. An aliquot of the supernatant was used to determine the total antioxidant content, if necessary diluting.

The tables 1—4 show the arithmetic mean values and standard deviations.

Results and discussions

Dark-colored A. tricolor seeds of Valentina cultivar germinated with an interval of two days. A slower increase in seedling height was observed in the stage of the first pair of true leaves and a sharp increase in plant height with development of subsequent leaves. Seedlings having well-developed cotyledons and first pair of true leaves were transplanted into protected and open ground.

Analysis of morphometric parameters of plant before cutting revealed that amaranth seedlings grown in sheltered soil had significantly higher morphometric indicators compared to seedlings grown in open ground.

The photosynthetic productivity of aerial parts of plants was significantly higher in seedlings in protected soil. When growing amaranth plants in protected ground at seedling stage, a more massive main shoot (stem) with a smaller mass of leaves is formed compared to open ground plants. Study of structure of seedlings' crop showed that aerial mass of seedlings grown in open ground have 52 % of leaves, 35 % of stems, while young plants in protected ground form 42 % of leaves and 44 % of stems.

Table 1

Determination of structure of Valentina seedlings grown in open and protected ground

Ground	Plant mass, g	Leaf mass, g Percentage by Plant mass, %	Stem mass, g Percentage by Plant mass	Root mass, g Percentage by Plant mass
Open	2.52	1.30	0.90	0.35
		52%	35%	13%
Protected	7.90	3.36	3.5	1.1
		42%	44%	14%

It is interesting to note that root mass of seedlings of both variants was 13…14 % of the aboveground mass. This suggests that photosynthetic metabolites in plants of open ground are accumulated in large quantities in leaves, while they are distributed almost evenly between leaves and stems in plants of protected ground.

Gluten-free protein, balanced for essential amino acids, and biologically active substances with antioxidant activity that affect physiological functions of the human body, effectively participating in metabolic and protective reactions comprise nutritional and pharmacopoeial value of Valentina leaves (amaranth *A. tricolor* L.) [17]. Ascorbic acid is a necessary component for human life. Some vegetable crops accumulate ascorbic acid in high concentrations: bell pepper — up to 200 mg%, leafy vegetable plants: vegetable chrysanthemum — up to 80 mg%, watercress and coriander — up to 150 mg% [18].

Table 2

Ascorbic acid in Valentina amaranth plant organs

Plant organs	Ascorbic acid content, mg%	
	Protected ground	Open ground
Leaves	108	167
Stems	19.8	44
Roots	25.12	22.8

Amaranth seedlings accumulate reduced ascorbic acid in all organs, but in different amounts. The data presented in table 2 indicate that the maximum amount of ascorbic acid accumulates in leaves of both variants, and the minimum — in stems of seedlings of protected soil and in roots of seedlings of open ground. The data obtained indicate that content of ascorbic acid in various plant organs depends on temperature conditions during cultivation. It is known that plant cell reactive oxygen species are formed at low temperature, where superoxide anion radical is the most dangerous. Ascorbic acid is able to neutralize O_2-. In open ground, a decrease in night temperature to 8 °C and lower for heat-loving seedlings is a stress factor that slows down their growth and development. Therefore, under these conditions, ascorbic acid, whose level in leaves of amaranth grown in open ground, was 1.5 fold higher than that in leaves of plants of protected ground, serves as protection from action of superoxide anion radicals. Compared to leaves, 5.45-fold decreased ascorbic acid was accumulated in stems of protected seedlings and 3.7-fold less accumulated ascorbic acid was in stems of open ground seedlings. An unequal amount of ascorbic acid was found in roots of young plants grown in open and protected ground. Ascorbic acid was accumulated in large quantities in leaves and stems of seedlings grown in open ground, which might indicate its active generation under conditions of weak low-temperature stress. In addition to ascorbic acid, red-colored pigment amaranthine with antioxidant activity comparable to that of superoxide dismutase is involved in the detoxification of the superoxide anion radical [19]. Leaves and inflorescences of amaranth seedlings of both cultivation variants contained practically comparable amounts of amaranthine, while stems of young open-ground plants accumulated 1.5 times more antioxidant than stems of seedlings of protected soil.

Table 3

Amaranthine level in organs of Valentina amaranth plants, mg per g wet weight

Sample	Protected ground	Open ground
Leaves	0.62± 0.03	0.59±0.03
Stems	0.22±0.01	0.34±0.02

The study of the total content of antioxidants in leaves and stems of amaranth seedlings grown in protected and open ground revealed the maximum content of antioxidants in water extracts, which was 2.5—3 fold higher than the level of antioxidants extracted in alcohol extract.

Table 4

Water- and alcohol-soluble antioxidants in Valentina amaranth, mg.Eq.GK/g

Sample	Protected Ground		Open Ground	
	Water	C_2H_5OH	Water	C_2H_5OH
Leaves	1.88± 0.09	0.65± 0.03	1.65± 0.08	0.63± 0.03
Stems	0.80± 0.04	0.38± 0.02	0.90± 0.05	0.23± 0.01
Roots	1.15± 0.06	0.30± 0.02	0.80±0.01	0.19±0.01

Moreover, a lower content of water- and alcohol-soluble antioxidants was found in stems of seedlings of both variants, but it was comparable with leaves.

Flavonoids have previously been shown to be contained in leaves of Valentina amaranth [19]. It should be noted that electrochemical oxidation of low molecular weight molecules with antioxidant activity of aqueous and alcoholic extracts can be described using flavonoids as an example by the following reaction: flavonoid –O–H – flavonoid –O+e+H^+.

Ability of ascorbic acid, amaranthine and flavonoid molecules to oxidize on electrode at a given potential indicates the ability of these molecules to capture free radicals [21]. The high total content of water and alcohol soluble antioxidants and ascorbic acid in the leaves of amaranth seedlings indicates a high antioxidant potential of seedlings, which actively protects young open ground plants from low temperature stress factors.

Conclusions

Low night temperature (8…10 °C) in open ground has a positive effect on photosynthetic productivity, growth and development of seedlings. Moreover, their leaf mass is characterized by the maximum amount of ascorbic acid, a comparable amount of amaranthine and the total content of water- and alcohol-soluble antioxidants. At a lower night temperature, more ascorbic acid and hydrophilic antioxidants are accumulated in stems of open-ground plants compared to protected-seedlings grown at optimum temperature.

In the roots of seedlings grown in open ground, less ascorbic acid and a lower content of hydrophilic and hydrophobic antioxidants were found, which suggests a more active outflow of metabolites — antioxidants from roots to plant's aboveground organs.

The data obtained allows to recommend use of amaranth seedlings grown in open and protected ground as a dietary antioxidant product for preventive purposes.

Введение

В жарких и влажных регионах мира востребованными овощными культурами считаются листовые амаранты рода Amaranthus съедобных видов: *A. tricolor, A. blitum, A. dubius, A. cruentus* и *A. Viridis* [1—3]. Во многих странах Африки и Юго-Восточной Азии, в Индии и Южном Китае в пищу широко

употребляют листовую зелень амаранта, выращивая ее как петрушку, листья и стебли которой применяют на пищевые цели. Молодые растения овощных видов амаранта используют при приготовлении разнообразных салатов, закусок, гарниров, супов, начинок для кондитерских изделий, напитков, макаронных изделий и даже народной медицине [4—7].

Столь широкое использование надземной части овощного амаранта в качестве пищевого продукта объясняется рядом причин, в т.ч. тем, что в этих странах практически не выращивают пряно-ароматические растения, а зелень во многих блюдах восполняет амарант [8, 9]. Популярность овощных амарантов обусловлена их мягким пикантным вкусом и высокой питательной ценностью, их листья отличаются богатым содержанием безглютенового белка, витаминов, минералов, особенно кальция и железа, а также биологически активных веществ [10]. Кроме того, в целом ряде стран существует дефицит животного белка, а листья овощных видов амаранта содержат до 20 % полноценного белка, сбалансированного по незаменимым аминокислотам. Поэтому население этих регионов пополняет недостаток пищевого белка путем сбора листьев дикорастущих овощных амарантов или выращивания сеянцев и приготовления на их основе разнообразных рационов [11].

В некоторых странах сеянцы (молодые растения) амаранта являются коммерческим товаром. В Индонезии овощной амарант выращивают на площади 2000 га. В тропических странах амарант высевают круглый год. Благодаря короткому циклу развития сеянцев (7-8 недель) их срезают на пищевое использование несколько раз за год.

Выращивание овощного амаранта в нечерноземной зоне на витаминную зелень сопряжено с рядом проблем. Например, возвратные холода в весенние месяцы апрель-май не позволяют провести сев семян амаранта в открытом грунте раньше конца мая или начала июня; дефицит влаги в засушливые годы может погубить урожай, поскольку сеянцы амаранта в большей степени нуждаются в поливе в отличие от взрослых растений. Помимо этого, всходы амаранта в открытом грунте могут страдать от сорной травы, скорость роста которой намного превосходит рост проростков амаранта, а позже молодые сеянцы могут уничтожить улитки [12]. При доращивании рассады амаранта в защищенном грунте таких проблем не возникает. Поэтому важно представлять закономерности изменения основных морфологических и биохимических показателей, определяющих продуктивность и питательную и фармакопейную ценность сеянцев, доращиваемых в открытом и защищенном грунте.

Цель работы — исследование морфологических и биохимических показателей сеянцев амаранта при доращивании их рассадным способом в открытом и защищенном грунте на пищевое использование.

Материалы и методы

Объектом исследования являются растения амаранта овощного вида *Amaranthus tricolor* L., сорта Валентина селекции ВНИИ селекции и семеноводства овощных культур (Московская область). Для посева семян использовали кассеты с предварительно увлажненной торфяной смесью. Посев проводили на глубину 0,5…1 см. После достижения 4-недельного возраста проростки пересаживали

в почву в защищенном и открытом грунте [13]. Растения выращивали в открытом грунте на дерново-подзолистой почве с тяжелым механическим составом при ночной температуре 8…14 °С и дневной 9…25 °С, а в защищенном грунте при ночной температуре 14…17 °С и 22…30 °С при дневной.

Молодые растения в возрасте 6-7 недель срезали и изучали морфометрические показатели: высоту растений, массу растений, листьев, стебля, корня, длину и ширину листовой пластинки. В опыте сравнивали растения амаранта сорта Валентина, выращенные в условиях защищенного и открытого грунта. С каждого опытного участка было собрано по 15 растений.

Биохимические исследования проводили в лаборатории физиологии и биохимии интродукции и функциональных продуктов ФГБНУ ФНЦО в 2018 г. В опытах использовали водные и спиртовые экстракты листьев, стеблей и корней сеянцев.

Экстракцию измельченных листьев и других органов растения проводили дистиллированной водой при комнатной температуре (гидромодуль 1:10) с последующим центрифугированием при 10000 об/мин. Количество амарантина в водных экстрактах определяли спектрофотометрическим методом с учетом молярного коэффициента экстинкции $5{,}66 \cdot 10^4 \cdot М^{-1} \cdot см^{-1}$ и молярного веса 726,6 [14]. Содержание восстановленной формы аскорбиновой кислоты (АК) определяли йодометрическим методом, основанным на титровании аскорбиновой кислоты в окрашенных экстрактах йодатом калия в кислой среде в присутствии йодистого калия и крахмала [15]. Суммарное содержание антиоксидантов определяли амперометрическим методом, результат выражали в эквивалентах галловой кислоты мг-экв. ГК/г. Измерения проводили на приборе «Цвет-Яуза 01-АА» в постоянном токовом режиме [16]. Измельчение образцов проводили в присутствии определенного объема экстрагирующей жидкости (бидистиллированная вода, 96% этиловый спирт) на гомогенизаторе при температуре 20…25 °С. Далее гомогенизат центрифугируется при 10000g 15 мин при 4 °С. Аликвоту супернатанта использовали для определения суммарного содержания антиоксидантов, при необходимости, разбавляя.

В табл. 1—4 приведены средние арифметические значения величины и стандарты отклонения.

Результаты и обсуждения

Из темноокрашенных семян амаранта, A. tricolor сорта Валентина высеянных в кассеты, всходы появились с интервалом в двое суток. Наблюдали замедленный прирост высоты проростка в стадии развития первой пары настоящих листьев и резкое увеличение высоты растения с развитием последующих листьев. При получении хорошо развитых семядольных и первой пары настоящих листьев рассаду пересаживали в защищенный и открытый грунт.

Анализ морфометрических показателей растения перед срезкой выявил, что сеянцы амаранта, выращенные в защищенном грунте, отличались существенно более высокими морфометрическими показателями по сравнению с сеянцами, выращенными в открытом грунте.

Фотосинтетическая продуктивность надземной массы растений была существенно выше у сеянцев защищенного грунта. При выращивании растений амаранта

в закрытом грунте на стадии сеянцев формируется более массивный главный побег (стебель) с меньшей массой листьев по сравнению с растениями открытого грунта. Изучение структуры урожая сеянцев показало, что надземная масса сеянцев, выращенных в открытом грунте, на 52 % состоит из листьев, на 35 % — из стеблей от общей массы растения, в то время как в защищенном грунте у молодых растений формируется листьев — 42 % и стеблей 44 % от общей массы растения (табл. 1).

Таблица 1

Определение структуры сеянцев амаранта сорта Валентина, выращенных в открытом и защищенном грунте

Грунт	Масса растения, г	Масса листьев, г	Масса стеблей, г	Масса корней, г
		Доля от массы растения, %	Доля от массы растения, %	Доля от массы растения, %
Открытый	2,52	1,30	0,90	0,32
		52 %	35 %	13 %
Защищенный	7,96	3,36	3,5	1,1
		42 %	44 %	14 %

Интересно отметить, что масса корней у сеянцев обоих вариантов составляла 13…14 % от надземной массы. Это позволяет предположить, что фотосинтетические метаболиты в растениях открытого грунта накапливаются в большем количестве в листьях, тогда как в растениях защищенного грунта распределяются практически равномерно между листьями и стеблями.

Пищевую и фармакопейную ценность листьев амаранта A. tricolor L. сорта Валентина составляет не только безглютеновый белок, сбалансированный по незаменимым аминокислотам, но и биологически активные вещества с антиоксидантной активностью, которые воздействуют на физиологические функции организма человека, эффективно участвуя в метаболических и защитных реакциях [17]. Из эссенциальных нутриентов необходимым компонентом жизнедеятельности человека является аскорбиновая кислота. Из овощных культур болгарский перец накапливает аскорбиновую кислоту в высокой концентрации до 200 мг%, а также листовые овощные растения: хризантема овощная до 80 мг%, а водяной кресс и кориандр до 150 мг% [18].

Таблица 2

Содержание аскорбиновой кислоты в органах растения амарант сорта Валентина, выращенных в условиях защищенного и открытого грунта

Органы растения	Содержание аскорбиновой кислоты	
	Защищенный грунт	Открытый грунт
Листья	108	167
Стебли	19,8	44
Корни	25,12	22,8

Сеянцы амаранта аккумулируют восстановленную аскорбиновую кислоту во всех органах, однако в неодинаковом количестве. Данные, приведенные в табл. 2, указывают, что максимальное количество аскорбиновой кислоты накапливается в листьях обоих вариантов, а минимальное — в стеблях сеянцев защищенного грунта и в корнях сеянцев открытого грунта. Полученные данные указывают, что содержание аскорбиновой кислоты в различных органах растения зависит от температурных условий выращивания. Известно, что при низкой

положительной температуре в растительной клетке образуются активные формы кислорода, из которых наибольшую опасность представляет супероксидный анион-радикал. Аскорбиновая кислота способна обезвреживать O_2^-. В открытом грунте снижение ночной температуры до 8 °С и ниже для теплолюбивых сеянцев является стресс-фактором, замедляющим их рост и развитие. В этих условиях защитой от действия супероксидных анион-радикалов служит аскорбиновая кислота, уровень которой в листьях амаранта, выращенного в открытом грунте, в 1,5 раза превышает таковой в листьях растений защищенного грунта. В стеблях сеянцев защищенного грунта аккумулируется меньше аскорбиновой кислоты по сравнению с листьями в 5,45 раза, а в стеблях сеянцев открытого грунта накапливается аскорбиновой кислоты в 3,7 раза меньше, чем в листьях. Неодинаковое количество аскорбиновой кислоты обнаружено в корнях молодых растений, выращенных в открытом и защищенном грунте. Аскорбиновая кислота накапливается в большем количестве в листьях и стеблях сеянцев, выращенных в открытом грунте, что может указывать на ее активную генерацию в условиях слабого низкотемпературного стресса. Помимо аскорбиновой кислоты в обезвреживании супероксидного аниона-радикала участвует краснокрашенный пигмент амарантин с антиоксидантной активностью, сравнимой с активностью супероксид дисмутазы [19]. Листья и соцветия сеянцев амарантов обоих вариантов выращивания содержат практически сравнимые количества амарантина, в то время как стебли молодых растений открытого грунта накапливают в 1,5 раза больше антиоксиданта, чем стебли сеянцев защищенного грунта.

Таблица 3

Определение амарантина в различных органах растений амаранта A. tricolor L. сорта Валентина, мг на г сыр. массы

Образец	В защищенном грунте	В открытом грунте
Листья	0,62± 0,03	0,59±0,03
Стебли	0,22±0,01	0,34±0,02

Изучение суммарного содержания антиоксидантов в листьях и стеблях сеянцев амаранта, выращенных в защищенном и открытом грунте, выявило максимальное содержание антиоксидантов в водных экстрактах, которое в 2,5…3 раза превышало уровень антиоксидантов, экстрагированных в спиртовом экстракте.

Таблица 4

Определение суммы водо- и спирторастворимых антиоксидантов в различных органах растений амарант A. tricolor L. сорта Валентина, выращенных в защищенном и открытом грунте, мг. экв. ГК/г

Образец	В защищенном грунте		В открытом грунте	
	Вода	C_2H_5OH	Вода	C_2H_5OH
Листья	1,88± 0,09	0,65± 0,03	1,65± 0,08	0,63± 0,03
Стебли	0,80± 0,04	0,38± 0,02	0,90± 0,05	0,23± 0,01
Корни	1,15± 0,06	0,30± 0,02	0,80±0,01	0,19±0,01

При этом меньшее содержание водо- и спирторастворимых антиоксидантов обнаружено в стеблях сеянцев обоих вариантов, но сравнимо с листьями.

Ранее мы показали, что в листьях амаранта сорта Валентина содержатся флавоноиды. Следует отменить, что электрохимическое окисление низкомолекулярных молекул с антиоксидантной активностью водных и спиртовых экстрактов

может быть описано на примере флавоноидов следующей реакцией: флавоноид –O–H → флавоноид –O+e+H^+ [20].

Способность молекул аскорбиновой кислоты, амарантина, флавоноидов окисляться на электроде при заданном потенциале свидетельствует о способности данных молекул улавливать свободные радикалы [21]. Высокое суммарное содержание водо- и спирторастворимых антиоксидантов и аскорбиновой кислоты в листьях сеянцев амаранта указывает на высокий антиоксидантный потенциал сеянцев, который активно защищает молодые растения открытого грунта от низкотемпературного стресс-фактора.

Вывод

Низкая ночная температура (8…10 °C) в открытом грунте положительно сказывается на фотосинтетической продуктивности, росте и развитии сеянцев. При этом их листовая масса отличается максимальным количеством аскорбиновой кислоты, сравнимым количеством амарантина и суммарным содержанием водо- и спирторастворимых антиоксидантов. При пониженной ночной температуре в стеблях растений открытого грунта накапливается больше аскорбиновой кислоты и гидрофильных антиоксидантов по сравнению с сеянцами защищенного грунта, выращенными при оптимальной температуре.

В корнях сеянцев, выращенных в открытом грунте, обнаружено меньшее количество аскорбиновой кислоты и более низкое содержание гидрофильных и гидрофобных антиоксидантов, что позволяет предположить более активный отток метаболитов – антиоксидантов из корней в надземные органы растения.

Полученные данные позволяют рекомендовать использование сеянцев амаранта, выращенных в открытом и защищенном грунте, в качестве диетического антиоксидантного продукта профилактического назначения.

References

1. Rastogi A, Shukla S. Amaranth: A new millennium crop of nutraceutical values. *Critical reviews in food science and nutrition*. 2013; 53(2):109—125. doi: 10.1080/10408398.2010.517876
2. Bhattacharyya B, Johri BM. Amaranthaceae. In: Bhattacharyya B, Johri BM. (eds.) *Flowering plants: Taxonomy and phylogeny*. Delphi: Narosa Publishing House; 1998. p. 113.
3. Das S. Systematics and taxonomic delimitation of vegetable, grain and weed Amaranthus: a morphological and biochemical approach. *Genet Resour Crop Evol.* 2012; 59(2):289-303. doi: 10.1007/s10722-011-9684-7
4. Peter K, Gandhi P. Rediscovering the therapeutic potential of amaranthus species: A review. *Egyptian Journal of Basic and applied Sciences.* 2017; 4(3):196-205. Available from: doi: 10.1016/ j.ejbas. 2017.05.001
5. Rahmatullah M, Hosain M, Rahman S, Akter M. Antyhyperglycaemic and antinociceptive activity evaluation of methanolic extract of whole plant of Amaranthus tricolor L. (Amaranthaceae). *Afr. J. Trad. Complement Altern Med*. 2013; 10(5):408-411. doi: 10.4314/ajtcam.v10i5.31
6. Aneja S, Vats M, Aggarwal S, Sardana S. Phytochemistry and hepatoprotective activity of aqueous extract of Amaranthus tricolor Linn. roots. *J. Ayurveda Integr. Med.* 2013; 4(4):211-215. doi: 10.4103/0975-9476.123693
7. Clemente AC, Desai PV. Evaluation of the hematological, hypoglycemic, hypolipidemic and antioxidant properties of Amaranthus tricolor leaf extract in rat. *Tropical Journal of Pharmaceutical Research*. 2011; 10(5):595-602. doi: doi.org/10.4314/tjpr.v10i5.8

8. Sarker U, Islam MT, Rabbani MG, Oba S. Variability, heritability and genetic association in vegetable amaranth (Amaranthus tricolor). *Spanish Journal of Agricultural Research.* 2015; 13(2):1–8. doi: 10.5424/sjar/2015132-6843
9. Sarker U, Islam MT, Rabbani MG, Oba S. Genotype variability in composition of antioxidant vitamins and minerals in vegetable amaranth. *Genetika*. 2015; 47(1):85–96. doi: 10.2298/GENSR1501085S
10. Robbins RJ. Phenolic acids in foods: an overview of analytical methodology. *J. Agric Food Chem*. 2003; 51(10):2866-2887. doi: 10.1021/jf026182t
11. Berberich S. History of amaranth. *Agric. Res.* 1980; 29(4):14.
12. Gins MS, Gins VK, Kononkov PF. *Introduktsiya amaranta v Rossii* [Amaranth introduction in Russia]. Moscow. 2019. (In Russ).
13. Gins MS, Gins VK, Kononkov PF, Pivovarov VF, Romanova EV, Osokin IE, Gins EM. *Tekhnologiya vyrashchivaniya ovoshchnogo (listovogo) amaranta* [Technology for growing vegetable (leaf) amaranth]. Moscow: RUDN Publ.; 2017. (In Russ).
14. Baiwas M, Dey S, Sen R. Betalains from Amaranthus tricolor L. *Journal of Pharmacognosy and Phytochemistry*. 2013; 1(5):87-95.
15. Sapozhnikova EV, Dorofeeva LS. Determination of ascorbic acid content in colored plant extracts by iodometric method. *Food industry*. 1966; (5):29—31. (In Russ).
16. Gins M.S., Gins V.K., Baykov A.A., Romanova E.V., Kononkov P.F., Torres M.K., Lapo O.A. *Metodika analiza summarnogo soderzhaniya antioksidantov v listovykh i listostebel'nykh ovoshchnykh kul'turakh* [Methodology for the analysis of the total content of antioxidants in leafy and leafy vegetable crops]. Moscow: RUDN Publ.; 2013. (In Russ).
17. Gins MS, Gins VK. *Fiziko-biokhimicheskie osnovy introduktsii i selektsii ovoshchnykh kul'tur* [Physico-biochemical fundamentals of the introduction and selection of vegetable crops]. Moscow: RUDN Publ.; 2011. (In Russ).
18. Sarker U, Oba S. Drought stress effects on growth, ROS markers, compatible solutes, phenolics, flavonoids, and antioxidant activity in Amaranthus tricolor. *Appl Biochem Biotechnol*. 2018; 186(4):999-1016. doi.org/10.1007/s12010-018-2784-5
19. Piattelli M. Betalains. In: Goodwin TW. (ed.) *Chemistry and biochemystry of plant pigments*. N.Y.: Acad. Press; 1976. p.560-596.
20. Blokhina O, Fagerstedt KV. Oxidative metabolism, ROS and NO under oxygen deprivation. *Plant Physiology and Biochemistry.* 2010; 48(5):359–373. doi.org/10.1016/j.plaphy.2010.01.007
21. Vladimirov YA, Proskurnina EV, Demin EM, Matveeva NS, Lyubitsky OB, Novikov AA. et al. Dihydroquercetin (taxifolin) and other flavonoids as inhibitors of the formation of free radicals at key stages of apoptosis. *Biochemistry*. 2009; 74(3):372-379.

Библиографический список

1. *Rastogi A., Shukla S.* Amaranthus: A new millennium crop of nutraceutical values // Critical reviews in food science and nutrition. 2013. Vol. 53. P. 109—125. doi:10.1080/10408398.2010.517876
2. *Bhattacharyya B., Johri B.M.* Amaranthaceae // Bhattacharyya B. Johri B.M (Eds.), Flowering plants: Taxonomy and phylogeny. Delphi : Narosa Publishing House, 1998. P. 113—113.
3. *Das S.* Systematic and taxonomic delimitation of vegetable, grain and weed Amaranthus: a morphological and biochemical approach // Genet Resour crop Evol. 2012. № 59. P. 289—303.
4. *Kavita Peter, Puneet Gandhi.* Rediscovering the therapeutic potential of amaranthus species: A review // Egyptian Journal of Basic and applied Sciences. 2017. http: dx.doi.org/10.1016/j.ejbas.2017.05.001
5. *Rahmatullah M., Hosain M., Rahman S. et all.* Antyhyperglycaemic and antinociceptive activity evaluation of methanolic extract of whole plant of Amaranthus tricolor L. (Amaranthaceae) // Afr. J. Trad. Complement Altern Med. 2013. № 10. P. 408—411.

6. *Aneja S., Vats M., Aggarwal S., Sardana S.* Phytochemistry and hepatoprotective activity of aqueous extract of Amaranthus tricolor Linn. Roots // J. Ayurveda Integr. Med. 2013. 4. P. 211—215.
7. *Clemente A.C., Desai P.V.* Evaluation of the hematological, hypoglycemic, hypolipidemic and antioxidant properties of Amaranthus tricolor leaf extract in Rat // Tropical Journal of Pharmaceutical Research. 2011. № 10. P. 595—602.
8. *Sarker U., Islam M.T., Rabbani M.G., Oba S.* Variability, heritability and genetic association in vegetable amaranth (Amaranthus tricolor) // Spanish Journal of Agricultural Research. 2015. Vol. 13. № 2. P. 1—8. doi: 10.5424/sjar/2015132-6843
9. *Sarker U., Islam M.T., Rabbani M.G., Oba S.* Genotype variability in composition of antioxidant vitamins and minerals in vegetable amaranth // Genetika. 2015. Vol. 47. P. 1. P. 85–96. doi: 10.2298/GENSR1501085S
10. *Robbins R.J.* Phenolic acids in foods: an overview of analytical methodology // J. Agric Food Chem. 2003. Vol. 51. P. 2866—2887.
11. *Berberich S.* History of amaranth // Agric. Res. US. 1980. Vol. 29. № 4. P. 14.
12. *Гинс М.С., Гинс В.К. Кононков П.Ф.* Интродукция амаранта в России. М., 2019.
13. *Гинс М.С., Гинс В.К., Кононков П.Ф., Пивоваров В.Ф., Романова Е.В., Осокин И.Е., Гинс Е.М.* Технология выращивания овощного (листового) амаранта. М., 2017.
14. *Mousumi Baiwas, Satyahari Dey, Ramkrishna Sen.* Betalains from Amaranthus tricolor L. // Journal of Pharmacognosy and Phytochemistry. 2013. Vol. 1 № 5. P. 87—95.
15. *Сапожникова Е.В., Дорофеева Л.С.* Определение содержания аскорбиновой кислоты в окрашенных растительных экстрактах йодометрическим методом // Консервная и овощесушильная промышленность. 1966. № 5. С. 29—31.
16. *Гинс М.С., Гинс В.К., Байков А.А., Романова Е.В., Кононков П.Ф., Торрес М.К., Лапо О.А.* Методика анализа суммарного содержания антиоксидантов в листовых и листостебельных овощных культурах. М., 2013.
17. *Гинс М.С., Гинс В.К.* Физико-биохимические основы интродукции и селекции овощных культур. М. : РУДН, 2011, 128 с.
18. *Sarker U., Oba S.* Drought stress effects on growth, ROS markers, compatible solutes, phenolics, flavonoids, and antioxidant activity in Amaranthus tricolor. Appl Biochem Biotechnol. 2018. Vol. 186. № 4. P. 999—1016. doi.org/10.1007/s12010-018-2784-5
19. *Piattelli M.* Betalains // Chemistry and bioche-mystry of plant pigments / Goodwin TW., ed. N.Y. : Acad. Press, 1976. P. 560—596.
20. *Blokhina O., Fagerstedt K.V.* Oxidative metabolism, ROS and NO under oxygen deprivation // Plant Physiology and Biochemistry. 2010. Vol. 48. № 5. P. 359–373. doi.org/10.1016/j.plaphy.2010.01.007
21. *Владимиров Ю.А., Проскурина Е.В., Демин Е.М., Матвеева Н.С., Любицкий О.Б., Новиков А.А., Измайлов Д.Ю., Осипов А.Н., Тихонов В.П., Каган В.Е.* Дигидрокверцетин (таксифолин) и другие флавоноиды как ингибиторы образования свободных радикалов на ключевых стадиях апоптоза // Биохимия. 2009. Т. 74. № 3. С. 372—379.

About authors:

Sergiev Iskren Georgiev — Associate professor, PhD, Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences; 21 Acad. Georgi Bonchev St., Sofia, Bulgaria, 1113; ORCID ID: 0000-0002-2420-9146; Scopus ID: 6603230596; e-mail: iskren@bio21.bas.bg

Todorova Desislava Aleksandrova — Associate professor, PhD, Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences; 21 Acad. Georgi Bonchev St., Sofia, Bulgaria, 1113; ORCID ID: 0000-0001-7100-834X; Scopus ID: 55569034700; e-mail: dessita@bio21.bas.bg

Gins Valentina Karlovna — Doctor of Biological Sciences, professor, chief researcher at Laboratory of Plant Physiology and Biochemistry and Introduction of Federal Scientific Vegetable Center; 14 Selektsionnaya st., VNIISSOK village, Moscow region, Odintsovo, Russian Federation, 143072; e-mail: anirr@bk.ru; ORCID ID: 0000-0002-7053-4345

Motyleva Svetlana Mikhailovna — Candidate of Agricultural Science, Associate Professor, Head of Laboratory of Physiology and Biochemistry of All-Russian Horticultural Institute for Breeding, Agrotechnology and Nursery; 4 Zagoryevskaya st., Moscow, Russian Federation; ORCID ID: 0000-0003-3390-1958; SCOPUS ID: 55996650700

Gins Ekaterina Muratovna — Junior Researcher, Lorch Potato Research Institute; 23 Lorha st., Kraskovo village, Lyubertsy district, Moscow region, 140051, Russian Federation; ORCID ID: 0000-0002-5685-6305

Moskalev Evgeny Aleksandrovich — Junior Researcher, Lorch Potato Research Institute; 23 Lorha st., Kraskovo village, Lyubertsy district, Moscow region, 140051, Russian Federation; ORCID ID: 0000-0002-4904-1159

Об авторах:

Сергиев Искрен Георгиев — доцент, доктор наук, Институт физиологии и генетики растений, Болгарская академия наук, Болгария, 1113, София, ул. Георгия Бончева, корп. 21, e-mail: iskren@bio21.bas.bg, ORCID ID: 0000-0002-2420-9146 SCOPUS ID: 6603230596

Тодорова Десислава Александрова — доцент, доктор наук, руководитель (регуляторы роста и развития растений), Институт физиологии и генетики растений, Болгарская академия наук, Болгария, 1113, София, ул. Георгия Бончева, корп. 21, e-mail: dessita@bio21.bas.bg, ORCID ID: 0000-0001-7100-834X Scopus ID 55569034700

Гинс Валентина Карловна — доктор биологических наук, профессор, главный научный сотрудник лаборатории физиологии и биохимии растений и интродукции Федерального государственного бюджетного научного учреждения «Федеральный научный центр овощеводства», Российская Федерация, 143072, Московская обл., Одинцовский р-н, п. ВНИИССОК, ул. Селекционная, д. 14; e-mail: anirr@bk.ru, ORCID ID: 0000-0002-7053-4345

Мотылева Светлана Михайловна — кандидат сельскохозяйственных наук, доцент, заведующий лаборатории физиологии и биохимии Федерального государственного бюджетного научного учреждения «Всероссийский селекционно-технологический институт садоводства и питомниководства», Российская Федерация, 115598, г. Москва, ул. Загорьевская, д. 4, ORCID ID:0000-0003-3390-1958 Scopus ID: 55996650700

Гинс Екатерина Муратовна — младший научный сотрудник Федерального государственного бюджетного научного учреждения «Всероссийский научно-исследовательский институт картофельного хозяйства имени А.Г. Лорха», Российская Федерация, 140051, Московская область, Люберецкий район, п. Красково, ул. Лорха, д. 23, литера «В» ORCID ID: 0000-0002-5685-6305

Москалев Евгений Александрович — младший научный сотрудник Федерального государственного бюджетного научного учреждения «Всероссийский научно-исследовательский институт картофельного хозяйства имени А.Г. Лорха», Российская Федерация, 140051, Московская область, Люберецкий район, п. Красково, ул. Лорха, д. 23, литера «В» ORCID ID: 0000-0002-4904-1159

RUDN Journal of Agronomy and Animal Industries
Вестник РУДН. Серия: АГРОНОМИЯ И ЖИВОТНОВОДСТВО
2019; 14(3):239—254
http://agrojournal.rudn.ru

ПОЧВОВЕДЕНИЕ И АГРОХИМИЯ
SOIL SCIENCE AND AGROCHEMISTRY

Research article

DOI 10.22363/2312-797X-2019-14-3-239-254

Geospatial analysis and assessment of garden soil contamination in New York City

Anna A. Paltseva*[1,2], Zhongqi Cheng[1,2]

[1]Brooklyn College of The City University of New York,
New York City, United States

[2]Graduate Center of The City University of New York,
New York City, United States

**Corresponding author: anyapaltseva@gmail.com*

Abstract. Elevated trace metal concentrations, in particular, lead (Pb), are prevalent in urban soils and it is one of the main hurdles for urban agriculture. The growing popularity of gardening in urban areas could also mean increased public health risk. In this study, the spatial distribution of Pb in New York City gardens was analyzed and visualized by Geographic Information System (GIS) tools. Pollution level and ecological risks of gardens and overall New York City (NYC) were evaluated with different indices. The degree of the contamination factors was ranked as follows: Pb >Cu > Zn > Cr>As>Ni>Cd. The single ecological risk index and potential ecological index indicated that Pb had moderate to significantly high risk to the local garden ecosystems. Based on the pollution load index, soil quality of the majority of NYC gardens were characterized as polluted. Geostatistical, geoprocessing, and spatial tools were used to create color-coded maps to support decision making related to gardening and to estimate potential human health risks from gardening, living, or working in/or near these gardens. These findings have important implications for the development of pollution prevention and mitigation strategies to reduce public health risk from garden soil trace metal contamination.

Key words: trace metals, GIS map, ecological index, lead, digital soil mapping, urban gardening

Notes. Authors declare no competing interests.

ACKNOWLEDGMENTS
The authors thank Tatiana Morin, George Lozefski, Zulema Blanco Garcia, Michael Grinshtein, Igor Bronz, Kayo Green, Donna Cao, Kristen Walsh, Norma Sutton, Ololade Thomas for their lab assistance and data collection, Rocky Lee Garcia and Dr. Viacheslav Vasenev for GIS consultation.

Article history:
Received: 14 August 2019. Accepted: 10 September 2019

For citation:
Paltseva AA, Cheng Z. Geospatial analysis and assessment of garden soil contamination in New York City. *RUDN Journal of Agronomy and Animal Industries*, 2019; 14(3):239—254. doi: 10.22363/2312-797X-2019-14-3-239-254

Introduction

Soil contamination in urban environments has only started to receive attention over the past few decades, while water and air pollution have been widely recognized and federal and state legislation were developed in the US since the early part of 20th century [1]. Significant health risks to urban residents and particularly to gardeners can come from interaction with contaminated soil and consumion of garden produce. Urban gardening has increased significantly in recent years; therefore, the inherent risks of gardening in contaminated soil has become an important issue of public health as more urban residents become affected by soil contamination.

Urban soil is a sink for anthropogenic Pb and other contaminants. Trace metals are among the most recalcitrant and lasting contaminants in cities, posing major health concerns [2]. In urban gardening, principal contaminant exposure pathways to human body consist of ingestion and inhalation of soil particles (including those lodged in vegetables through splash and local re-deposition), as well as ingestion of trace metal-contaminated vegetables [3—5]. Lead is a known neurotoxin affecting nearly all bodily systems [1, 6, 7]. Common health consequences for children are behavioral or learning issues, decreased IQ, hyperactivity, delayed growth, hearing problems, anemia, and in rare cases, Pb exposure can lead to seizures, coma, or death [8, 9].

Soil trace metal contamination is mainly the result of historical deposition from past land use and proximity to polluting sources, such as power plants, incinerators, old houses, and vehicular traffic [10, 11], as well as geogenic sources [12,13]. According to an EPA report (14), three main sources responsible for the elevated soil-lead levels have been identified: (1) lead-based paint; (2) point source emitters; and (3) leaded gasoline emissions. Many studies cite more than one source as commonly responsible for elevated soil-lead levels at a given location.

Starting in 1973, the U.S. federal government initiated a gradual phase-out of Pb in gasoline, and by 1996, banned the sale completely [15]. However, gardens near busy streets may have accumulated higher levels of Pb in the topsoil. Today, Pb is still emitted from some manufacturing sites such as metal smelting, battery manufacturing, and other factories that use Pb in industrial processes. Although the Toxic Substances Control Act (TSCA) banned the use of Pb-based paint in 1978, flakes of lead-based paint on the outside of the old buildings can also get into the soil close to the foundation of buildings. Contaminated soil dust can be re-suspended by wind, and mobilized into homes and yards. Lead contaminated soil has been recognized as one of the major sources of Pb exposure in urban settings [16].

Urban soils are known to be very spatially heterogeneous, varying in parent material and biological, chemical, and physical properties [17]. High concentrations of trace metals are often reported around the world with high degree of variability. Trace metals in a soil vary in their availability to plants, soil creatures, and humans depending how these characteristics spatially fluctuate in the urban landscape due to functional zoning, proximity to roads, emissions, etc. [17]. Soil Pb distribution in many

large cities has been investigated (e.g., [18—21], including New York City [22—25]. Previous studies have called for further detailed geospatial analysis of the data using large-scale Geographic Information Systems (GIS) for a better health-based assessment [26], as well as the evaluation of soil contamination in the context of risk to human health and threat to ecological systems. Thus, the aims of this study were: 1) to analyze the spatial distribution of Pb in NYC gardens, and 2) to assess pollution and ecological risk indices using available trace metal data.

Materials and methods

Data sources

The data on soil trace metal concentrations have been collected by the Brooklyn College Urban Soils Lab since 2009and the NYC Urban Soils Institute since 2016.This is part of a soil screening and testing service provided by the Labs to the public. Gardeners were instructed to collect soil from the surface down to depths of 14 to 20 cm (i.e., 6 inches) and composite soils collected from 5 to 10 locations around the garden. Each sample was recorded with a unique identification number, location, type of garden, soil trace metal concentration, and other soil characteristics such as pH, salts, organic matter content, and soil texture.

Soils are mostly screened by a portable X-ray fluorescence (pXRF) analyzer Innov-X Delta Classic [27], with some samples analyzed by Inductively coupled plasma mass spectrometry (ICP-MS) Perkin Elmer, Elan DRCe (EPA Method 6020a) following acid digestion with EPA Method 3052 [28, 29]. The pXRF scans were done directly on zip lock bags containing air-dried soil sent in by gardeners. Each sample was scanned three times with 90 sec exposure time. Samples were thoroughly mixed again between scans. Mean concentrations from the three scans were then recorded.

For external quality control for the ICP-MS analyzed samples reference standards SRM-2702, SRM-2586, SRM-2587, and SRM-2702a were used. Each batch digestion of up to 20samples always included at least three of these reference standards. Germanium was used as an internal standard for instrumental drift correction in all analyses. For the samples with both ICP-MS and pXRF analyses performed, there was good agreement between the two sets of Pb concentration data, with correlation coefficient of 0.94. It should be noted that Pb data include both ICP-MS data and pXRF data, but for other trace metals only ICP-MS data were used for this study.

The first map of Pb contamination for garden soils in New York City(NYC) was published in 2015 based on data for 1,652 garden soil samples, collected during the 2009—2014 period [23]. Li et al. (2017) added data from other land uses and from various sources and published a more comprehensive Pb distribution map for NYC. New data have been continuously collected, georeferenced and added to the original database. In total, there are 2322 garden soil samples in this study, collected during the 2009—2017 period (Fig. 3). A few samples could represent one garden with different soil management practices.

Shape files of Green Thumb Gardens, parks, schoolyards, playgrounds, NYC borough, and zip codes boundaries were downloaded from NYC Open data

(https://nycopendata.socrata.com/). The list of NYC neighborhoods (Table 2) was found at the NYC Department of Health and Mental Hygiene Environment & Health Data Portal. Boundaries for 42 neighborhoods were retrieved from the Official Website of the City of New York (http://www.health.ny.gov/).

Geospatial analysis and visualization

ESRI ArcGIS 10.5 was used for geospatial analysis and visualization of the trace metal data. Lead concentration was interpolated by ordinary kriging (Fig. 3). Kriging allows predicting the value in unmeasured points based on the known data in neighboring points and spatial relationships between the points. Ordinary kriging uses dimensionless points to estimate other dimensionless points, e.g. Pb contour plots.

The Pb levels are shown in mg/kg and are classified into four categories (0 – 149 mg/kg, 150 – 399 mg/kg, 400 – 1200 mg/kg and > 1,200 mg/kg). The 1200 mg/kg threshold reflects USEPA standard for non-children play areas and the 400 mg/kg threshold reflects USEPA standard for children play areas (USEPA 2001). The 150 mg/kg value is an estimated threshold for soil Pb, reflective of the new Center for Disease Control and Prevention (CDC) guidance. Based on research conducted by the Toxics Cleanup Program Policy and Technical Support Unit, 2010, a level of 150 mg/kg of Pb in soil can lead to an approximate blood Pb level of 5μg/dl [31].

Soil pollution level and ecological risk assessment

To assess pollution levels and ecological risk of the garden ecosystems the following indices were used:

1. The contamination factors CF_i for the same metal was determined as $CF_i=C_m/B_m$, where C_m is the measured concentration of the examined metals in the soil samples, and B_m is the background concentration in unpolluted soils [32]. The following values used in this study Cd=0.5, Pb=19, Zn=65, As=5, Ni=17, Cu=14, Cr=13 were adapted from New York State Department of Environmental Conservation Rural Soil Background Survey [33].

2. The single ecological risk index $E_i=T_i \cdot CF_i$, where T_i is the toxic-response factor for a given metal (e.g. Cd=30, Pb=5, Zn=1, As=10, Ni=5, Cu=5, Cr=2) [32];

3. The potential ecological risk index (PERI)=$\sum(E_i)$ posed by multiple element pollution was originally proposed by Hakanson (1980) to assess heavy metal contamination of sediments. Later, it was adopted to evaluate heavy metal contamination in soils and to relate ecological and environmental effects with their toxicology and the toxic-response factor [34, 35].

4. The pollution load index introduced by Tomlinson et al. (1980): $PLI=(CF_1 \cdot CF_2 \cdot \ldots \cdot CF_n)^{(1/n)}$, where is the number of metals studied), gives simple comparative means for assessing a site quality. The PLI shows the number of times by which the metal concentration in the soil exceeds the average natural background content. It provides a total indication of the overall level of trace metal toxicity in a given sample. The PLI value of > 1 is considered as polluted, PLI <1 — no pollution and PLI=1 means that trace metal load is close to the background level [37].

Results and discussion

Assessment of soil quality using pollution and ecological risk indices

To assess quality of soils and their contamination levels different indices were used. Using the contamination factors (CF) showed in Table 1, it was possible to rank the following degree of contamination factors based on the mean values for 746 samples analyzed with the ICP-MS: Pb > Cu > Zn > Cr > As > Ni > Cd. The contamination factors were classified as follows: low (CF<1); moderate (1<CF<3); considerable (3<CF<6); and very high (CF>6). This shows that Pb had the highest CF, followed by Cu, Cr and Zn, and all of them fall into the "very high" contaminant factor category. In comparison, overall Cd and Ni fall into the "low" category. It should be noted that, however, the CF values for individual samples are highly variable, and sometimes can differ by 2-3 orders of magnitude. This is consistent with the extreme heterogeneities commonly found for urban soils.

Table 1

Summary statistics of the contamination factors (CF) for *n* = 746

	Cr	Ni	Cu	Zn	As	Cd	Pb
Mean	7	3	11	7	4	2	32
Max	254	131	342	265	120	96	379
Min	0.49	0.01	0.01	0.51	0.02	0.04	0.08
Std Dev	11	6	16	11	5	9	41

The calculated E_i — Single ecological risk index of the individual contaminants is represented on logarithmic scale in Fig. 1. It indicates that Pb had moderate to significantly high risk to the local ecosystem, while Zn, Cr, and Ni indicated low risks and other elements (As and Cd) showed low to moderate risk or moderate risk (Cu).

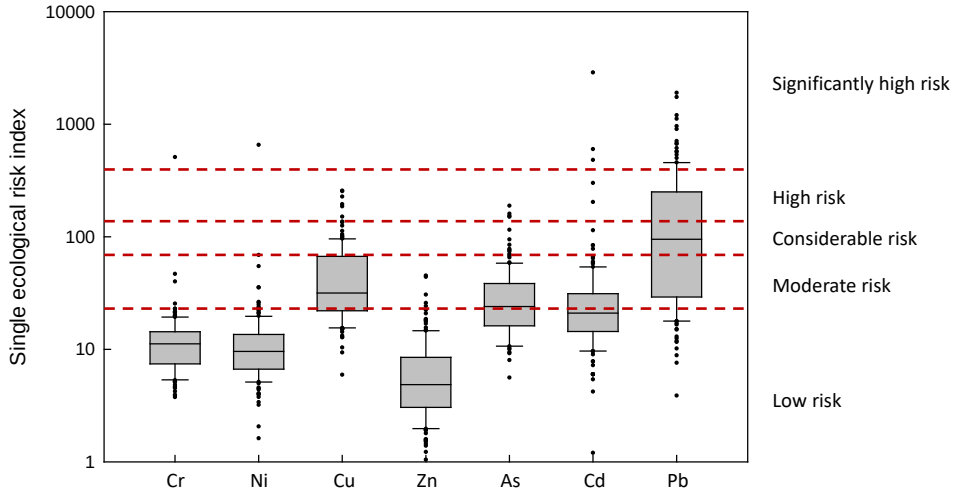


Fig. 1. Single ecological risk indices of the individual contaminants represented on the logarithmic scale

Contributions of individual trace elements to the overall potential ecological risk of the soil are represented on Fig. 2. The ecological risk comes mainly from soil pollution with Pb (46 %), consistent with the single ecological index (Ei) and the contaminant factor (CF). When the overall potential ecological risk (PERI) to the local ecosystems is considered, 12 % of the studied samples had very high PERI (>600), 28 % had considerable PERI (300-600), 30 % had moderate PERI (150-300), and another 30 % of the samples had low PERI (<150).

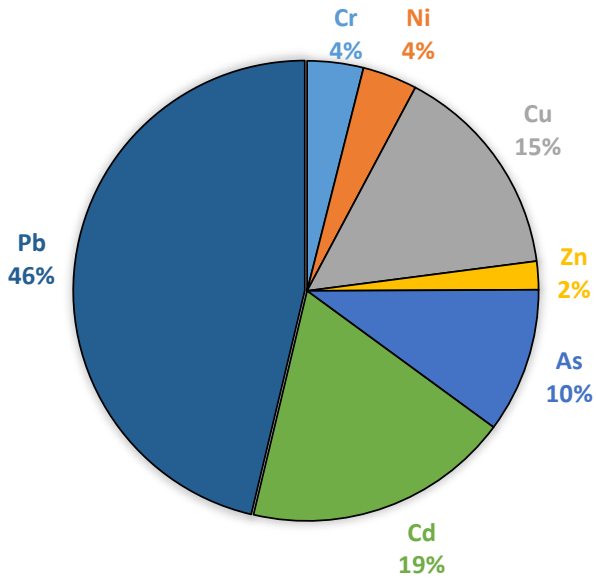


Fig. 2. Makeup of the mean potential ecological risk index calculated as the sum of the mean risk factors of the trace elements (351). The number next to the element represents percent contributions of individual trace elements to the mean potential ecological risk of the soils

The pollution load index gives simple comparative means for assessing a site quality. For the 746 samples, PLI ranged from 0.59 to 50 with mean of 4.6.PLI > 1 (polluted soil quality) indicates progressive deterioration [37]. Only 9 gardens or 1.2 % of samples were below 1.

Distribution of soil Pb in NYC gardens

Spatial patterns in Pb distribution was mapped and analyzed based on 2322 samplepoints from the compiled database (Fig. 3, Table 2). Each point on the map is a garden and may represent multiple samples that are from the same street address. Total Pb concentrations ranged from 3.3 to 45,076 mg/kg (mean 630 mg/kg and median 344 mg/kg). The highest Pb concentrations vary among different neighborhoods of northern and central Brooklyn. Two gardens were identified with Pb concentration over 10,000 mg/kg. The garden from the 11205 zip code (Downtown-Heights-Park Slope) had the highest Pb concentration found in this study 45,076 mg/kg (Fig. 3). There were 282 soil samples from 245 gardens with Pb concentrations exceeding 1,200 mg/kg. The largest number of samples (219) was from the 11238 zip code (Prospect Heights). If neighborhood (a district comprised of several zip code areas) is considered, the most number of samples (486) was collected from the Downtown-Heights-Park Slope area. The highest median Pb level among all zip codes (1,052 mg/kg) was

found in the 11211 zip code in Greenpoint. The highest median (1,019 mg/kg) among the neighborhoods was found also in Greenpoint (Brooklyn). Columbia University study of soils from about 50 homes in Greenpoint show that 92 % of the yards tested had at least one sample above the residential soil standard for New York (https://greenpointpost.com/nearly-85-of-greenpoint-backyard-soil-samples-show-unsafe-lead-levels-by-epa-standards-study). It should be noted that there were not enough samples collected in eastern Queens, the Bronx and throughout Staten Island to confident lymap and predict Pb distributions in those areas.

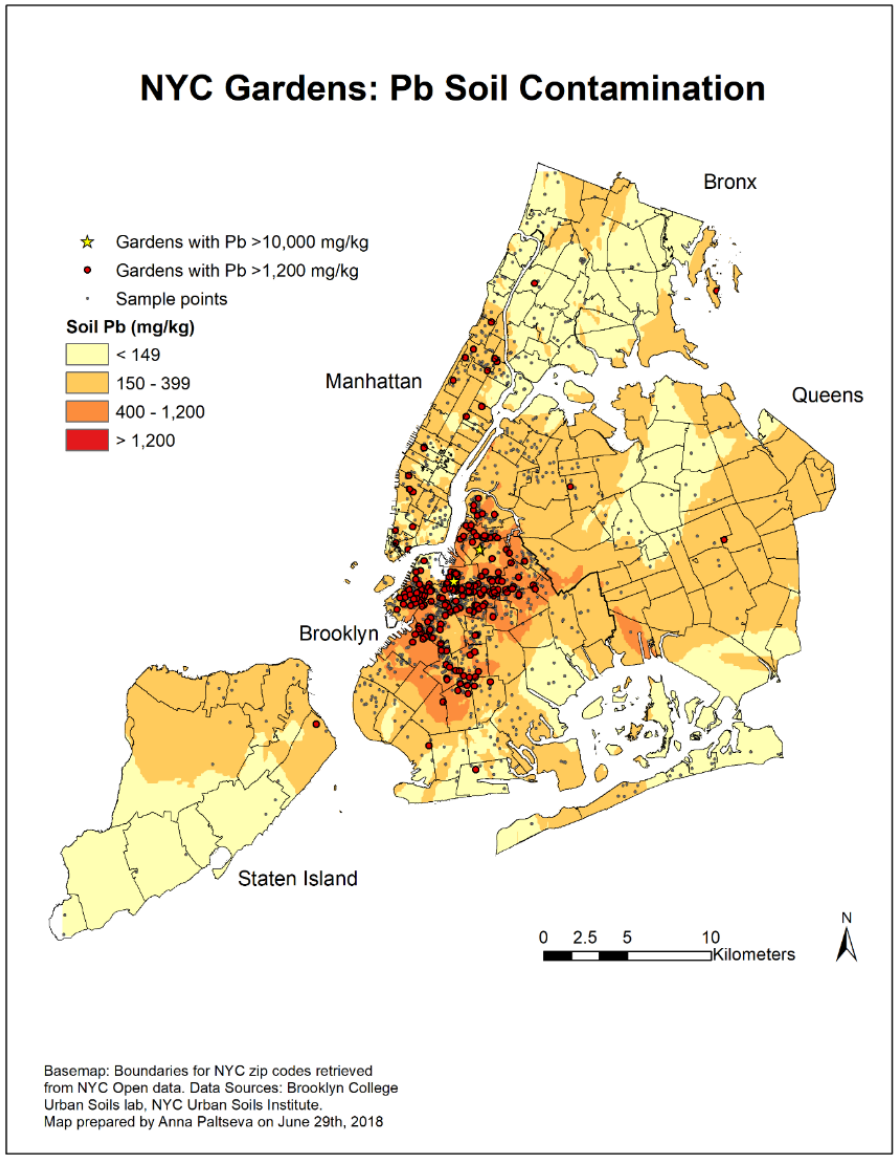


Fig. 3. NYC Gardens: Pb Soil Contamination map shows predicted soil Pb distribution by kriging based on 2322 garden soil samples. (Note: there were not enough samples collected in eastern Queens, the Bronx and throughout Staten Island to confidently map and predict Pb distributions in those areas)

Table 2

Lead concentrations in garden soils of NYC neighborhoods (mg/kg) (total n = 2322)

Neighborhood	Median	Max	Min	# of samples
Greenpoint	1019	15911	26	71
Williamsburg-Bushwick	586	3970	14	97
Downtown-Heights-Park Slope	558	45076	4	486
Bedford Stuyvesant-Crown Heights	488	4026	3	362
East New York	523	1863	57	24
Sunset Park	519	3515	57	48
Borough Park	498	5474	11	148
East Flatbush-Flatbush	302	9112	10	168
Canarsie-Flatlands	169	863	26	36
Bensonhurst-Bay Ridge	276	1609	11	36
Coney Island-Sheepshead Bay	111	1425	12	44
Kingsbridge-Riverdale	122	965	34	45
Northeast Bronx	147	515	21	16
Fordham-Bronx Park	110	761	23	25
Pelham-Throgs Neck	370	2015	29	5
Crotona-Tremont	97	1374	40	21
High Bridge-Morrisania	172	742	25	20
Hunts Point-Mott Haven	233	541	46	15
Washington Heights-Inwood	196	2439	38	37
Central Harlem-Morningside Heights	154	6395	29	77
East Harlem	92	2639	21	31
Upper West Side	215	2273	31	20
Upper East Side	131	1905	13	31
Chelsea-Clinton	155	2077	14	14
Gramercy Park-Murray Hill	107	1105	11	39
Greenwich Village-Soho	71	3478	10	15
Union Square-Lower East Side	222	1439	32	54
Lower Manhattan	240	3051	24	28
Long Island City-Astoria	295	1039	11	50
West Queens	288	2766	27	36
Flushing-Clearview	147	508	39	15
Bayside-Little Neck	417	673	86	2
Ridgewood-Forest Hills	196	550	19	24
Fresh Meadows	158	653	62	8
Southwest Queens	618	685	82	5
Jamaica	163	1726	54	15
Southeast Queens	67	82	52	2
Rockaway	148	780	14	21
Port Richmond	433	840	89	13
Stapleton-St. George	562	1418	27	17
Willowbrook	117	170	64	2
South Beach-Tottenville	44	155	12	8

Sources of Pb and other contaminants

The Pb distribution map (Fig. 3) shows that, in general, soil Pb content decreases from the inner city towards outskirts, which is commonly seen for cities with an industrial history [1]. Li et al. (2018) found a general correlation between Pb levels and historical land use, where highly elevated levels of soil Pb corresponded with industrialized areas. Specific hotspots of Pb were identified in neighborhoods with extensive industrial history such as Red Hook, Brooklyn Heights, Gowanus, Park Slope, Boerum Hill, Fort Greene, Williamsburg, and Bedford-Stuyvesant. These observations are consistent with our findings with the highest soil Pb found in the same neighborhoods.

Historically, leaded gasoline, lead-based paint, and many other lead-based products were widely used until around 1990's [38]. It has been recognized that these contributed to the widespread Pb deposition into the soil, especially in urban areas. An estimated 4-5 million metric tons of Pb from car exhaust released into the environment from 1929 to 1986 throughout the United States [39] is a big contributor to the soil legacy of Pb. Although lead-based paint is no longer being used, some old houses with leaded paint still serve as a source of Pb for soil. Moreover, solid waste incineration (common in the US and New York City during the 20th century) could be another source of trace metals (including Pb) causing excessive deposition of contaminants in soil (Walsh et al. 2001). Deposition of 34 million tons of refuse incineration throughout NYC landfills caused the release of 1 million tons of air pollutants, which eventually settled onto the topsoil. Manufacturing and smelting activities involving lead-bearing products, as point sources, also have contaminated soil with large quantities of Pb deposited into the topsoil within the industrial site and neighboring areas. It is highly likely that at many places both point and non-point sources of Pb contributed to the elevated levels of Pb in soil.

Lead distribution changes over time

Fig. 5 shows the spatial distribution of garden soil samples the two labs received from NYC gardeners between 2009 and 2017, color-coded for every three years. There are no noticeable changes in terms of spatial distributions of the samples received over time. This, on one hand, may suggest the spatial distribution of gardens in the city, and on the other hand may point to how effective the information regarding to soil contamination has been delivered.

It is very important to note, however, that over time the Pb levels we found in soil samples received have not shown any significant decline. One would expect that with increased awareness and educational campaign, remediation and mitigation actions had been taken (including replacing with new, clean soil), thus more soil samples would have lower Pb levels. Our study shows that there is only a slight increase in the % of samples < 400 ppm, comparing 2009—2011 (51 %) with 2015—2017 (55 %). In the 2015—2017 samples, there were still 13 % above 1,200 mg/kg and 44 % above 400 mg/kg, highlight significant health risk. The environmental challenge remains after nearly ten years of research and outreach, while more and more urban residents are getting involved in urban gardening and greening.

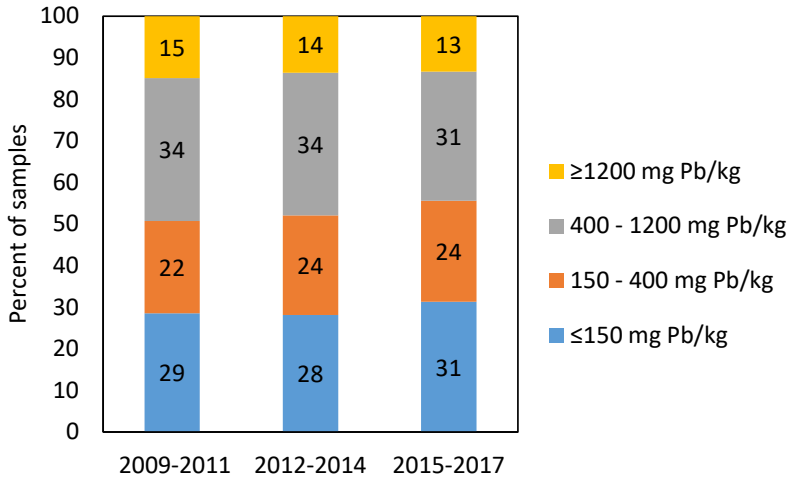


Fig. 4. Distribution of Pb levels in garden soil samples received

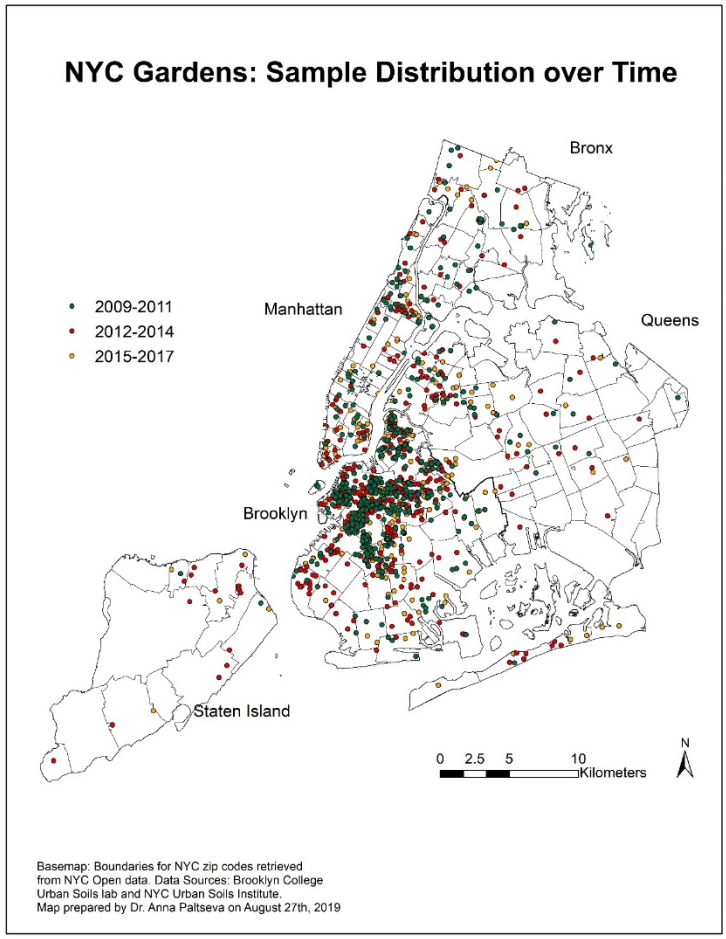


Fig. 5. Sample spatial disctribution over time between 2009 and 2017 (*n*= 2322 garden soil samples)

Conclusions

In this study, soil quality assessment indices were calculated based on individual metals (Pb, Zn, Cd, As, Cu, Cr, Ni) for 746 garden samples. The majority of soils is contaminated and poses significant risks to human health and ecological systems, particularly by Pb. A consolidated garden soil Pb database was compiled (total of 2,322 garden samples), from which color-coded map was created to visualize areas with potential health risk from soil contamination. The highest Pb levels were found in northern and central Brooklyn. Generally, Pb levels became lower toward the suburban areas. The Pb contamination map would be valuable not only to guide remediation efforts but also for urban planning such as developing gardens and green spaces or sitting of new parks.

References

1. Mielke HW. Lead in the inner cities. *Am Sci*. 1999; 87(1):62–73.
2. Meuser H. Introduction. In: *Contaminated Urban Soils.* Dordrecht: Springer; 2010; p.1–3. doi: 10.1007/978-90-481-9328-8
3. Brown SL, Chaney RL, Hettiarachchi GM. Lead in Urban Soils: A Real or Perceived Concern for Urban Agriculture? J Environ Qual. 2016; 45(1):26–36. doi: 10.2134/jeq2015.07.0376
4. Paltseva A, Cheng Z, Deeb M, Groffman PM, Shaw RK, Maddaloni M. Accumulation of arsenic and lead in garden-grown vegetables: Factors and mitigation strategies. *Sci Total Environ*. 2018; 640–641:273–283. doi: 10.1016/j.scitotenv.2018.05.296
5. Clark HF, Hausladen DM, Brabander DJ. Urban gardens: Lead exposure, recontamination mechanisms, and implications for remediation design. *Environ Res.* 2008; 107(3):312–319. doi: 10.1016/j.envres.2008.03.003
6. Ryan J, Scheckel KG, Berti WR, Brown SL, Casteel SW, Chaney RL, et al. Reducing children's risk from lead in soil. *Environ Sci Technol*. 2004; 38(1):18A-24A. doi: 10.1021/es040337r
7. Lanphear BP, Matte TD, Rogers J, Clickner RP, Dietz B, Bornschein RL, et al. The contribution of lead-contaminated house dust and residential soil to children's blood lead levels: a pooled analysis of 12 epidemiologic studies. Environ Res. 1998; 79(1):51–68. doi: 10.1006/enrs.1998.3859
8. U.S. EPA. *Reusing potentially contaminated landscapes: Growing Gardens in Urban Soils.* 2011.
9. Landes FC, Paltseva A, Sobolewski JM, Cheng Z, Ellis TK, Mailloux BJ, et al. A field procedure to screen soil for hazardous lead. *Anal Chem*. 2019; 91(13):8192-8198. doi: 10.1021/acs.analchem.9b00681
10. Alloway BJ. Contamination of domestic gardens and allotments. *L Contam Reclam*. 2004; 12(3):179–187.
11. Chaney RL, Sterrett SB, Mielke HW. The potential for heavy metal exposure from urban gardens and soils. In: Preer JR. (ed.) *Proc. Symp. Heavy Metals in Urban Gardens. Univ. Dist.* Washington: Columbia Extension Service; 1984. p.37–84.
12. Jean-Soro L, Le Guern C, Bechet B, Lebeau T, Ringeard MF. Origin of trace elements in an urban garden in Nantes, France. *J Soils Sediments.* 2015; 15(8):1802–1812. doi: 10.1007/s11368-014-0952-y
13. Pouyat RV, Yesilonis ID, Russell-Anelli J, Neerchal NK. Soil chemical and physical properties that differentiate urban land-use and cover types. *Soil Sci Soc Am J.* 2007; 71(3):1010-1019. doi: 10.2136/sssaj2006.0164
14. U.S. EPA. Sources of Lead in Soil: A Literature Review Final Report. 1998; (February):5–28.
15. Smith WH. Lead Contamination of the Roadside Ecosystem. *J Air Pollut Control Assoc*. 1976;26(8):753–766. doi: 10.1080/00022470.1976.10470310
16. Summary of the Toxic Substances Control Act. 15 U.S.C. §2601 et seq. 1976. Available from: http://www2.epa.gov/laws-regulations/summary-toxic-substances-control-act

17. Pouyat RV, Szlavecz K, Yesilonis ID, Groffman PM, Schwarz K. Chemical, physical and biological characteristics of urban soils. *Agron Monogr*. 2010; 55:119–52. doi: 10.2134/agronmonogr55.c7
18. Levin R, Brown MJ, Kashtock ME, Jacobs DE, Whelan EA, Rodman J, et al. Lead exposures in U.S. children, 2008: Implications for prevention. *Environmental Health Perspectives*. 2008; 116(10):1285–1293. doi: 10.1289/ehp.11241
19. Mielke HW, Anderson JC, Berry KJ, Mielke PW, Chaney RL, Leech M. Lead concentrations in inner city soils as a factor in the child lead problem. *Am J Public Health*. 1983; 73(12):1366–1369. doi: 10.2105/AJPH.73.12.1366
20. Wu J, Edwards R, He X (Elaine), Liu Z, Kleinman M. Spatial analysis of bioavailable soil lead concentrations in Los Angeles, California. *Environ Res*. 2010; 110(4):309–317. doi: 10.1016/j.envres.2010.02.004
21. Yesilonis ID, Pouyat RV, Neerchal NK. Spatial distribution of metals in soils in Baltimore, Maryland: Role of native parent material, proximity to major roads, housing age and screening guidelines. Environ Pollut. 2008; 156(3):723–731. doi: 10.1016/j.envpol.2008.06.010
22. Li I, Cheng Z, Paltseva A, Morin T, Smith B, Shaw R. Lead in New York City Soils. In: Vasenev V.I., Dovletyarova E, Chen Z, Valentini R. (eds.) *Megacities 2050: Environmental Consequences of Urbanization: Proceedings of the VI International Conference on Landscape Architecture to Support City Sustainable Development*. Cham: Springer International Publishing; 2018. p.62–79. doi: 10.1007/978-3-319-70557-6_9
23. Cheng Z, Paltseva A, Li I, Morin T, Huot H, Egendorf S, et al. Trace metal contamination in New York City garden soils. *Soil Sci*. 2015; 180(4/5):167–174. doi: 10.1097/SS.0000000000000126
24. Burt R, Hernandez L, Shaw R, Tunstead R, Ferguson R, Peaslee S. Trace element concentration and speciation in selected urban soils in New York City. *Environ Monit Assess*. 2014; 186(1):195–215. doi: 10.1007/s10661-013-3366-1
25. Paltseva A, Cheng Z, Deeb M, Groffman PM, Maddaloni M. Variability of Bioaccessible Lead in Urban Garden Soils. *Soil Sci*. 2018; 183(4):123–131. doi: 10.1097/SS.0000000000000232
26. Datko-Williams L, Wilkie A, Richmond-Bryant J. Analysis of U.S. soil lead (Pb) studies from 1970 to 2012. *Sci Total Environ*. 2014; 468–469:854–863. doi: 10.1016/j.scitotenv.2013.08.089
27. U.S. EPA. Method 6200: Field portable X-ray fluorescence spectrometry for the determination of elemental concentrations in soil and sediment. Test Methods For Evaluating Solid Waste. 2006.
28. U.S. EPA. Method 3052: microwave assisted acid digestions of siliceous and organically based matrices. 1996.
29. U.S. EPA. Method 6020A: Inductively coupled plasma-mass spectrometry. Test Methods For Evaluating Solid Waste. 1998.
30. U.S. EPA. Lead. Identification of Dangerous Levels of Lead. Vol. 40 CFR 745, Federal Register. 2001 Jan.
31. Centers for Disease Control and Prevention. Low level lead exposure harms children: a renewed call of primary prevention. Rep Advis Comm Child Lead Poisoning Prev CDC. 2012; 1–54. Available from: http://www.cdc.gov/nceh/lead/ACCLPP/Final_Document_030712.pdf.
32. Hakanson L. An ecological risk index for aquatic pollution control. A sedimentological approach. *Water Res*. 1980; 14(8):975–1001. doi: 10.1016/0043-1354(80)90143-8
33. New York State Department of Environmental Conservation. *Concentrations of Selected Analytes in Rural New York State Surface Soils: a Summary Report on the Statewide Rural Surface Soil Survey*. 2005.
34. Qingjie G, Jun D, Yunchuan X, Qingfei W, Liqiang Y. Calculating Pollution Indices by Heavy Metals in Ecological Geochemistry Assessment and a Case Study in Parks of Beijing. *J China Univ Geosci*. 2008; 19(3):230–241. doi: 10.1016/S1002-0705(08)60042-4
35. Mugoša B, Đuroviā D, Nedović-Vuković M, Barjaktarović-Labović S, Vrvić M. Assessment of ecological risk of heavy metal contamination in coastal municipalities of montenegro. *Int J Environ Res Public Health*. 2016; 13(4):393. doi: 10.3390/ijerph13040393
36. Tomlinson DL, Wilson JG, Harris CR, Jeffrey DW. Problems in the assessment of heavy-

metal levels in estuaries and the formation of a pollution index. *Helgoländer Meeresuntersuchungen.* 1980; 33(1–4):566–575. doi: 10.1007/BF02414780
37. Cabrera F, Clemente L, Díaz Barrientos E, López R, Murillo JM. Heavy metal pollution of soils affected by the Guadiamar toxic flood. *Sci Total Environ*. 1999; 242(1–3):117–129. doi: 10.1016/S0048-9697(99)00379-4
38. U.S. EPA. 40 CFR Part 745 Lead: Requirements for Lead-Based Paint Activities in Target Housing and Child-Occupied Facilities. Final Rule. Federal Registrar. 45,777–45,825. 1996.
39. Sheets RW, Kyger JR, Biagioni RN, Probst S, Boyer R, Barke K. Relationship between soil lead and airborne lead concentrations at Springfield, Missouri, USA. *Sci Total Environ*. 2001; 271(1–3):79–85. doi: 10.1016/S0048-9697(00)00832-9

About authors:
Paltseva Anna Alexandrovna — PhD, Adjunct Assistant Professor, Department of Earth and Environmental Sciences, Brooklyn College of The City University of New York, 2900 Bedford Avenue, Brooklyn, New York, 11210, United States; e-mail: anyapaltseva@gmail.com; ORCID ID: 0000-0002-1828-7164

Cheng Zhongqi — PhD, Professor, Department of Earth and Environmental Sciences, Brooklyn College of The City University of New York, 2900 Bedford Avenue, Brooklyn, New York, 11210, United States; e-mail: ZCheng@brooklyn.cuny.edu; ORCID ID: 0000-0002-2486-9662

Научная статья

DOI 10.22363/2312-797X-2019-14-3-239-254

Геопространственный анализ и оценка загрязнения садовых почв в Нью-Йорке

А.А. Пальцева [*1,2], Чж. Ченг[1,2]

[1]Бруклинский колледж Городского университета Нью-Йорка,
Нью-Йорк, Соединенные Штаты

[2]Аспирантура Городского университета Нью-Йорка,
Нью-Йорк, Соединенные Штаты

* *anyapaltseva@gmail.com*

Аннотация. Повышенные концентрации микроэлементов, в частности, свинца (Pb), распространены в городских почвах, и это является одним из основных препятствий для городского сельского хозяйства. Растущая популярность садоводства в городах также может означать повышение риска для здоровья населения. Пространственное распределение свинца в садах Нью-Йорка было проанализировано и визуализировано с помощью инструментов географической информационной системы (ГИС). Уровень загрязнения и экологические риски садов и Нью-Йорка в целом оценивались по разным показателям. Степень загрязнения была ранжирована следующим образом: Pb> Cu> Zn> Cr> As> Ni> Cd. Единый индекс экологического риска и потенциальный экологический индекс указывают на то, что Pb умеренно или значительно повышал риск для местных садовых экосистем. На основе индекса нагрузки загрязнения качество почвы большинства садов Нью-Йорка было охарактеризовано как загрязненное. Геостатистические, геообрабатывающие и пространственные инструменты использовались для создания карт с цветовой кодировкой для поддержки принятия решений, связанных с садоводством, и для оценки потенциальных рисков для здоровья человека, связанных с садоводством, проживанием или работой в / или вблизи садов. Эти выводы имеют большое

значение для разработки стратегий предотвращения загрязнения, смягчения его последствий и снижения риска для здоровья населения от загрязнения почвогрунтами садовых почв.

Ключевые слова: микроэлементы, ГИС-карта, экологический индекс, свинец, цифровое картирование почвы, садоводство городское

Конфликт интересов отсутствует.

БЛАГОДАРНОСТИ

Авторы выражают свою благодарность Татьяне Морин, Джорджу Лозефски, Зулеме Бланко Гарсиа, Майклу Гринштейн, Игорю Бронз, Кайо Грин, Донне Цао, Кристен Уолш, Норме Саттон, Оололад Томас за помощь в проведении лабораторных анализов и сбору данных, а также Рокки Ли Гарсиа и Вячеславу Васеневу за консультирование по работе в системе ГИС.

История статьи:

Поступила в редакцию: 14 августа 2019 г. Принята к публикации: 10 сентября 2019 г.

Для цитирования:

Paltseva A.A., Cheng Z. Geospatial analysis and assessment of garden soil contamination in New York City // Вестник Российского университета дружбы народов. Серия: Агрономия и животноводство. 2019. Т. 14. № 3. С. 239—254. doi: 10.22363/2312-797X-2019-14-3-239-254

Библиографический список

1. *Mielke H.W.* Lead in the inner cities // Am Sci. 1999. Vol. 87. № 1. C. 62–73.
2. *Meuser H.* Introduction // Contaminated Urban Soils. Dordrecht : Springer, 2010. P. 1–3. Available from: doi: 10.1007/978-90-481-9328-8
3. *Brown S.L., Chaney R.L., Hettiarachchi G.M.* Lead in Urban Soils: A Real or Perceived Concern for Urban Agriculture? // J Environ Qual. 2016. Vol. 45. № 1. C. 26–36. doi: 10.2134/jeq2015.07.0376
4. *Paltseva A., Cheng Z., Deeb M., Groffman P.M., Shaw R.K., Maddaloni M.* Accumulation of arsenic and lead in garden-grown vegetables: Factors and mitigation strategies // Sci Total Environ. 2018. Vol. 640–641. P. 273–283. doi: 10.1016/j.scitotenv.2018.05.296
5. *Clark H.F., Hausladen D.M., Brabander D.J.* Urban gardens: Lead exposure, recontamination mechanisms, and implications for remediation design // Environ Res. 2008. Vol. 107. № 3. P. 312–319. doi: 10.1016/j.envres.2008.03.003
6. *Ryan J., Scheckel K.G., Berti W.R., Brown S.L., Casteel S.W., Chaney R.L., et al.* Reducing children's risk from lead in soil // Environ Sci Technol. 2004. Vol. 38 № 1. P. 18A-24A. doi: 10.1021/es040337r
7. *Lanphear B.P., Matte T.D., Rogers J., Clickner R.P., Dietz B., Bornschein R.L.,* et al. The contribution of lead-contaminated house dust and residential soil to children's blood lead levels: a pooled analysis of 12 epidemiologic studies // Environ Res. 1998. Vol. 79. № 1. P. 51–68. doi: 10.1006/enrs.1998.3859
8. U.S. EPA. Reusing potentially contaminated landscapes: Growing Gardens in Urban Soils. 2011.
9. *Landes F.C., Paltseva A., Sobolewski J.M., Cheng Z., Ellis T.K., Mailloux B.J.,* et al. A field procedure to screen soil for hazardous lead // Anal Chem. 2019. Vol. 91. № 13. P. 8192—8198. doi: 10.1021/acs.analchem.9b00681
10. *Alloway B.J.* Contamination of domestic gardens and allotments // L Contam Reclam. 2004. Vol. 12. № 3. P. 179–187.
11. *Chaney R.L., Sterrett S.B., Mielke H.W.* The potential for heavy metal exposure from urban gardens and soils // Preer JR. (ed.) Proc. Symp. Heavy Metals in Urban Gardens. Univ. Dist. Washington: Columbia Extension Service, 1984. P. 37–84.

12. *Jean-Soro L., Le Guern C., Bechet B., Lebeau T., Ringeard M.F.* Origin of trace elements in an urban garden in Nantes, France // J Soils Sediments. 2015. Vol. 15. № 8. P. 1802–1812. doi: 10.1007/s11368-014-0952-y
13. *Pouyat R.V., Yesilonis I.D., Russell-Anelli J., Neerchal N.K.* Soil chemical and physical properties that differentiate urban land-use and cover types // Soil Sci Soc Am J. 2007. Vol. 71. № 3. P. 1010-1019. doi: 10.2136/sssaj2006.0164
14. U.S. EPA. Sources of Lead in Soil: A Literature Review Final Report. 1998; (February):5–28.
15. *Smith W.H.* Lead Contamination of the Roadside Ecosystem // J Air Pollut Control Assoc. 1976. Vol. 26. № 8. P. 753–766. doi: 10.1080/00022470.1976.10470310
16. Summary of the Toxic Substances Control Act. 15 U.S.C. §2601 et seq. 1976. Режим доступа: http://www2.epa.gov/laws-regulations/summary-toxic-substances-control-act
17. *Pouyat R.V., Szlavecz K., Yesilonis I.D., Groffman P.M., Schwarz K.* Chemical, physical and biological characteristics of urban soils // Agron Monogr. 2010. №. 55. С. 119–52. doi: 10.2134/agronmonogr55.c7
18. *Levin R., Brown M.J., Kashtock M.E., Jacobs D.E., Whelan E.A., Rodman J.,* et al. Lead exposures in U.S. children, 2008: Implications for prevention // Environmental Health Perspectives. 2008. Vol. 116. № 10. P. 1285–1293. doi: 10.1289/ehp.11241
19. *Mielke H.W., Anderson J.C., Berry K.J., Mielke P.W., Chaney R.L., Leech M.* Lead concentrations in inner city soils as a factor in the child lead problem // Am J Public Health. 1983. Vol. 73. № 12. P. 1366–1369. doi: 10.2105/AJPH.73.12.1366
20. *Wu J., Edwards R., He X. (Elaine), Liu Z., Kleinman M.* Spatial analysis of bioavailable soil lead concentrations in Los Angeles, California // Environ Res. 2010. Vol. 110. № 4. P. 309–317. doi: 10.1016/j.envres.2010.02.004
21. *Yesilonis I.D., Pouyat R.V., Neerchal N.K.* Spatial distribution of metals in soils in Baltimore, Maryland: Role of native parent material, proximity to major roads, housing age and screening guidelines // Environ Pollut. 2008. Vol. 156. № 3. P. 723–731. doi: 10.1016/j.envpol.2008.06.010
22. *Li I., Cheng Z., Paltseva A., Morin T., Smith B., Shaw R.* Lead in New York City Soils // Megacities 2050: Environmental Consequences of Urbanization: Proceedings of the VI International Conference on Landscape Architecture to Support City Sustainable Development / Vasenev V.I., Dovletyarova E., Chen Z., Valentini R. (eds.). Cham : Springer International Publishing, 2018. P. 62–79. doi: 10.1007/978-3-319-70557-6_9
23. *Cheng Z., Paltseva A., Li I., Morin T., Huot H., Egendorf S.,* et al. Trace metal contamination in New York City garden soils // Soil Sci. 2015. Vol. 180. № 4/5. P. 167–174. doi: 10.1097/SS.0000000000000126
24. *Burt R., Hernandez L., Shaw R., Tunstead R., Ferguson R., Peaslee S.* Trace element concentration and speciation in selected urban soils in New York City // Environ Monit Assess. 2014. Vol. 186. № 1. P. 195–215. doi: 10.1007/s10661-013-3366-1
25. *Paltseva A., Cheng Z., Deeb M., Groffman P.M., Maddaloni M.* Variability of Bioaccessible Lead in Urban Garden Soils // Soil Sci. 2018. Vol. 183. № 4. P. 123–131. doi: 10.1097/SS.0000000000000232
26. *Datko-Williams L., Wilkie A., Richmond-Bryant J.* Analysis of U.S. soil lead (Pb) studies from 1970 to 2012 // Sci Total Environ. 2014. Vol. 468–469. P. 854–863. doi: 10.1016/j.scitotenv.2013.08.089
27. U.S. EPA. Method 6200: Field portable X-ray fluorescence spectrometry for the determination of elemental concentrations in soil and sediment. Test Methods For Evaluating Solid Waste. 2006.
28. U.S. EPA. Method 3052: microwave assisted acid digestions of siliceous and organically based matrices. 1996.
29. U.S. EPA. Method 6020A: Inductively coupled plasma-mass spectrometry. Test Methods For Evaluating Solid Waste. 1998.
30. U.S. EPA. Lead. Identification of Dangerous Levels of Lead. Vol. 40 CFR 745, Federal Register. 2001 Jan.

31. Centers for Disease Control and Prevention. Low level lead exposure harms children: a renewed call of primary prevention. Rep Advis Comm Child Lead Poisoning Prev CDC. 2012. P. 1–54. Режим доступа: http://www.cdc.gov/nceh/lead/ACCLPP/Final_Document_030712.pdf
32. *Hakanson L.* An ecological risk index for aquatic pollution control. A sedimentological approach // Water Res. 1980. Vol. 14. № 8. P. 975–1001. doi: 10.1016/0043-1354(80)90143-8
33. New York State Department of Environmental Conservation. Concentrations of Selected Analytes in Rural New York State Surface Soils: a Summary Report on the Statewide Rural Surface Soil Survey. 2005.
34. *Qingjie G., Jun D., Yunchuan X., Qingfei W., Liqiang Y.* Calculating Pollution Indices by Heavy Metals in Ecological Geochemistry Assessment and a Case Study in Parks of Beijing // J China Univ Geosci. 2008. Vol. 19. № 3. P. 230–241. doi: 10.1016/S1002-0705(08)60042-4
35. *Mugoša B., Đuroviā D., Nedović-Vuković M., Barjaktarović-Labović S., Vrvić M.* Assessment of ecological risk of heavy metal contamination in coastal municipalities of Montenegro // Int J Environ Res Public Health. 2016. Vol. 13. № 4. P. 393. doi: 10.3390/ijerph13040393
36. *Tomlinson D.L., Wilson J.G., Harris C.R., Jeffrey D.W.* Problems in the assessment of heavy-metal levels in estuaries and the formation of a pollution index // Helgoländer Meeresuntersuchungen. 1980. Vol. 33. № 1–4. P. 566–575. doi: 10.1007/BF02414780
37. *Cabrera F., Clemente L., Díaz Barrientos E., López R., Murillo J.M.* Heavy metal pollution of soils affected by the Guadiamar toxic flood // Sci Total Environ. 1999. Vol. 242. № 1–3. P. 117–129. doi: 10.1016/S0048-9697(99)00379-4
38. U.S. EPA. 40 CFR Part 745 Lead: Requirements for Lead-Based Paint Activities in Target Housing and Child-Occupied Facilities. Final Rule. Federal Registrar. 45,777–45,825. 1996.
39. *Sheets R.W., Kyger J.R., Biagioni R.N., Probst S., Boyer R., Barke K.* Relationship between soil lead and airborne lead concentrations at Springfield, Missouri, USA // Sci Total Environ. 2001. Vol. 271. № 1–3. P. 79–85. doi: 10.1016/S0048-9697(00)00832-9

Об авторах:

Пальцева Анна Александровна — PhD, доцент, кафедра наук о Земле и окружающей среде, Бруклинский колледж Городского университета Нью-Йорка, 11210, Соединенные Штаты, Нью-Йорк, Бруклин, Бедфорд-авеню, 2900; e-mail: anyapaltseva@gmail.com; ID ORCID: 0000-0002-1828-7164

Ченг Чжунци — PhD, профессор кафедры наук о Земле и окружающей среде, Бруклинский колледж Городского университета Нью-Йорка, 11210, Соединенные Штаты, Нью-Йорк, Бруклин, Бедфорд-авеню, 2900; e-mail: ZCheng@brooklyn.cuny.edu; ID ORCID: 0000-0002-2486-9662

RUDN Journal of Agronomy and Animal Industries
Вестник РУДН. Серия: АГРОНОМИЯ И ЖИВОТНОВОДСТВО
2019; 14(3):255—265
http://agrojournal.rudn.ru

ВЕТЕРИНАРИЯ
VETERINARY SCIENCE

Research article

DOI 10.22363/2312-797X-2019-14-3-255-265

Assessment of vital organ histopathology and plasma oxidative conditions of rainbow trout *Oncorhynchus mykiss* reared in earthen saltwater pond

Seyyed Morteza Hoseini*, Behrooz Gharavi, Yousef Iri

Inland Waters Aquatic Resources Research Center,
Iranian Fisheries Sciences Research Institute, Agricultural Research,
Education and Extension Organization, *Gorgan, Iran*
** Corresponding author: seyyedmorteza.hoseini@gmail.com*

Abstract. The aim of this study was to compare gill, kidney, liver and gut histopathology, and plasma antioxidant markers of rainbow trout *Oncorhynchus mykiss* reared in saltwater earthen ponds in Gomishan, Iran. To this, 10000 fish were distributed in a three-ha earthen pond and 150 fish in three fiberglass tanks (2000L). Blood samples were taken after 3 months rearing with same commercial feed. The source of fish and feed was similar between the saltwater pond and fiberglass tanks. After the 3-month rearing, gill, kidney, liver and gut samples were taken from the pond fish; whereas, blood samples were taken from both the pond and tank fish. There was no significant difference in water temperature, dissolved oxygen and pH between the pond and tanks; however, water salinity and ammonia was higher in the pond compared to the tanks. Plasma superoxide dismutase and glutathione peroxidase activity of the fish in earthen ponds were significantly higher than those fish reared in fiberglass tanks; however, there was no significant in thiobarbituric acid reactive substances between the pond and tanks. The fish had various histopathological symptoms including primary and lamella hyperplasia, lamellar fusion and epithelial lifting. In the kidney section, the fish showed glomerulus shrinkage and/or disappearance, melanomacrophage aggregates and hematopoietic tissue necrosis. These fish showed necrosis and melanomacrophage aggregates in liver and goblet cell hypertrophy in gut. The results suggest that the fish in the earthen pond faced stressful conditions, which might be due to water salinity and ammonia; however, other possible factors, such as pollutants and different feeding regimen must be considered.

Key words: trout, earthen pond, antioxidant system, saltwater, histopathology, Gomishan, Iran

Article history:
Received: 4 August 2019. Accepted: 2 September 2019

For citation:
Hoseini SM, Gharavi B, Iri Y. Assessment of vital organ histopathology and plasma oxidative conditions of rainbow trout *Oncorhynchus mykiss* reared in earthen saltwater pond. *RUDN Journal of Agronomy and Animal Industries*, 2019; 14(3):255—265. doi: 10.22363/2312-797X-2019-14-3-255-265

Introduction

Gomishan site of shrimp culture is important in economy of the Goletan province, Iran. The site area is near 4000 ha and had total production of 2000 tons of shrimp in 2018. Despite its importance, a problem of the site is seasonality of work period, because the water temperature is suitable for shrimp culture only four months a year. As a result, the employees hire workers seasonally, which is considered as a social crisis [1]. A solution for this problem is to introduce an aquatic species to be cultured in the second term of year (autumn and winter). In this case, rainbow trout (*Oncorhynchus mykiss*) seems to be fitted with the environmental conditions of the site (temperature and salinity); however, it is necessary to monitor the fish health in these earthen ponds [2].

Histopathological studies are reliable tools to assess fish health, illustrating the fish responses to toxicant exposure and water salinity [3]. In this case, fish gill is the major organ for hydromineral control, responding morphologically to water salinity [4]. Liver is the main site of metabolism and the target of toxicants, showing wide range of morphological and histological alteration in response to toxicants [5]. Fish kidney is involved in salinity adaptation, which responds morphologically to toxicants [5]. Fish gut is an important organ in immune defense, which responds to toxicant and dietary compounds [3]. Monitoring of these organs provides useful information about the fish overall health.

Oxidative stress is a condition in when pro-oxidant compounds exceed antioxidant defense [6, 7]. In this case, stressful conditions lead to oxidative stress. Superoxide dismutase (SOD) and glutathione peroxidase (GPx) are antioxidant enzymes collectively converting superoxide ions to water and oxygen [7, 8]. Therefore, they are important in monitoring oxidative conditions of fish. However, these enzymes show different either elevation or demotion under stressful conditions. As a result, thiobarbituric acid reactive substances (TBARS) are measured as a relevant marker of lipid peroxidation and oxidative stress [9].

According to above, the aim of the present study was to investigate rainbow trout health (histopathology and antioxidant responses) in earthen ponds of the Gomishan site.

Materials and methods

This study was conducted in an earthen pond in the Gomishan site of shrimp culture. The pond was filled with the Caspian Sea water in spring, and used for shrimp culture during spring and summer. Then the pond water was drained and the pond bottom was dried for 2 months. Thereafter, the pond was refilled with the sea water for trout culture. 10000 rainbow trout (~35 g) were stocked in a 3-ha pond. For comparison of blood antioxidant marker with a reference, 150 rainbow trout, with the same origin of the pond fish, were socked in three fiberglass tanks (2000 L). The fish in the pond and fiberglass tanks were reared for three months using a commercial feed (Faradaneh Co., Tehran, Iran; 40—44 % protein, 12—16 % fat). Feeding was performed twice

a day based on 3 % of biomass. Water of the pond was static; whereas, water flow rate in the tanks was 0.3 L/min. kg fish. Water physiochemical parameters were measured in the pond and tanks monthly.

At the end of the three-month rearing, 12 fish were sampled from either the pond or tanks and immediately anesthetized with 100 mg/L eugenol. Immediately after the anesthesia, blood samples were taken from caudal vein using heparinized syringes and collected in 2-mL tubes. The blood samples were centrifuged for 10 min for plasma separation. The resultant plasma was kept at –20 for further analyses. After the blood sampling, gill, liver, kidney and gut samples were taken from the pond fish. The organs were fixed in 10 % buffered formalin for histopathological examination.

Plasma SOD, GPx and MDA were determined using commercial kits (ZellBio, GmbH, Veltinerweg, Germany), as previously reported M.A. Taheri et al. [10]. The fish organs were parafinized, sectioned (4μm thickness) and stained (hematoxylin-eosin) according to A. Hedayati et al. [11]. Histopathological damages were determined according to Haschek et al. [3] and R.J. Roberts [12].

Plasma and water physiochemical parameters were subjected to t-test. Data were presented as mean ± SD. All analyses were performed using SPSS v. 22.

Results and discussion

Water physiochemical parameters in pond and tanks are presented in Table 1. The pond water had significantly higher salinity and unionized ammonia compared to the tanks. Nevertheless, there was no significant difference in water temperature, dissolved oxygen and pH.

Table 1

Water physiochemical characteristics in the pond and tank. Different letters show significant difference between the pond and tank

Physiochemical characteristics	Pond	Tank
Temperature (ºC)	13 ± 1*a*	14 ± 1*a*
Dissolved oxygen (mg/L)	8.88 ± 0.55*a*	8.56 ± 0.84*a*
Salinity (g/L)	20.7 ± 1.12*b*	2.88 ± 0.12*a*
pH	8.65 ± 0.55*a*	7.52 ± 0.69*a*
Unionized ammonia (μg/L)	10.0 ± 1.0*b*	2.0 ± 0.40*a*

Plasma antioxidant parameters are presented in Table 2. The fish reared in the tanks had significantly lower SOD and GPx activities; but there was no significant difference in plasma TBARS levels between the fish.

Table 2

Plasma SOD, GPx and MDA in the fish reared in the pond and tanks. Different letters show significant difference between the pond and tank

	SOD (U/L)	GPx (U/L)	TBARS (μM/L)
Pond	35.1 ± 5.89*b*	45.5 ± 7.32*b*	6.85 ± 1.98*a*
Tank	23.7 ± 4.55*a*	30.0 ± 5.21*a*	5.21 ± 1.57*a*

Gill section of the fish reared in Gomishan pond is presented in Figure 1. The fish had various histopathological symptoms including primary and lamella hyperplasia, lamellar fusion and epithelial lifting. In the kidney section, the fish showed glomerulus shrinkage and/or disappearance, melanomacrophage aggregates and hematopoietic tissue necrosis (Figure 2). These fish showed necrosis and melanomacrophage aggregates in liver (Figure 3) and goblet cell hypertrophy in gut (Figure 4).

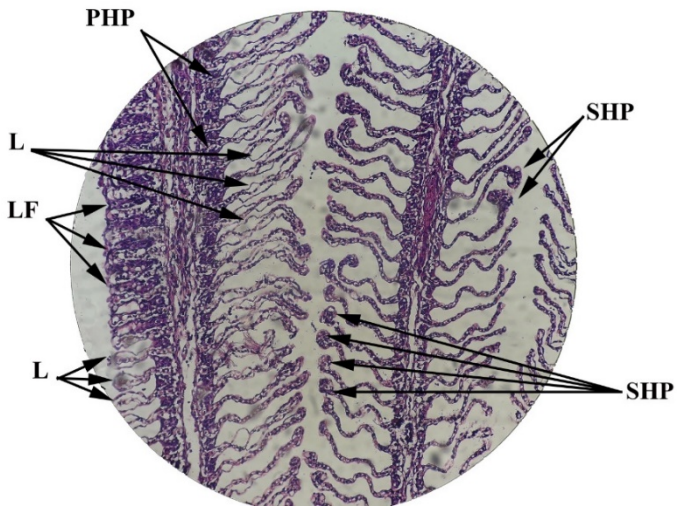


Fig. 1. Gill section of the fish reared in the pond. PHP: primary lamella hyperplasia; L: epithelial lifting; LF: lamellar fusion; SHP: secondary lamella hyperplasia. Hematoxylin-eosin staining (20×)

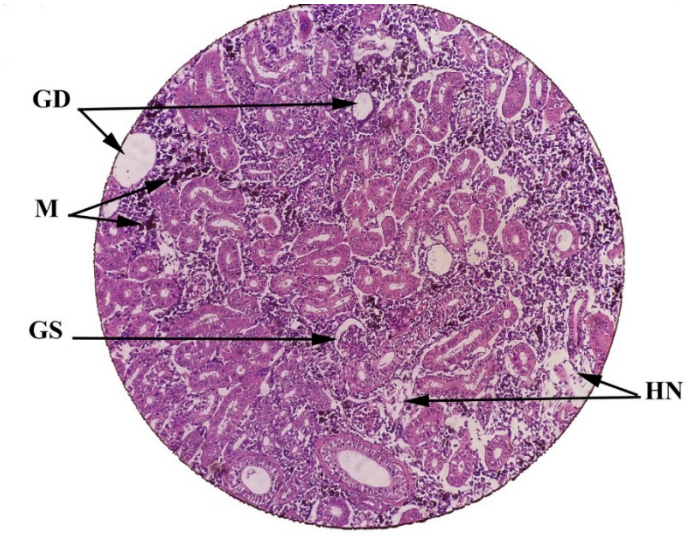


Fig. 2. Kidney section of the fish reared in the pond. GD: glomerulus disappearance; M: melanomacrophage aggregates; GS: glomerulus shrinkage; HN: hematopoietic tissue necrosis. Hematoxylin-eosin staining (20×)

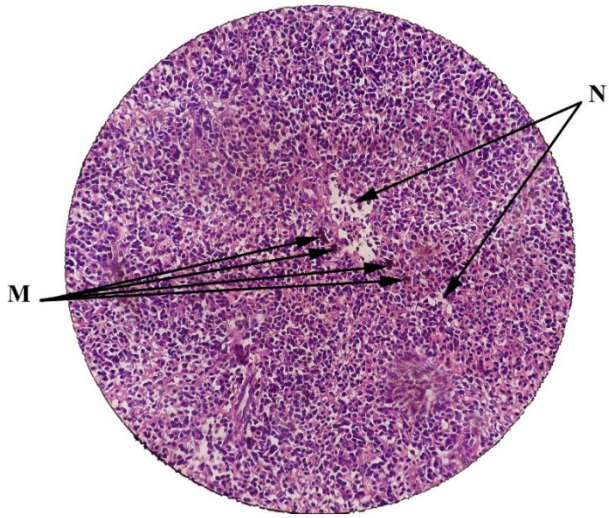


Fig. 3. Liver section of the fish reared in the pond. N: necrosis; M: melanomacrophage aggregates. Hematoxylin-eosin staining (20×)

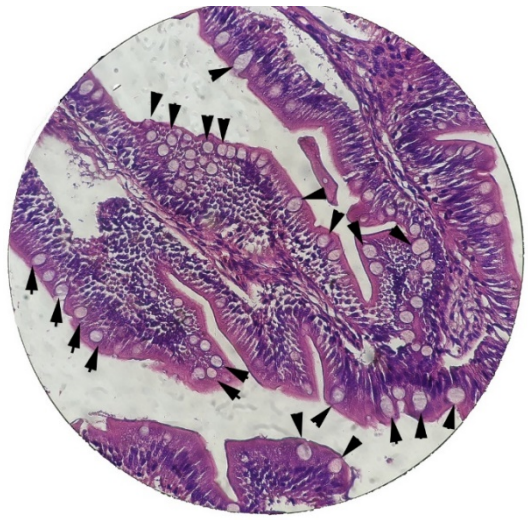


Fig. 4. Gut section of the fish reared in the pond. Arrow heads show hypertrophy of the goblet cells. Hematoxylin-eosin staining (40×)

The present study showed that saltwater earthen ponds of Gomishan site had different environment conditions compared to freshwater farms of rainbow trout culture. In these ponds, due to higher pH and static water [13—15], unionized ammonia is higher than the freshwater systems. Higher ammonia along with saltwater are stressful for rainbow trout, thus, affect the fish health [13—16].

The pond fish showed higher antioxidant enzymes' activity but did not elevated TBARS, compared to the tank fish. This suggests activation of antioxidant system, but not oxidative stress occurrence [17]. Several factors might be involved in antioxidant system activation. Ammonia was found to induce oxidative stress in fish leading to elevated TBARS [18]. Accordingly, oxidative conditions were not so severe as to cause oxidative stress in the pond fish, and activation of antioxidant system counteracted such

mild oxidative condition. Beside ammonia, osmotic stress causes oxidative condition, triggering the fish antioxidant system [19, 20]. Therefore, higher water ammonia and salinity contributed to activation of antioxidant system of the pond fish. Moreover, considering the wide difference between the pond and tank media, other possibility such as pathogens, pollutants, etc. could be considered.

Gill is the main organ for hydromineral control in freshwater fish [4]. It has been reported that freshwater fish exposed to saltwater expressed some morphological changes in gill structure [21]. Hyperplasia is a defensive mechanism to thicken gill membrane for reducing exchange capacity between the internal and external media of the fish body. This reduces the entrance of harmful substances, including hyperosmotic water and ammonia, from water to the fish body [22]. Epithelial lifting provides distance between the fish internal body and ambient water to reduce harmful substances entrance to the fish body [22]. However, lamellar fusion occurs in adjacent secondary lamella and extensively decreases respiratory capacity [22].

Beside gill, kidney is involved in water and ions regulation in fish [21]. When freshwater fish enter hyperosmotic media, water is passively excreted from the fish body and the animal must actively drink along with suppressing glomerulus filtration. Accordingly, fish have small glomerulus in freshwater [23]. The fish reared in Gomishan ponds had both shrinked and disappeared glomerulus to adapt higher water salinity. However, melanomacrophage aggregates and necrosis in hematopoietic tissues suggest that there was other problem in these fish. The liver section showed similar symptoms, supporting such hypothesis. One of the reasons might be higher water ammonia, which causes damage in fish kidney [24]. On the other hand, other toxicants such as metals might contribute to these damages, as they are present in the Caspian Sea water [25].

The pond-reared fish showed increased number and size of goblet cells in gut. These cells are responsible for mucus secretion and increase in their number and size translated to higher mucus secretion [26]. The reason for these changes is not clear, however, the pond water has microbial community different from the tank water. It is possible some microbes stimulate the fish gut and lead to higher mucus secretion [27]. On the other hand, despite the tank fish that eat solely pellet feeds, the pond fish eat on both pellet feed and natural foods in pond (e.g. insects, worms, etc.). Thus, it is expected the pod fish have gut structure different from the tank fish.

In conclusion, the fish in the earthen pond faced stressful conditions, which might be due to water salinity and ammonia. It is necessary to apply managerial practice to suppress such problems and augment the fish health and welfare.

References

1. Hoseini SM, Kor A, Gharavi B, Iri Y. Investigation of immunological characteristics of rainbow trout in Gomishan saltwater ponds with those reared in freshwater. *Journal of aquatic animal exploitation and rearing*. 2019; 8:41—46. (In Persian).
2. Hoseini SM. Comparison of stress markers of rainbow trout reared in earthen saltwater pond and fiberglass freshwater tanks. *Advanced aquaculture sciences journal*. 2019; (2):33—39.
3. Haschek WM, Rousseaux CG, Wallig MA. (eds). *Fundamentals of toxicologic pathology*. 3rd ed. San Diego: Academic Press; 2009.

4. Evans DH, Piermarini PM, Choe KP. The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. *Physiol. Rev*. 2005; 85(1):97—177.
5. Ghelichpour M, Taheri Mirghaed A, Mirzargar SS, Joshaghani H, Ebrahimzadeh Mousavi H. Plasma proteins, hepatic enzymes, thyroid hormones and liver histopathology of Cyprinus carpio (Linnaeus, 1758) exposed to an oxadiazin pesticide, indoxacarb. *Aquac. Res*. 2017; 48(11):5666—5676. doi: 10.1111/are.13390
6. Yousefi M, Hoseini SM, Vatnikov YA, Nikishov AA, Kulikov EV. Thymol as a new anesthetic in common carp (Cyprinus carpio): Efficacy and physiological effects in comparison with eugenol. *Aquaculture*. 2018; 495: 376—383. doi: 10.1016/j.aquaculture.2018.06.022
7. Yousefi M, Hoseinifar SH, Ghelichpour M, Hoseini SM, Anesthetic efficacy and biochemical effects of citronellal and linalool in common carp (Cyprinus carpio Linnaeus, 1758) juveniles. *Aquaculture*. 2018; 493:107—112. doi: 10.1016/j.aquaculture.2018.04.054
8. Hoseini SM, Mirghaed AT, Iri Y, Ghelichpour M. Effects of dietary cineole administration on growth performance, hematological and biochemical parameters of rainbow trout (Oncorhynchus mykiss). *Aquaculture*. 2018; 495: 766—772. doi: 10.1016/j.aquaculture.2018.06.073
9. Pérez Jiménez A, Hidalgo MC, Morales AE, Arizcun M, Abellan E, Cardenete G. Antioxidant enzymatic defenses and oxidative damage in Dentex dentex fed on different dietary macronutrient levels. *Comparative biochemistry and physiology Part C: toxicology and pharmacology*. 2009; 150:537—545. doi: 10.1016/j.cbpc.2009.07.011
10. Taheri Mirghaed A, Hoseini SM, Ghelichpour M. Effects of dietary 1, 8-cineole supplementation on physiological, immunological and antioxidant responses to crowding stress in rainbow trout (Oncorhynchus mykiss). *Fish Shellfish Immunol*. 2018; 81,182—188. doi: 10.1016/j.fsi.2018.07.027
11. Hedayati A, Hoseini SM, Ghelichpour M. Acute toxicity of waterborne manganese to Rutilus caspicus (Yakovlev, 1870)–gill histopathology, immune indices, oxidative condition, and saltwater resistance. *Toxicol. Environ. Chem.* 2014; 96(10):1535—1545. doi: 10.1080/ 02772248.2015.1028408
12. Roberts RJ (ed.) *Fish pathology*. 4th ed. Oxford: Wiley-Blackwell; 2012.
13. Taheri Mirghaed A, Fayaz S, Hoseini SM. Dietary 1, 8-cinoele affects serum enzymatic activities and immunological characteristics in common carp (Cyprinus carpio) exposed to ambient ammonia. *Aquac. Res*. 2018;. 50:146—153. doi: 10.1111/are.13877
14. Hoseini SM, Yousefi M, Hoseinifar SH, Van Doan H. Antioxidant, enzymatic and hematological responses of common carp (Cyprinus carpio) fed with myrcene- or menthol-supplemented diets and exposed to ambient ammonia. *Aquaculture*. 2019; 506:246—255. doi: 10.1016/j.aquaculture.2019.03.048
15. Taheri Mirghaed A, Fayaz S, Hoseini SM. Effects of dietary 1, 8-cineole supplementation on serum stress and antioxidant markers of common carp (Cyprinus carpio) acutely exposed to ambient ammonia. *Aquaculture*. 2019; 509:8—15. doi: 10.1016/j.aquaculture.2019.04.071
16. Morgan JD, Iwama GK. Effects of salinity on growth, metabolism, and ion regulation in juvenile rainbow and steelhead trout (Oncorhynchus mykiss) and fall chinook salmon (Oncorhynchus tshawytscha). *Canadian journal of fisheries and aquatic sciences*. 1991; 48(11):2083—2094. doi: 10.1139/f91-247
17. Hoseini SM, Yousefi M. Beneficial effects of thyme (Thymus vulgaris) extract on oxytetracycline-induced stress response, immunosuppression, oxidative stress and enzymatic changes in rainbow trout (Oncorhynchus mykiss). *Aquacult Nutr*. 2019; 25(2):298—309. doi: 10.1111/anu.12853

18. Hegazi MM, Attia ZI, Ashour OA. Oxidative stress and antioxidant enzymes in liver and white muscle of Nile tilapia juveniles in chronic ammonia exposure. *Aquat. Toxicol*. 2010; 99(2):118—125. doi: 10.1016/j.aquatox.2010.04.007
19. An KW, Kim NN, Shin HS, Kil GS, Choi CY. Profiles of antioxidant gene expression and physiological changes by thermal and hypoosmotic stresses in black porgy (Acanthopagrus schlegeli). *Comparative biochemistry and physiology Part A: molecular & integrative physiology*. 2010; 156(2):262—268. doi: 10.1016/j.cbpa.2010.02.013
20. Kim JH, Park HJ, Kim KW, Hwang IK, Kim DH, Oh CW, Lee JS, Kang JC. Growth performance, oxidative stress, and non-specific immune responses in juvenile sablefish, Anoplopoma fimbria, by changes of water temperature and salinity. *Fish Physiol. Biochem*. 2017; 43(5):1421—1431. doi: 10.1007/s10695-017-0382-z
21. Baldisserotto B, Mancera JM, Kapoor BG eds. Fish osmoregulation. CRC Press. 2019.
22. Ghelichpour M, Rajabiesterabadi H, Hoseini SM. Gill histopathological characteristics of Caspian roach (Rutilus rutilus caspicus) fingerlings treated with potassium permanganate and formalin. *Aquac. Res*. 2016; 47(1):276—282. doi: 10.1111/are.12489
23. Chenari F, Morovvati H, Ghazilou A, Savari A, Ronagh M. Rapid variation in kidney histology in spotted scat Scatophagus argus on exposed to abrupt salinity changes. *Iranian journal of veterinary research*. 2011; 12(3):256—261.
24. Benli AÇK, Köksal G, Özkul A. Sublethal ammonia exposure of Nile tilapia (Oreochromis niloticus L.): Effects on gill, liver and kidney histology. *Chemosphere*. 2008; 72(9):1355-1358. doi: 10.1016/j.chemosphere.2008.04.037
25. Saghali M, Hoseini S, Hosseini S, Baqraf R. Determination of heavy metal (Zn, Pb, Cd and Cr) concentration in benthic fauna tissues collected from the Southeast Caspian Sea, Iran. B*ull. Environ. Contam. Toxicol*. 2014; 92(1):57—60. doi: 10.1007/s00128-013-1133-1
26. Khojasteh SB, Sheikhzadeh F, Mohammadnejad D, Azami A. Histological, histochemical and ultrastructural study of the intestine of rainbow trout (Oncorhynchus mykiss). *World applied sciences journal*. 2009; 6(11):1525—1531.
27. Merrifield DL, Carnevali O. Probiotic modulation of the gut microbiota of fish. *Aquaculture nutrition: Gut health, probiotics and prebiotics*. 2014; 185—222. doi: 10.1002/9781118897263.ch8

About authors:

Hoseini Seyyed Morteza — Assistant professor, Head of stock rehabilitation devision, Inland Waters Aquatic Resources Research Center Iranian Fisheries Sciences Research Institute, Agricultural Research, Education and Extension Organization, Blv. Shahid Shekari, Gorgan, Iran; e-mail: seyyedmorteza.hoseini@gmail.com; ORCID 0000-0002-9824-5430. Scopus ID 35386383300. ResearcherID: B-7386-2017

Gharavi Behrooz — Researcher assistant, Head of aquatic animal health ad disease group, Inland Waters Aquatic Resources Research Center, Iranian Fisheries Sciences Research Institute, Agricultural Research, Education and Extension Organization, Blv. Shahid Shekari, Gorgan, Iran. ResearcherID B-3256-2017. ORCID 0000-0001-8552-7781

Iri Yousef — Researcher assistant, Head of fisheries research station of Gharasoo, Inland Waters Aquatic Resources Research Center, Iranian Fisheries Sciences Research Institute, Agricultural Research, Education and Extension Organization, Blv. Shahid Shekari, Gorgan, Iran; Gharasoo village, 5km of Bandar Turkman to Bandar Gaz road, Golestan province, Iran. Scopus ID 55837163000. ResearcherID B-4934-2017. ORCID 0000-0002-0802-3966

Научная статья

DOI 10.22363/2312-797X-2019-14-3-255-265

Оценка гистопатологии жизненно важных органов и условий окислительных процессов цитоплазмы радужной форели *Oncorhynchus mykiss*, выращенной в глиняном пруду с морской водой

С.М. Хосейни*, Б. Гарави, Ю. Ири

Центр изучения ресурсов гидробионтов во внутренних водах,
Иранский институт изучения рыбного хозяйства,
Организация сельскохозяйственных исследований и образования, *Горган, Иран*

**seyyedmorteza.hoseini@gmail.com*

Аннотация. Цель данного исследования заключалась в сравнении гистопатологий жабр, почек, печени и кишечника, и оценке активности системы антиоксидантной защиты радужной форели (*Oncorhynchus mykiss*), выращенной в глиняных прудах с морской водой в г. Гомишан в Иране. Для этого в глиняный пруд площадью 3 га поместили 10000 особей и 150 рыб распределили в трех резервуарах из стекловолокна (2000 л). Форель выращивали в течение трех месяцев, после чего у рыб, культивируемых в пруду и резервуарах, были взяты образцы крови, в то время как образцы тканей жабр, почек, печени и кишечника были взяты для гистологического анализа только у прудовых рыб. Температура воды, содержание растворенного в воде кислорода и значение pH в пруду и резервуарах имели несущественные различия; однако такие параметры как соленость воды и содержание аммиака имели более высокие показатели в пруду. Активность цитоплазматической супероксиддисмутазы и глутатионпероксидазы у рыб, культивируемых в глиняных прудах, была значительно выше по сравнению с особями, выращенными в стекловолоконных резервуарах. Тем не менее, между прудом и резервуарами не было значительной разницы по количеству веществ, реагирующих с тиобарбитуровой кислотой. После проведения гистологического анализа у рыб были выявлены такие патоморфологические изменения как первичная гиперплазия ламелл, слияние ламелл и эпителиальный лифтинг. На поперечном сечении почек у рыб наблюдали сморщивание или исчезновение клубочков, скопления меланомакрофагов и некроз гемопоэтической ткани. Кроме того, у этих рыб были обнаружены следы некрозов и скопления меланомакрофагов в печени, а также гипертрофия бокаловидных клеток кишечника. Результаты исследований показывают, что в глиняном пруду рыба оказалась под воздействием стрессовых факторов, среди которых могут быть параметры солености воды и содержания аммиака. Но необходимо учитывать и другие возможные причины, например, загрязняющие вещества и различные режимы кормления.

Ключевые слова: форель, глиняный пруд, система антиоксидантной защиты, морская вода, гистопатология, Гомишан, Иран

История статьи:
Поступила в редакцию: 4 августа 2019 г. Принята к публикации: 2 сентября 2019 г.

Для цитирования:
Hoseini S.M., Gharavi B., Iri Y. Assessment of vital organ histopathology and plasma oxidative conditions of rainbow trout *Oncorhynchus mykiss* reared in earthen saltwater pond // Вестник Российского университета дружбы народов. Серия: Агрономия и животноводство. 2019. Т. 14. № 3. С. 225—265. doi: 10.22363/2312-797X-2019-14-3-255-265

Библиографический список

1. *Hoseini S.M., Kor A., Gharavi B., Iri Y.* Investigation of immunological characteristics of rainbow trout in Gomishan saltwater ponds with those reared in freshwater // Journal of aquatic animal exploitation and rearing. 2019. № 8. P. 41—46. (In Persian).
2. *Hoseini S.M.* Comparison of stress markers of rainbow trout reared in earthen saltwater pond and fiberglass freshwater tanks // Advanced aquaculture sciences journal. 2019. № 2. P. 33—39.
3. Fundamentals of toxicologic pathology / W.M. Haschek, C.G. Rousseaux, M.A. Wallig, eds. 3rd ed. San Diego : Academic Press, 2009.
4. *Evans D.H., Piermarini P.M., Choe K.P.* The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste // Physiol. Rev. 2005. Vol. 85. № 1. P. 97—177.
5. *Ghelichpour M., Taheri Mirghaed A., Mirzargar S.S., Joshaghani H., Ebrahimzadeh Mousavi H.* Plasma proteins, hepatic enzymes, thyroid hormones and liver histopathology of Cyprinus carpio (Linnaeus, 1758) exposed to an oxadiazin pesticide, indoxacarb // Aquac. Res. 2017. Vol. 48. № 11. P. 5666—5676. doi: 10.1111/are.13390
6. *Yousefi M., Hoseini S.M., Vatnikov Y.A., Nikishov A.A., Kulikov E.V.* Thymol as a new anesthetic in common carp (Cyprinus carpio): Efficacy and physiological effects in comparison with eugenol // Aquaculture. 2018. № 495. P. 376—383. doi: 10.1016/j.aquaculture.2018.06.022
7. *Yousefi M., Hoseinifar S.H., Ghelichpour M., Hoseini S.M.* Anesthetic efficacy and biochemical effects of citronellal and linalool in common carp (Cyprinus carpio Linnaeus, 1758) juveniles // Aquaculture. 2018. № 493. P. 107—112. doi: 10.1016/j.aquaculture.2018.04.054
8. *Hoseini S.M., Mirghaed A.T., Iri Y., Ghelichpour M.* Effects of dietary cineole administration on growth performance, hematological and biochemical parameters of rainbow trout (Oncorhynchus mykiss) // Aquaculture. 2018. № 495. P. 766—772. doi: 10.1016/j.aquaculture.2018.06.073
9. *Pérez Jiménez A., Hidalgo M.C., Morales A.E., Arizcun M., Abellan E., Cardenete G.* Antioxidant enzymatic defenses and oxidative damage in Dentex dentex fed on different dietary macronutrient levels // Comparative biochemistry and physiology Part C: toxicology and pharmacology. 2009. № 150. P. 537—545. doi: 10.1016/j.cbpc.2009.07.011
10. *Taheri Mirghaed A., Hoseini S.M., Ghelichpour M.* Effects of dietary 1, 8-cineole supplementation on physiological, immunological and antioxidant responses to crowding stress in rainbow trout (Oncorhynchus mykiss) // Fish Shellfish Immunol. 2018. № 81. P. 182—188. doi: 10.1016/j.fsi.2018.07.027
11. *Hedayati A., Hoseini S.M., Ghelichpour M.* Acute toxicity of waterborne manganese to Rutilus caspicus (Yakovlev, 1870)–gill histopathology, immune indices, oxidative condition, and saltwater resistance // Toxicol. Environ. Chem. 2014. Vol. 96. № 10. P. 1535—1545. doi: 10.1080/02772248.2015.1028408
12. Fish pathology / R.J. Roberts, ed. 4th ed. Oxford : Wiley-Blackwell, 2012.
13. *Taheri Mirghaed A., Fayaz S., Hoseini S.M.* Dietary 1, 8-cinoele affects serum enzymatic activities and immunological characteristics in common carp (Cyprinus carpio) exposed to ambient ammonia // Aquac. Res. 2018. № 50. P. 146—153. doi: 10.1111/are.13877
14. *Hoseini S.M., Yousefi M., Hoseinifar S.H., Van Doan H.* Antioxidant, enzymatic and hematological responses of common carp (Cyprinus carpio) fed with myrcene- or menthol-supplemented diets and exposed to ambient ammonia // Aquaculture. 2019. № 506. P. 246—255. doi: 10.1016/j.aquaculture.2019.03.048
15. *Taheri Mirghaed A., Fayaz S., Hoseini S.M.* Effects of dietary 1, 8-cineole supplementation on serum stress and antioxidant markers of common carp (Cyprinus carpio) acutely exposed to ambient ammonia // Aquaculture. 2019. № 509. P. 8—15. doi: 10.1016/j.aquaculture.2019.04.071
16. *Morgan J.D., Iwama G.K.* Effects of salinity on growth, metabolism, and ion regulation in juvenile rainbow and steelhead trout (Oncorhynchus mykiss) and fall chinook salmon (Oncorhynchus tshawytscha) // Canadian journal of fisheries and aquatic sciences. 1991. Vol. 48. № 11. P. 2083—2094. doi: 10.1139/f91-247

17. *Hoseini S.M., Yousefi M.* Beneficial effects of thyme (Thymus vulgaris) extract on oxytetracycline-induced stress response, immunosuppression, oxidative stress and enzymatic changes in rainbow trout (Oncorhynchus mykiss) // Aquacult Nutr. 2019. Vol. 25. № 2. P. 298—309. doi: 10.1111/anu.12853
18. *Hegazi M.M., Attia Z.I., Ashour O.A.* Oxidative stress and antioxidant enzymes in liver and white muscle of Nile tilapia juveniles in chronic ammonia exposure // Aquat. Toxicol. 2010. Vol. 99. № 2. P. 118—125. doi: 10.1016/j.aquatox.2010.04.007
19. *An K.W., Kim N.N., Shin H.S., Kil G.S., Choi C.Y.* Profiles of antioxidant gene expression and physiological changes by thermal and hypoosmotic stresses in black porgy (Acanthopagrus schlegeli) // Comparative biochemistry and physiology Part A: molecular & integrative physiology. 2010. Vol. 156. № 2. P. 262—268. doi: 10.1016/j.cbpa.2010.02.013
20. *Kim J.H., Park H.J., Kim K.W., Hwang I.K., Kim D.H., Oh C.W., Lee J.S., Kang J.C.* Growth performance, oxidative stress, and non-specific immune responses in juvenile sablefish, Anoplopoma fimbria, by changes of water temperature and salinity // Fish Physiol. Biochem. 2017. Vol. 43. № 5. P. 1421—1431. doi: 10.1007/s10695-017-0382-z
21. Fish osmoregulation / B. Baldisserotto, J.M. Mancera, B.G. Kapoor, eds. CRC Press. 2019.
22. *Ghelichpour M., Rajabiesterabadi H., Hoseini S.M.* Gill histopathological characteristics of Caspian roach (Rutilus rutilus caspicus) fingerlings treated with potassium permanganate and formalin // Aquac. Res. 2016. Vol. 47. № 1. P. 276—282. doi: 10.1111/are.12489
23. *Chenari F., Morovvati H., Ghazilou A., Savari A., Ronagh M.* Rapid variation in kidney histology in spotted scat Scatophagus argus on exposed to abrupt salinity changes // Iranian journal of veterinary research. 2011. Vol. 12. № 3. P. 256—261.
24. *Benli A.Ç.K., Köksal G., Özkul A.* Sublethal ammonia exposure of Nile tilapia (Oreochromis niloticus L.): Effects on gill, liver and kidney histology // Chemosphere. 2008. Vol. 72. № 9. P. 1355—1358. doi: 10.1016/j.chemosphere.2008.04.037
25. *Saghali M., Hoseini S., Hosseini S., Baqraf R.* Determination of heavy metal (Zn, Pb, Cd and Cr) concentration in benthic fauna tissues collected from the Southeast Caspian Sea, Iran // Bull. Environ. Contam. Toxicol. 2014. Vol. 92. № 1. P. 57—60. doi: 10.1007/s00128-013-1133-1.
26. *Khojasteh S.B., Sheikhzadeh F., Mohammadnejad D., Azami A.* Histological, histochemical and ultrastructural study of the intestine of rainbow trout (Oncorhynchus mykiss) // World applied sciences journal. 2009. Vol. 6. № 11. P. 1525—1531.
27. *Merrifield D.L., Carnevali O.* Probiotic modulation of the gut microbiota of fish // Aquaculture nutrition: Gut health, probiotics and prebiotics. 2014. P. 185—222. doi: 10.1002/9781118897263.ch8

Об авторах:

Хосейни Сейед Мортеза — доцент, руководитель отдела по восстановлению запасов, Центр изучения ресурсов гидробионтов во внутренних водах, Иранский институт изучения рыбного хозяйства, Организация сельскохозяйственных исследований и образования, Иран, Горган, бул. Шахид Шекари, e-mail: seyyedmorteza.hoseini@gmail.com. ORCID 0000-0002-9824-5430. Scopus ID 35386383300. ResearcherID: B-7386-2017

Гарави Белруз — научный сотрудник, руководитель группы по защите здоровья водных животных, Центр изучения ресурсов гидробионтов во внутренних водах, Иранский институт изучения рыбного хозяйства, Организация сельскохозяйственных исследований и образования, Иран, Горган, бул. Шахид Шекари. ResearcherID B-3256-2017. ORCID 0000-0001-8552-7781

Ири Юсеф — научный сотрудник, начальник научно-исследовательской станции рыболовства в Гарасоо, Центр изучения ресурсов гидробионтов во внутренних водах, Иранский институт изучения рыбного хозяйства, Организация сельскохозяйственных исследований и образования, Иран, провинция Голестан, д. Гарасоо, 5 км от Бандар Туркмен до Бандар Газ. Scopus ID 55837163000. ResearcherID B-4934-2017. ORCID 0000-0002-0802-3966

RUDN Journal of Agronomy and Animal Industries
Вестник РУДН. Серия: АГРОНОМИЯ И ЖИВОТНОВОДСТВО
2019; 14(3):266—278
http://agrojournal.rudn.ru

Research article

DOI 10.22363/2312-797X-2019-14-3-266-278

Galactooligosaccharide effects as prebiotic on intestinal microbiota of different fish species

Seyed H. Hoseinifar[1,2*], Hien V. Doan[2], Ghasem Ashouri[1]

[1]Polytechnic University of Marche, *Ancona, Italy*
[2]Chiang Mai University, *Chiang Mai, Thailand*
**Corresponding author: hossein.hoseinifar@gmail.com*

Abstract. Manipulation of the gut microbiota toward potentially beneficial bacteria (probiotics) has beneficial effects on fish physiology and health. The effects of prebiotics on gut microbiota are species specific. The present study aimed at investigation of the effects of galactooligosaccharide (GOS) as prebiotic on intestinal microbiota of Caspian roach and Caspian white fish fingerlings. which are among the most economically valuable species in the Caspian Sea. The study was conducted in a completely randomized design with two set of experiment each of them include three treatments in triplicates in which 0 (control), 1 and 2% GOS were used in diet for 6 weeks. At the end of the period, changes in the intestinal microbiota, including total bacterial count, lactic acid count and lactic acid bacteria (LAB) levels and dominance of LAB in the intestinal microbiota, were measured by culture-based method. Dietary GOS had no significant effect on total bacterial count in both species ($P < 0.05$). The LAB levels in the intestinal microbiota in the treatments fed with prebiotics was significantly higher than the control group ($P < 0.05$). LAB bacteria showed the highest increase and dominance in treatments fed with 2% GOS. Also, the highest ratio of lactic acid bacteria to the total number of viable bacteria was observed in the treatment with 2% GOS treatment ($P < 0.05$). The results of this study indicated the possibility of alterations in the bacterial communities of Caspian roach and Caspian white fish fingerlings gut toward beneficial bacterial communities using GOS as prebiotic.

Key words: prebiotic, Caspian white fish, Caspian roach, galactooligosaccharide, gut microbiota

ACKNOWLEDGMENTS
The author wishes to thank Friesland Foods Domo managers (Zwolle, The Netherlands) which kindly provide prebiotic. In addition, we would like to thank the staff of Ghareshsoo research station for their help during the experiment.

Article history:
Received: 15 July 2019. Accepted: 22 August 2019

For citation:
Hoseinifar SH, Doan HV, Ashouri G. Galactooligosaccharide Effects as prebiotic on intestinal microbiota of different fish species. *RUDN Journal of Agronomy and Animal Industries*, 2019; 14(3):266—278. doi: 10.22363/2312-797X-2019-14-3-266-278

Introduction

The study of gut microbiota is of high importance not only regarding disease but also regarding the status of fish physiology and immunity [1]. Establishment of a healthy microbiome in intestine has direct immune-physiological effects on host. It is now well-demonstrated that there is a direct cross talk between gut microbiota and immune response of fish [2, 3]. With the identification of lactic acid bacteria (LAB) in the intestinal microbiota of different fish and shrimp shellfish species in the last decade and determination of their role in the health, welfare and growth performance of the host, the importance of this group of bacteria has become increasingly clear [4, 5]. Although the presence of LAB in the intestinal microbiota of many fish, including Atlantic cod (*Gadus morhua*), rainbow trout (*Oncorhynchus mykiss*), Beluga (*Huso huso*), Persian sturgeon (*Acipenser persicus*) and Arctic charr (*Salvelinus alpinus*) has been proven, they are not the dominant microbiota and constitute a very limited portion of gut microbiota of these species [6]. In addition, it was not possible to isolate LAB bacteria from several fish species [5, 6]. Given this fact, it has been attempted to increase the number of these bacteria through dietary approaches [7, 8]. One of the most important compounds suggested in this regard are prebiotics, which are compounds that are not absorbed by host organism and consumed by potentially beneficial intestinal bacteria (such as LAB) and increase their numbers [9, 10].

Despite recent studies on the effects of prebiotics on fish growth, immunity and physiological indices, many aspects of their potential for alteration of gut microbiota in aquatic and increasing dominance of beneficial bacteria remained unknown [11]. The previous studies revealed that different prebiotics had different effects on LAB levels and also a single prebiotic had different effects on different fish species. Even in some cases, using high levels of more complex prebiotics (higher degree of polymerization) resulted in adverse effects on total bacterial counts and LAB levels [12]. The contradictory of a prebiotic on different host can be due to difference in intestinal microbiota, physiological condition of digestive tract, etc. [13]. Therefore, determination of a prebiotic effect on intestinal microbiota of different species based on comparative studies will help to identify the best prebiotic to change the gut microbiota for that species.

Galactooligosaccharide (GOS) is one of the most promising prebiotic which previous studies revealed that it could exerts positive effects in different fish species [14—16]. In spite of extensive researches on administration of GOS in fish [17—21], to the best of our knowledge there was no published study the effects of GOS on gut microbiota of different fish species using comparative study. Therefore, in the present study we decided to determine the possible effects of GOS on intestinal microbiota of Caspian roach and Caspian white fish.

Materials and methods

Experimental diets

A commercial feed (Dansu, Iran) was used as a control diet (non-supplemented diet). To prepare experimental diet the basal diet was supplemented with two levels of GOS as prebiotic (1 and 2 %). The ingredients were blended thoroughly in a mixer. Then, water was added and made into pellets. The pellets were air-dried, ground and

sieved to produce a suitable crumble (ca. 500μm). The experimental diets were stored in plastic bags at −2 °C for further use.

Fish husbandry

The present study was conducted at the Gharasu Fisheries Research Station. The Caspian white (*Rutilus kutum*) fish and Caspian roach (*Rutilus caspicus*) fingerlings were supplied by Sijowal Caspian Sea Teleost Fish Propagation & Cultivation Centre (Golestan province, Iran). Fish with mean weight of 1.3 g were stocked in nine separate tanks for each species (totally 18 tanks) at density of 30 fish per tank. The fish were acclimated to lab condition for 2 weeks and then feeding with experimental diets were started. During acclimation, fish were fed with control diet. The culture system water was closed with constant aeration. To maintain water quality every 2 days 50 % of water was exchanged. The water quality parameters were controlled and maintained at optimum levels.

Prebiotic

The prebiotic used in the presents study was GOS that was kindly supplied by Friesland Foods Domo Company (Zwolle, The Netherlands). The commercial product name was Vivinal-GOS® and obtained through the enzymatic conversion of lactose and mainly consists of galactose and glucose molecules.

Evaluation of gut microbiota

Total viable autochthonous heterotrophic aerobic bacteria and LAB levels were determined at the start of trial from 15 specimens from the initial pool of fish. Also, at the end of the feeding trial (week 8) microbiological studies were performed. Fish were starved for 24 h to study the autochthonous microbiota. Three specimen were randomly selected from each tank (i.e. $n = 9$ per treatment).The intestine of fish were assessed and prepared for bacteria culture as we described on previous study [12]. Briefly, the surface bacteria were killed before dissection using 0.1 % benzalkonium chloride. With utmost care to be aseptic, the intestine of samples obtained, washed with sterile saline and homogenized using tissue homogenizer (*Potter-Elvehjem, USA)*. The homogenized intestine was serially diluted to 10^{-7} by using sterile saline (0.85 % NaCl). Then, to dermine the level of total bacteria and LAB a portion of the diluates (100 μL) was spread onto plate count agar (PCA) (Merck, Germany) and de Man, Rogosa and Sharpe (MRS) agar (Merck, Germany), respectively. The seeded plates were incubated at room temperature (25ºC) for 5 days [22]. Thereafter, the colony forming units (CFU) g^{-1} were counted from statistically viable plates (i.e. plates containing 30–300 colonies)[23].

Statistical analysis

Prior to statistical analysis, the normality of data and homogeneity of variance were checked and confirmed. Then, the statistically significant difference (at $P < 0.05$) between treatments was checked using One-way analysis of variance (ANOVA) followed by Duncan's multiple range tests (36). All statistical analysis were performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). The figures were drawn using Excel software (Micorsoft Office ver. 2016).

Results and discussion

The total viable autochthonous heterotrophic aerobic bacteria (THAB) level (Log CFU/g) in the intestine of Caspian roach and Caspian white fish fingerlings fed with different levels of galactaligosaccharide (GOS) as prebiotics is shown in Figure 1. At the beginning of the feeding trial, the THAB of intestinal microbiota was 5.10 ± 0.24 log CFU/g. As shown in Figure 1 A, dietary administration of 1 or 2% GOS in diet had no significant effect on THAB counts in the gut microbiota of Caspian roach ($P > 0.05$). Similar result was noticed in case of the gut microbiota of Caspian white fish ($P > 0.05$) (Figure 1 B).

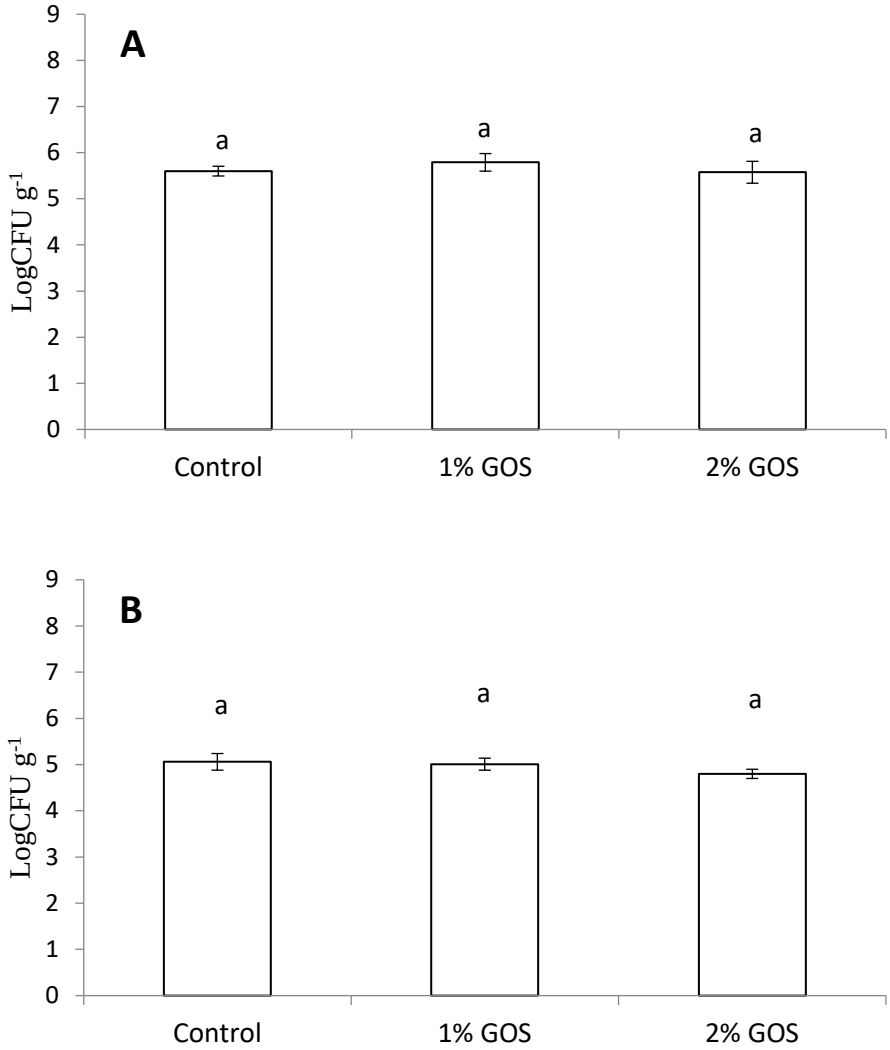


Fig. 1. The effects of different levels of galactoaligosaccharide (GOS) as prebiotic on total bacterial counts (log CFU/g) in Caspian roach (A) and Caspian white fish (B) fingerlings.
The bars (mean ±SD) assigned similar letters indicate no significant difference ($P > 0.05$)

The effects of different levels of GOS prebiotics on the level of LAB of lactic acid bacteria (Log CFU/g) in the gut microbiota of Caspian roach and Caspian white fish fingerling sare summarized in Figure 2. At the beginning of the period, no lactic acid bacteria were isolated from the gut microbiota of both fish species. Indeed, the number of LAB in the intestinal microbiota were statistically too few to count (TFTC; lower than 30 colonies in the first dilution). Similarly, at the end of trial in case of both fish species the LAB levels were TFTC in the control treatment. While, feeding with GOS caused significant increase of LAB level in gut microbiota of the Caspian roach and Caspian white fish fingerlings. In both species, the highest LAB level was noticed in gut microbiota of fish fed with 2% GOS. There were significant difference between 1% GOS and 2% GOS treatment in case of gut microbiota LAB level in Caspian roach ($P < 0.05$). However, no significant difference was noticed in case of 1 and 2% GOS in Caspian white fish ($P > 0.05$).

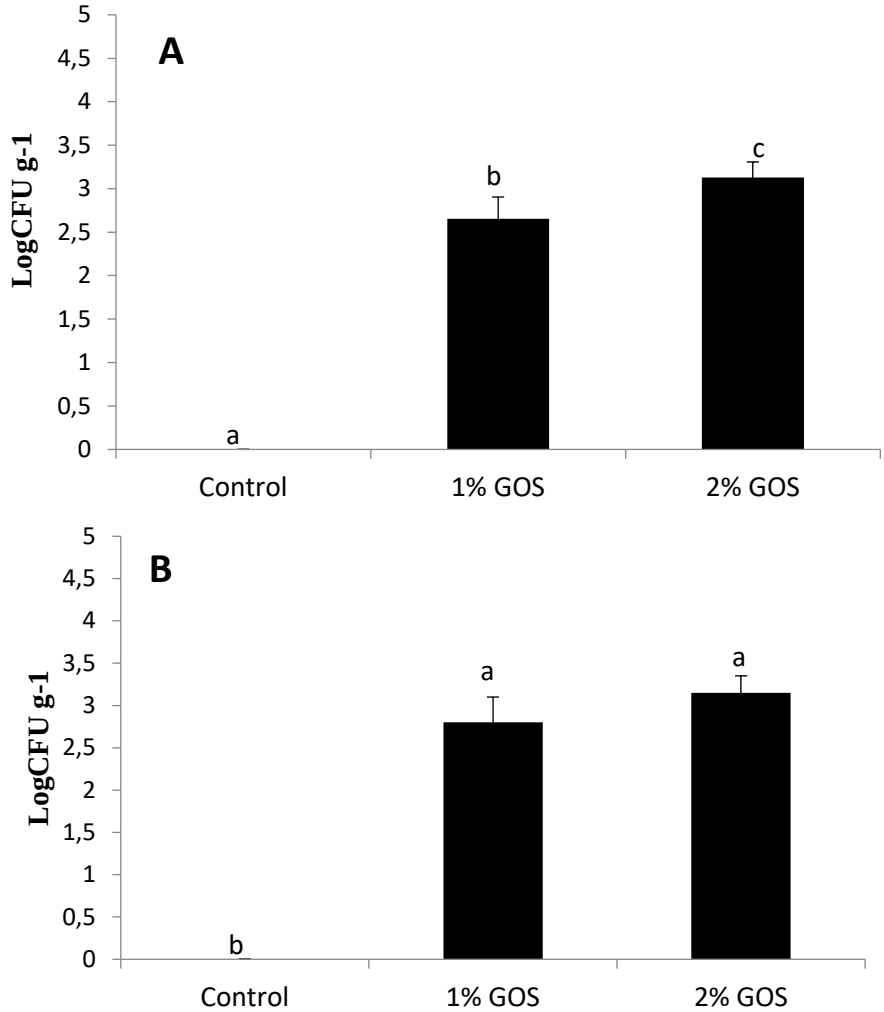


Fig. 2. The effects of different levels of galactoaligosaccharide (GOS) as prebiotic on Lactic acid bacteria levels (log CFU/g) in Caspian roach (A) and Caspian white fish (B) fingerlings. The bars (mean ±SD) assigned with different letters indicate significant difference ($P < 0.05$)

In addition, we calculated the ratio of LAB (potentially useful probiotic bacteria) to THAB in the gut microbiota of both species to see the alteration in the dominance of LAB in gut microbiota (Table 1). The obtained results showed that the ratio of LAB to THAB in all prebiotic treatments was significantly higher than the control treatment ($P < 0.05$). The highest increase in the ratio of lactic acid bacteria to the total number of viable bacteria was observed in the 2% GOS treatment ($P < 0.05$). Although the addition of GOS to Caspian white fish diet significantly increased the ratio of lactic acid bacteria, this increment was not dose dependent; there was no significant difference between 1 and 2 % levels ($P < 0.05$).

Table 1

The ratio (%) of lactic acid bacteria to the total viable bacteria in the gut microbiota of Caspian roach and Caspian white fish fingerlings fed with different levels of GOS as prebiotic. The data in a row (mean ± SD) assigned with different letters indicate significant difference ($P < 0.05$)

Fish species	Treatments		
	Control	1% GOS	2% GOS
Caspian roach	TFTC[c]	1.70±0.34[b]	4.84 ± 0.81[a]
Caspian white fish	TFTC[b]	4.45±0.16[a]	4.12 ± 0.41[a]

The intestinal microbiota of the fish includes a complex and diverse community of aerobic and anaerobic bacteria. One of the group of bacteria in the gut microbiota are LAB that are of great importance nowadays as probiotics [24]. Although isolation of lactic acid bacteria from the gut microbiota of various species of fish has been reported, these bacteria are not among the predominant bacterial communities in the gut and are present in low abundance [5]. Lactic acid bacteria are capable of inhibiting the growth of pathogenic bacteria through excretion of bacteriocins and thereby can pose positive effects on the health status and disease resistance of fish [25]. Although identification of the gut microbiota of fish and its manipulating is complex and is not fully understood, providing knowledge regarding possible alternative for modulation of gut microbiota toward beneficial populations is of high importance and can be a promising strategy for enhancing immunity and disease resistance [26—28]. This strategy can help to reduce utilization of antibiotics in aquaculture which per results in sustainable aquaculture [11]. One of the proposed methods for modulation of the intestinal microbiota composition is the use of dietary supplements such as prebiotics [2, 7—9, 29]. To date, many studies have been conducted on the beneficial effects of prebiotics on humans and pets, and in recent years, the use of these supplements in the diet of fish and other aquatic animals has been considered. The efficacy and efficacy of prebiotics have been shown to be influenced by the degree of polymerization, fermentability, host species, resident gut microbiota [8]. Therefore, considering the inter-species variation, in order to ensure the beneficial effect of the prebiotic used in the diet and the optimal prebiotic selection, comparative studies should be done.

The results of the present study revealed no significant alteration in THAB in the gut microbiota of both Caspian roach and Caspian white fish (Figure 1). In line with the findings of the present study, dietary administration of inactive yeast (*Saccharomyces cerevisiae*)

and prebiotic fructoaligosaccharide (FOS) had no significant effects on THAB in the intestinal microbiota of Beluga sturgeon (*Huso huso*) [12, 30]. Similarly, feeding turbot with FOS supplemented diet exerts no significant effect on THAB [31]. On the other hand, negative effects on THAB level was reported in beluga fed with inulin [32]. The inability of dietary prebiotic to alter the THAB seems to be due to the limited binding sites in the gut [1, 33]. Indeed, the previous studies revealed that prebiotics seems to change the balance of gut microbiota by providing energy source for the beneficial bacteria rather than increasing the THAB. Therefore, the THAB cannot be altered very much due to the limited binding sites.

Concerning the effects of the tested prebiotic (GOS) on potentially useful intestinal bacteria, the results indicated a significant increase in the number of LAB in the intestinal microbiota of both Caspian white fish and roach compared to the control treatment. The highest increase was observed in fish fed with 2% GOS. Previous studies on the aquatic gut microbiota revealed despite the limited number of LAB in the gut microbiota, these potentially useful (probiotic) bacteria can be increased through administration of optimum prebiotics and become dominant bacterial communities [8]. Although there is no comparative study regarding the effects of prebiotics on the composition of the gut microbiota of Caspian white fish and roach, the results of this study are consistent with those of Hoseinifar, Mirvaghefi, Amoozegar, Merrifield, Ringø [34] that showed the use of GOS (as a prebiotic) in the diet significantly increased the number of LAB in the gut microbiota of rainbow trout. In addition, the use of FOS and yeast prebiotics significantly increased the number of LAB in the gut microbiota of Beluga [12, 30]. Similar results have been observed regarding the effects of FOS on the levels of probiotic bacteria in the intestinal microbiota of Turbot [31]. However, in contrast with these finding, inulin had no significant effect on LAB levels in the intestinal microbiota of the Beluga [35]. Despite the several reports on the prebiotic effects of GOS on physiological and health indices of fish, there are limited reports on the prebiotic effect on the intestinal microbiota composition of fish. According to the results of the present study, GOS is an effective prebiotic for modulation of gut microbiota of both species. The observed differences regarding the dose can be due to differences in the physiological characteristics of the gut, the prebiotic type and the microbiota composition of the gut of these species.

In conclusion, the results of this study showed that the use of GOS can be taken into account as an effective prebiotic in Caspian roach and Caspian white fish diet, aimed at modulation of the balance of gut microbiota toward beneficial bacteria. However, determining the possible effects on physiological parameters as well as mode of action needs further investigation.

References

1. Llewellyn MS, Boutin S, Hoseinifar SH, Derome N. Teleost microbiomes: the state of the art in their characterization, manipulation and importance in aquaculture and fisheries. *Frontiers in Microbiology*. 2014; 5:207. doi: 10.3389/fmicb.2014.00207
2. Nawaz A, Bakhsh javaid A, Irshad S, Hoseinifar SH, Xiong H. The functionality of prebiotics as immunostimulant: Evidences from trials on terrestrial and aquatic animals. *Fish & Shellfish Immunology*. 2018; 76:272—278. doi: 10.1016/j.fsi.2018.03.004

3. Lazado CC, Caipang CMA. Mucosal immunity and probiotics in fish. *Fish & Shellfish Immunology.* 2014; 39(1):78—89. doi: 10.1016/j.fsi.2014.04.015
4. Van Doan H, Hoseinifar SH, Ringo E, Angeles Esteban M, Dadar M, Dawood MA, et al. Host-associated probiotics: A key factor in sustainable aquaculture. *Reviews in Fisheries Science & Aquaculture.* 2019; 1—27. doi: 10.1080/23308249.2019.1643288
5. Ringo E, Hoseinifar SH, Ghosh K, Doan HV, Beck BR, Song SK. Lactic Acid bacteria in finfish—an update. *Frontiers in Microbiology.* 2018; 9:1818. doi: 10.3389/fmicb.2018.01818
6. Merrifield DL, Balcázar JL, Daniels C, Zhou Z, Carnevali O, Sun YZ et al. Indigenous lactic acid bacteria in fish and crustaceans. In: Merrifield DL, Ringo E. (eds.) *Aquaculture Nutrition: gut health, probiotics and prebiotics.* Oxford: John Wiley & Sons; 2014. p.128—168.
7. Doan HV, Hoseinifar SH, Esteban MÁ, Dadar M, Thu TTN. Mushrooms, seaweed, and their derivatives as functional feed additives for aquaculture: an updated view. *Studies in Natural Products Chemistry.* 2019; 62:41—90. doi: 10.1016/B978-0-444-64185-4.00002-2
8. Ringo E, Zhou Z, Vecino JLG, Wadsworth S, Romero J, Krogdahl A, et al. Effect of dietary components on the gut microbiota of aquatic animals. A never-ending story? *Aquaculture Nutrition.* 2016; 22(2):219—282. doi: 10.1111/anu.12346
9. Dawood MAO, Koshio S. Recent advances in the role of probiotics and prebiotics in carp aquaculture: A review. *Aquaculture.* 2016; 454:243—251. doi: 10.1016/j.aquaculture.2015.12.033
10. 1Hoseinifar SH, Esteban MÁ, Cuesta A, Sun Y-Z. Prebiotics and fish immune response: A review of current knowledge and future perspectives. *Reviews in Fisheries Science & Aquaculture.* 2015; 23(4):315—328. doi: 10.1080/23308249.2015.1052365
11. Lieke T, Meinelt T, Hoseinifar SH, Pan B, Straus DL, Steinberg CEW. Sustainable aquaculture requires environmental-friendly treatment strategies for fish diseases. *Reviews in Aquaculture.* 2019. doi: 10.1111/raq.12365
12. Hoseinifar SH, Mirvaghefi A, Mojazi Amiri B, Rostami HK, Merrifield DL. The effects of oligofructose on growth performance, survival and autochthonous intestinal microbiota of beluga (Huso huso) juveniles. *Aquaculture Nutrition.* 2011; 17(5):498—504. doi: 10.1111/j.1365-2095.2010.00828.x
13. Daniels C, Hoseinifar SH. Prebiotic Applications in shellfish. In: Merrifield LD, Ringo E (eds.) *Aquaculture Nutrition: gut health, probiotics and prebiotics.* Oxford: John Wiley & Sons; 2014. p.401—418.
14. Zhou Q-C, Buentello JA, Gatlin Iii DM. Effects of dietary prebiotics on growth performance, immune response and intestinal morphology of red drum (Sciaenops ocellatus). *Aquaculture.* 2010; 309(1—4):253—257. doi: 10.1016/j.aquaculture.2010.09.003
15. Burr G, Hume M, Ricke S, Nisbet D, Gatlin D. In Vitro and in vivo evaluation of the prebiotics GroBiotic®-A, inulin, mannanoligosaccharide, and galactooligosaccharide on the digestive microbiota and performance of hybrid striped bass (Morone chrysops × Morone saxatilis). *Microbial ecology.* 2010; 59(1):187—198. doi: 10.1007/s00248-009-9597-6
16. Grisdale-Helland B, Helland SJ, Gatlin III DM. The effects of dietary supplementation with mannanoligosaccharide, fructooligosaccharide or galactooligosaccharide on the growth and feed utilization of Atlantic salmon (Salmo salar). *Aquaculture.* 2008; 283(1—4):163—167. doi: 10.1016/j.aquaculture.2008.07.012
17. Yousefi S, Hoseinifar SH, Paknejad H, Hajimoradloo A. The effects of dietary supplement of galactooligosaccharide on innate immunity, immune related genes expression and growth performance in zebrafish (Danio rerio). *Fish & Shellfish Immunology.* 2018; 73:192—196. doi: 10.1016/j.fsi.2017.12.022
18. Hoseinifar SH, Ahmadi A, Khalili M, Raeisi M, Van Doan H, Caipang CM. The study of antioxidant enzymes and immune-related genes expression in common carp (Cyprinus carpio) fingerlings fed different prebiotics. *Aquaculture Research.* 2017; 48(11):5447—5454. doi: 10.1111/are.13359
19. Modanloo M, Soltanian S, Akhlaghi M, Hoseinifar SH. The effects of single or combined administration of galactooligosaccharide and Pediococcus acidilactici on cutaneous mucus immune parameters, humoral immune responses and immune related genes expression in

common *carp (Cyprinus carpio)* fingerlings. *Fish & Shellfish Immunology.* 2017; 70:391—397. doi: 10.1016/j.fsi.2017.09.032

20. Guerreiro I, Couto A, Machado M, Castro C, Pousão-Ferreira P, Oliva-Teles A, et al. Prebiotics effect on immune and hepatic oxidative status and gut morphology of white sea bream (Diplodus sargus). *Fish & Shellfish Immunology.* 2016; 50:168—174. doi: 10.1016/j.fsi.2016.01.023
21. Miandare HK, Farvardin S, Shabani A, Hoseinifar SH, Ramezanpour SS. The effects of galactooligosaccharide on systemic and mucosal immune response, growth performance and appetite related gene transcript in goldfish (Carassius auratus gibelio). *Fish & Shellfish Immunology.* 2016; 55:479—483. doi: 10.1016/j.fsi.2016.06.020
22. Hoseinifar SH, Khalili M, Sun YZ. Intestinal histomorphology, autochthonous microbiota and growth performance of the oscar (Astronotus ocellatus Agassiz, 1831) following dietary administration of xylooligosaccharide. *Journal of Applied Ichthyology.* 2016; 32(6):1137—1141. doi: 10.1111/jai.13118
23. Rawling M, Merrifield D, Kühlwein H, Snellgrove D, Gioacchini G, Carnevali O, et al. Dietary modulation of immune response and related gene expression profiles in mirror carp (Cyprinus carpio) using selected exotic feed ingredients. *Aquaculture.* 2014; 418:177—184. doi: 10.1016/j.aquaculture.2013.10.002
24. Van Doan H, Hoseinifar SH, Ringo E, Angeles Esteban M, Dadar M, Dawood MA, et al. Host-associated probiotics: a key factor in sustainable aquaculture. *Reviews in Fisheries Science & Aquaculture.* 2019; 1—27. doi: 10.1080/23308249.2019.1643288
25. Hoseinifar SH, Sun Y, Wang A, Zhou Z. Probiotics as means of diseases control in aquaculture, A Review of current knowledge and future perspectives. *Frontiers in Microbiology.* 2018; 9:2429. doi: 10.3389/fmicb.2018.02429
26. Van Doan H, Hoseinifar SH, Tapingkae W, Seel-audom M, Jaturasitha S, Dawood MA, et al. Boosted growth performance, mucosal and serum immunity, and disease resistance nile tilapia (Oreochromis niloticus) fingerlings using corncob-derived xylooligosaccharide and lactobacillus plantarum CR1T5. *Probiotics and antimicrobial proteins.* 2019; 1—12. doi: 10.1007/s12602-019-09554-5
27. Dawood MAO, Koshio S, Esteban MA. Beneficial roles of feed additives as immunostimulants in aquaculture: a review. *Reviews in Aquaculture.* 2018; 10(4):950—974. doi: 10.1111/raq.12209
28. Abdel-Tawwab M, Abdel-Rahman AM, Ismael NEM. Evaluation of commercial live bakers' yeast, Saccharomyces cerevisiae as a growth and immunity promoter for Fry Nile tilapia, Oreochromis niloticus (L.) challenged in situ with Aeromonas hydrophila. *Aquaculture.* 2008; 280(1—4):185—189. doi: 10.1016/j.aquaculture.2008.03.055
29. Ringo E, Dimitroglou A, Hoseinifar SH, Davies SJ. Prebiotics in Finfish: an update. In: Merrifield DL, Ringo E. (eds.) *Aquaculture nutrition: gut health, probiotics and prebiotics.* Oxford: John Wiley & Sons; 2014. p.360—400. doi: 10.1002/9781118897263.ch14
30. Hoseinifar SH, Mirvaghefi A, Merrifield DL. The effects of dietary inactive brewer's yeast Saccharomyces cerevisiae var. ellipsoideus on the growth, physiological responses and gut microbiota of juvenile beluga (Huso huso). *Aquaculture.* 2011; 318(1—2):90—94. doi: 10.1016/j.aquaculture.2011.04.043
31. Mahious AS, Gatesoupe FJ, Hervi M, Metailler R, Ollevier F. Effect of dietary inulin and oligosaccharides as prebiotics for weaning turbot, Psetta maxima (Linnaeus, C. 1758). *Aquaculture International.* 2006; 14(3):219—229. doi: 10.1007/s10499-005-9003-4
32. Akrami R, Ghelichi A, Manuchehri H. Effect of dietary inulin as prebiotic on growth performance and survival of juvenile rainbow trout (Oncorhynchus mykiss). *Journal of marine science and technology research.* 2009; 4(3):1—9.
33. Li X, Ringo E, Hoseinifar SH, Lauzon HL, Birkbeck H, Yang D. The adherence and colonization of microorganisms in fish gastrointestinal tract. *Reviews in Aquaculture.* 2019; 11(3):603—618. doi: 10.1111/raq.12248

34. Hoseinifar SH, Mirvaghefi A, Amoozegar MA, Merrifield D, Ringø E. In vitro selection of a synbiotic and in vivo evaluation on intestinal microbiota, performance and physiological response of rainbow trout (Oncorhynchus mykiss) fingerlings. *Aquaculture Nutrition.* 2015; 23(1):111—118. doi: 10.1111/anu.12373
35. Reza A, Abdolmajid H, Abbas M, Abdolmohammad AK. Effect of dietary prebiotic inulin on growth performance, intestinal microflora, body composition and hematological parameters of juvenile beluga, Huso huso (Linnaeus, 1758). *Journal of the World Aquaculture Society*. 2009; 40(6):771—779. Available from: doi: 10.1111/j.1749-7345.2009.00297.x

About authors:

Hoseinifar Seyed Hossein — Phd, Department of Life and Environmental Sciences, Polytechnic University of Marche, 22 Piazza Roma, Ancona, Italy, 60100; Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University, Su Thep, Mueang Chiang Mai District, Chiang Mai, Thailand, 50200; e-mail: hossein.hoseinifar@gmail.com; ORCID: 0000-0002-0210-9013

Doan Hien Van — Phd, Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University, 239 HuayKaew Road, Muang District, Chiang Mai, Thailand, 50200; WOS - N-9579-2019

Ashouri Ghasem — Department of Life and Environmental Sciences, Polytechnic University of Marche, 22 Piazza Roma, Ancona, Italy, 60100

Научная статья

DOI 10.22363/2312-797X-2019-14-3-266-278

Влияние галактоолигосахаридов в виде пребиотика на микрофлору кишечника различных видов рыб

С.Х. Хосейнифар*[1,2], Х.В. Доан[2], Г. Ашоури[1]

[1] Политехнический Университет Марке, *Италия, Анкона*

[2] Чиангмайский университет, *Чиангмай, Таиланд*

* *hossein.hoseinifar@gmail.com*

Аннотация. Обогащение кишечной микробиоты потенциально полезными бактериями (пробиотиками) оказывает благотворное влияние на физиологические процессы и здоровье рыб. Однако, воздействие пребиотиков на микрофлору кишечника является видоспецифичным. Настоящее исследование направлено на изучение влияния галактоолигосахаридов в качестве пребиотика на кишечную микробиоту каспийской плотвы и мальков каспийского кутума, являющихся одними из наиболее экономически ценных видов рыб, обитающих в Каспийском море. Исследование проводилось в течение 6 недель по полной рандомизированной схеме, в двух повторениях, каждое из которых включало три варианта обработки — 0 (контроль), 1 и 2 % ГОС, в трехкратной повторности. После этого с помощью культурального метода были изучены изменения в микробиоте кишечника рыб, включая общее количество бактерий, количество молочной кислоты и молочнокислых бактерий, а также влияние молочнокислых бактерий на микрофлору кишечника. Диетические галактоолигосахариды не оказали значительного влияния на общее количество бактерий у обоих видов ($P < 0.05$). Уровень молочнокислых бактерий в кишечнике был значительно выше при лечении пребиотиками, чем в контрольной группе ($P < 0.05$). Значительное увеличение количества молочнокислых бактерий и их преобладание было отмечено в варианте с использованием 2 % галактоолигосахаридов. Кроме

того, самое высокое количество молочнокислых бактерий по отношению к общему количеству жизнеспособных бактерий наблюдалось в варианте с использованием 2 % галактоолигосахаридов ($P < 0.05$). Результаты данного исследования доказывают возможность и эффективность использования галактоолигосахаридов в качестве пребиотика для обогащения кишечной бактериальной микрофлоры каспийской плотвы и мальков каспийского кутума.

Ключевые слова: пребиотик, каспийский кутум, каспийская плотва, галактоолигосахарид, кишечная микробиота

БЛАГОДАРНОСТИ

Авторы выражают благодарность сотрудникам Friesland Foods Domo (Зволле, Нидерланды), любезно предоставившим препараты-пребиотики. Также мы хотели бы поблагодарить сотрудников исследовательской станции Ghareshsoo за помощь в проведении научных экспериментов.

История статьи:

Поступила в редакцию: 15 июля 2019 г. Принята к публикации: 22 августа 2019 г.

Для цитирования:

Hoseinifar S.H., Doan H.V., Ashouri G. Galactooligosaccharide effects as prebiotic on intestinal microbiota of different fish species // Вестник Российского университета дружбы народов. Серия: Агрономия и животноводство. 2019. Т. 14. № 3. С. 266—278. doi: 10.22363/2312-797X-2019-14-3-266-278

Библиографический список

1. *Llewellyn M.S., Boutin S., Hoseinifar S.H., Derome N.* Teleost microbiomes: the state of the art in their characterization, manipulation and importance in aquaculture and fisheries // Frontiers in Microbiology. 2014. № 5. P. 207. doi: 10.3389/fmicb.2014.00207
2. *Nawaz A., Bakhsh javaid A., Irshad S., Hoseinifar S.H., Xiong H.* The functionality of prebiotics as immunostimulant: Evidences from trials on terrestrial and aquatic animals // Fish & Shellfish Immunology. 2018. № 76. P. 272—278. doi: 10.1016/j.fsi.2018.03.004
3. *Lazado C.C., Caipang C.M.A.* Mucosal immunity and probiotics in fish // Fish & Shellfish Immunology. 2014. Vol. 39. № 1. P. 78—89. doi: 10.1016/j.fsi.2014.04.015
4. *Van Doan H., Hoseinifar S.H., Ringo E., Angeles Esteban M., Dadar M., Dawood M.A.,* et al. Host-associated probiotics: A key factor in sustainable aquaculture // Reviews in Fisheries Science & Aquaculture. 2019. P. 1—27. doi: 10.1080/23308249.2019.1643288
5. *Ringo E., Hoseinifar S.H., Ghosh K., Doan H.V,. Beck B.R., Song S.K.* Lactic Acid bacteria in finfish — an update // Frontiers in Microbiology. 2018. № 9. P. 1818. doi: 10.3389/fmicb.2018.01818
6. *Merrifield D.L., Balcázar J.L., Daniels C., Zhou Z., Carnevali O., Sun Y.Z.* et al. Indigenous lactic acid bacteria in fish and crustaceans // Aquaculture Nutrition: gut health, probiotics and prebiotics / Merrifield D.L., Ringo E., eds. Oxford : John Wiley & Sons, 2014. P. 128—168.
7. *Doan H.V., Hoseinifar S.H., Esteban M.Á., Dadar M., Thu T.T.N.* Mushrooms, seaweed, and their derivatives as functional feed additives for aquaculture: an updated view // Studies in Natural Products Chemistry. 2019. № 62. P. 41—90. doi: 10.1016/B978-0-444-64185-4.00002-2
8. *Ringo E., Zhou Z., Vecino J.L.G., Wadsworth S., Romero J., Krogdahl A., et al.* Effect of dietary components on the gut microbiota of aquatic animals. A never-ending story? // Aquaculture Nutrition. 2016. Vol. 22. № 2. P. 219—282. doi: 10.1111/anu.12346
9. *Dawood M.A.O., Koshio S.* Recent advances in the role of probiotics and prebiotics in carp aquaculture: A review // Aquaculture. 2016. № 454. P. 243—251. doi: 10.1016/j.aquaculture.2015.12.033
10. *Hoseinifar S.H., Esteban M.Á., Cuesta A., Sun Y.-Z.* Prebiotics and fish immune response: A review of current knowledge and future perspectives // Reviews in Fisheries Science & Aquaculture. 2015. Vol. 23. № 4. P. 315—328. doi: 10.1080/23308249.2015.1052365

11. *Lieke T., Meinelt T., Hoseinifar S.H., Pan B., Straus D.L., Steinberg C.E.W.* Sustainable aquaculture requires environmental-friendly treatment strategies for fish diseases // Reviews in Aquaculture. 2019. doi: 10.1111/raq.12365
12. *Hoseinifar S.H., Mirvaghefi A., Mojazi Amiri B., Rostami H.K., Merrifield D.L.* The effects of oligofructose on growth performance, survival and autochthonous intestinal microbiota of beluga (Huso huso) juveniles // Aquaculture Nutrition. 2011. Vol. 17. № 5. P. 498—504. doi: 10.1111/j.1365-2095.2010.00828.x
13. *Daniels C., Hoseinifar S.H.* Prebiotic Applications in shellfish // Aquaculture Nutrition: gut health, probiotics and prebiotics / Merrifield L.D., Ringo E., eds. Oxford : John Wiley & Sons, 2014. P. 401—418.
14. *Zhou Q.-C., Buentello J.A., Gatlin Iii D.M.* Effects of dietary prebiotics on growth performance, immune response and intestinal morphology of red drum (Sciaenops ocellatus). Aquaculture. 2010. Vol. 309. № 1—4. P. 253—257. doi: 10.1016/j.aquaculture.2010.09.003
15. *Burr G., Hume M., Ricke S., Nisbet D., Gatlin D.* In Vitro and in vivo evaluation of the prebiotics GroBiotic®-A, inulin, mannanoligosaccharide, and galactooligosaccharide on the digestive microbiota and performance of hybrid striped bass (Morone chrysops × Morone saxatilis) // Microbial ecology. 2010. Vol. 59. № 1. P. 187—198. doi: 10.1007/s00248-009-9597-6
16. *Grisdale-Helland B., Helland S.J., Gatlin III D.M.* The effects of dietary supplementation with mannanoligosaccharide, fructooligosaccharide or galactooligosaccharide on the growth and feed utilization of Atlantic salmon (Salmo salar) // Aquaculture. 2008. Vol. 283. № 1—4. P. 163—167. doi: 10.1016/j.aquaculture.2008.07.012
17. *Yousefi S., Hoseinifar S.H., Paknejad H., Hajimoradloo A.* The effects of dietary supplement of galactooligosaccharide on innate immunity, immune related genes expression and growth performance in zebrafish (Danio rerio) // Fish & Shellfish Immunology. 2018. № 73. P. 192—196. doi: 10.1016/j.fsi.2017.12.022
18. *Hoseinifar S.H., Ahmadi A., Khalili M., Raeisi M., Van Doan H., Caipang C.M.* The study of antioxidant enzymes and immune-related genes expression in common carp (Cyprinus carpio) fingerlings fed different prebiotics // Aquaculture Research. 2017. Vol. 48. № 11. P. 5447—5454. doi: 10.1111/are.13359
19. *Modanloo M., Soltanian S., Akhlaghi M., Hoseinifar S.H.* The effects of single or combined administration of galactooligosaccharide and Pediococcus acidilactici on cutaneous mucus immune parameters, humoral immune responses and immune related genes expression in common *carp (Cyprinus carpio)* fingerlings // Fish & Shellfish Immunology. 2017. № 70. P. 391—397. doi: 10.1016/j.fsi.2017.09.032
20. *Guerreiro I., Couto A., Machado M., Castro C., Pousão-Ferreira P., Oliva-Teles A., et al.* Prebiotics effect on immune and hepatic oxidative status and gut morphology of white sea bream (Diplodus sargus) // Fish & Shellfish Immunology. 2016. № 50. P. 168—174. doi: 10.1016/j.fsi.2016.01.023
21. *Miandare H.K., Farvardin S., Shabani A., Hoseinifar S.H., Ramezanpour S.S.* The effects of galactooligosaccharide on systemic and mucosal immune response, growth performance and appetite related gene transcript in goldfish (Carassius auratus gibelio) // Fish & Shellfish Immunology. 2016. № 55. P. 479—483. doi: 10.1016/j.fsi.2016.06.020
22. *Hoseinifar S.H., Khalili M., Sun Y.Z.* Intestinal histomorphology, autochthonous microbiota and growth performance of the oscar (Astronotus ocellatus Agassiz, 1831) following dietary administration of xylooligosaccharide // Journal of Applied Ichthyology. 2016. Vol. 32 № 6. P. 1137—1141. doi: 10.1111/jai.13118
23. *Rawling M., Merrifield D., Kühlwein H., Snellgrove D., Gioacchini G., Carnevali O.,* et al. Dietary modulation of immune response and related gene expression profiles in mirror carp (Cyprinus carpio) using selected exotic feed ingredients // Aquaculture. 2014. № 418. P. 177—184. doi: 10.1016/j.aquaculture.2013.10.002
24. *Van Doan H., Hoseinifar S.H., Ringo E., Angeles Esteban M., Dadar M., Dawood M.A.,* et al. Host-associated probiotics: a key factor in sustainable aquaculture // Reviews in Fisheries Science & Aquaculture. 2019. P. 1—27. doi: 10.1080/23308249.2019.1643288

25. *Hoseinifar S.H., Sun Y., Wang A., Zhou Z.* Probiotics as means of diseases control in aquaculture, A Review of current knowledge and future perspectives // Frontiers in Microbiology. 2018. № 9. P. 2429. doi: 10.3389/fmicb.2018.02429
26. *Van Doan H., Hoseinifar S.H., Tapingkae W., Seel-audom M., Jaturasitha S., Dawood M.A.,* et al. Boosted growth performance, mucosal and serum immunity, and disease resistance nile tilapia (Oreochromis niloticus) fingerlings using corncob-derived xylooligosaccharide and lactobacillus plantarum CR1T5 // Probiotics and antimicrobial proteins. 2019. P. 1—12. doi: 10.1007/s12602-019-09554-5
27. *Dawood M.A.O., Koshio S., Esteban M.A.* Beneficial roles of feed additives as immunostimulants in aquaculture: a review // Reviews in Aquaculture. 2018. Vol. 10. № 4. P. 950—974. doi: 10.1111/raq.12209
28. *Abdel-Tawwab M., Abdel-Rahman A.M., Ismael N.E.M.* Evaluation of commercial live bakers' yeast, Saccharomyces cerevisiae as a growth and immunity promoter for Fry Nile tilapia, Oreochromis niloticus (L.) challenged in situ with Aeromonas hydrophila // Aquaculture. 2008. Vol. 280. № 1—4. P. 185—189. doi: 10.1016/j.aquaculture.2008.03.055
29. *Ringo E., Dimitroglou A., Hoseinifar S.H., Davies S.J.* Prebiotics in Finfish: an update // Aquaculture nutrition: gut health, probiotics and prebiotics / Merrifield D.L, Ringo E., eds. Oxford : John Wiley & Sons, 2014. P. 360—400. doi: 10.1002/9781118897263.ch14
30. *Hoseinifar S.H., Mirvaghefi A., Merrifield D.L.* The effects of dietary inactive brewer's yeast Saccharomyces cerevisiae var. ellipsoideus on the growth, physiological responses and gut microbiota of juvenile beluga (Huso huso) // Aquaculture. 2011. Vol. 318. № 1—2. P. 90—94. doi: 10.1016/j.aquaculture.2011.04.043
31. *Mahious A.S., Gatesoupe F.J., Hervi M., Metailler R., Ollevier F.* Effect of dietary inulin and oligosaccharides as prebiotics for weaning turbot, Psetta maxima (Linnaeus, C. 1758) // Aquaculture International. 2006. Vol. 14. № 3. P. 219—229. doi: 10.1007/s10499-005-9003-4
32. *Akrami R., Ghelichi A., Manuchehri H.* Effect of dietary inulin as prebiotic on growth performance and survival of juvenile rainbow trout (Oncorhynchus mykiss) // Journal of marine science and technology research. 2009. Vol. 4. № 3. P. 1—9.
33. *Li X., Ringo E., Hoseinifar S.H., Lauzon H.L., Birkbeck H., Yang D.* The adherence and colonization of microorganisms in fish gastrointestinal tract // Reviews in Aquaculture. 2019. Vol. 11. № 3. P. 603—618. doi: 10.1111/raq.12248
34. *Hoseinifar S.H., Mirvaghefi A., Amoozegar M.A., Merrifield D., Ringø E.* In vitro selection of a synbiotic and in vivo evaluation on intestinal microbiota, performance and physiological response of rainbow trout (Oncorhynchus mykiss) fingerlings // Aquaculture Nutrition. 2015. Vol. 23. № 1. P. 111—118. doi: 10.1111/anu.12373
35. *Reza A., Abdolmajid H., Abbas M., Abdolmohammad A.K.* Effect of dietary prebiotic inulin on growth performance, intestinal microflora, body composition and hematological parameters of juvenile beluga, Huso huso (Linnaeus, 1758) // Journal of the World Aquaculture Society. 2009. Vol. 40. № 6. P. 771—779. doi: 10.1111/j.1749-7345.2009.00297.x

Об авторах:

Хосейнифар Сейед Хоссейн — факультет наук о жизни и окружающей среде, Политехнический университет Марке, Италия, 60100, Анкона, Пьяцца Рома, 22; отделение животных и водных наук, факультет сельского хозяйства, Университет Чиангмая, Таиланд, 50200, Чиангмай, Су Тхеп; e-mail: hossein.hoseinifar@gmail.com; ORCID: 0000-0002-0210-9013

Доан Хиен Ван — факультет наук о животных и водных животных, сельскохозяйственный факультет, Университет Чиангмая, Таиланд, 50200, Чиангмай, Су Тхеп

Ашури Гасем — факультет наук о жизни и окружающей среде, Политехнический университет Марке, Италия, 60100, Анкона, Пьяцца Рома, 22

RUDN Journal of Agronomy and Animal Industries
Вестник РУДН. Серия: АГРОНОМИЯ И ЖИВОТНОВОДСТВО
2019; 14(3):279—288
http://agrojournal.rudn.ru

Research article

DOI 10.22363/2312-797X-2019-14-3-279-288

LC50 determination and intoxication symptoms of a new pyridine carboxamide pesticide Flonicamid on common carp *Cyprinus carpio*

Melika Ghelichpour*[1,2], Ali T. Mirghaed[1,2], Amalia P. Jimenez[3,4]

[1]University of Tehran, *Tehran, Iran*

[2]Center for International Scientific Studies & Collaboration (CISSC), *Tehran, Iran*

[3]University of Granada, *Granada, Spain*

[4]University of Porto, *Porto, Portugal*

[*]*Corresponding author: m.ghelichpour@ut.ac.ir*

Abstract. The present study was undertaken to evaluate the lethal toxicity and stress signs of Flonicamid toward juvenile common carp (*Cyprinus carpio*). The 96 h LC50 values, determined 43.17 mgL^{-1} by probit analysis in a semi-static system. LC50 24, 48 and 72 h were 47.54, 41.83 and 43.51 mgL^{-1}, respectively. Behavioral changes include hyperexcitement, erratic swimming, dark coloration, loss of equilibrium and lethargy were observed with different intensities. Consequently, mortality rate, stress signs and behavioral changes observed in response to the Flonicamid are dependent to dose and time exposure.

Key words: Flonicamid, lethal concentration, *Cyprinus carpio*, intoxication symptoms, behavior

ACKNOWLEDGMENTS
This work has been supported the Center for International Scientific Studies & Collaboration (CISSC), Ministry of Science Research Technology.

Article history:
Received: 1 July 2019. Accepted: 1 August 2019

For citation:
Ghelichpour M, Mirghaed AT, Jimenez AP. LC50 Determination and intoxication symptoms of a new pyridine carboxamide pesticide Flonicamid on common carp *Cyprinus carpio*. *RUDN Journal of Agronomy and Animal Industries*, 2019; 14(3):279—288. doi: 10.22363/2312-797X-2019-14-3-279-288

Introduction

Flonicamid is the approved common name for the chemical composition, IKI220; N-cyanomethyl-4-trifluoromethyl nicotinamide. It is a novel systemic pyridine carboxamide pesticide discovered by Ishihara Sangyo Kaisha, Ltd (Tokyo, Japan). It is used for

controlling hemipterous pests, and thysanopterous pests that are resistant to other insecticides. It is a useful pesticide for the control of sucking pests such as aphids, whiteflies, trips, some micro lepidoptera, and a number of coleopteran species [1]. Flonicamid causes starvation in sucking insects via inhibit their feeding. The mechanism is stop feeding by blocking the ion channel. After spraying Flonicamid, although insects are able to attach to the plant, their salivation and sap feeding are limited or completely blocked [2].

The high rate of human population growth and the rapid pace of industrialization have caused the problem of sewage disposal. The domestic wastes and untreated or partially treated industrial sewages, may have pollutants like heavy metals, pesticides and many organic compounds, affect the fish and other aquatic organisms with several negative side effect on non-target organisms such as fish disease or death. Actually, it is a well-known fact that indiscriminate use of pesticides in agriculture has resulted in extensive distribution in the environment and also has a direct impact on non-target organisms [3].

Investigation the behavior is considered as a promising tool in ecotoxicology [4]. Behavior of toxicant exposed fish is the integrative measures of neurotoxicity and reflecting biochemical and physiological reactions to the toxicant. Also, behavior is a unified result of endogenous and exogenous processes and low level of exposures have been involved in various behavioral and physiological impairments [5]. Monitoring fish behavior which exposed to pesticides performed in several investigations [4, 6].

Using pesticides is common in the extensive agriculture farms of southern region of the Caspian Sea in Iran which located nearby the fish farms. Therefore, the fish in this region, either in the sea or fish farms are endangered by pesticides [4]. As Flonicamid has a favorable toxicological profile, with low toxicity to mammals, recently, it has been widely used as a safe insecticide in the agricultural farms of this region.

The present study has aimed to determine the lethal toxicity of Flonicamid and its effects on some behavioral changes of juvenile common carp (*Cyprinus carpio*). Common carp is a highly edible fish and preferred for culture due to its high growth rate and prolific breeding in confined water. Also, it is commercial and an important aquaculture fish species in the southern Caspian Sea. Common carp is cultured for food consumption and also restoration of fish stocks.

Materials and methods

Fish maintenance & pesticide preparation

Total number of 200 juvenile common carp with average weight and length of 48 ± 4.25 g and 11 ± 1.5 cm, respectively, were stored in a 2000 L capacity fiberglass tank. During two weeks acclimation, the fish were fed 1.5 % of the body weight with commercial carp feed, twice a day (Mazandaran Animal & Aquatic Feed Co., Sari, Mazandaran, Iran) and aerated continuously. 50 % water exchanging performed daily. Leftover food in the tank was removed daily when water of the tank was changed. Physicochemical properties of water were monitored during the experiment. Water temperature, dissolved oxygen, salinity, and pH were measured by Hach HQ40d

portable apparatus (Loveland, Colorado, USA). Also, photometer (Wagtech 7100, Berkshire, UK) was used to calculate total hardness, alkalinity, and calcium.

Behavior monitoring

The fish behaviors, clinical and apparent characteristics were monitored during Flonicamid exposure daily. Changes in fish swimming and coloration and any other apparent symptoms were reported for each group to compare the effect of concentration and time of Flonicamid exposure on experimental fish.

Determination of lethal concentration

150 common carp were distributed into 15 fiberglass tanks with 160 L volume. The tanks were considered as five groups for five different Flonicamid concentrations. Three replicate were taken for each concentration. 0 (control), 30, 40, 50 and 60 mg L^{-1} were determined according to the pre range finding test. The fish were exposed to the distinct concentrations of Flonicamid (with specifications in Table 1) for 96 h to determine LC50 values after 24, 48, 72 and 96 h. They were starved 24 h prior to exposing and during assay no food was given to the fish. The mortality was recorded in each group and dead fish were removed from tanks. Simultaneously renewed water, some amount of the pesticide were added into tanks to keep the concentration of the treatments, daily.

Table 1

Specifications of Flonicamid

Pesticide	CAS number	Supplier	Grade	Chemical name	Alternative name
Flonicamid	158062-67-0	Ishiharab Sangyo Kaisha, Ltd Tokyo, Japan	Commercial formulation (50 % WG)	IKI220; N-cyanomethyl-4-trifluoromethyl nicotinamide	Teppeki

Analysis

The fish behavior was monitored immediately after exposure to Flonicamid and continued every one hour in the first day. Then, the fish behavior was investigated three times a day in every 24 h up to 96 h after Flonicamid exposure. Data on mortality of fish were investigated according to time by probit method in statistical SPSS software (version 17). Based on probit analysis method, LC50 values and 95 % lower and upper confidence limits determined. In Finney's method, a regression equation and graphical interpolation are used to determine the LC50 value. To draw the graphical interpolation, logarithms of the chemical compound concentration and the probit value of percentage mortality are used on the *X* axis and *Y* axis, respectively. The 95 % confidence limits of the LC50 values were calculated by the formula of Mohapatra, Rengarajan [7] in Finney's method.

Results and discussion

Physicochemical characteristics of water in the experiment were checked daily to establish the environmental parameters. Table 2 shows these parameters.

Table 2

Physicochemical characteristics of water

Characteristics	Unit	Mean ± SD
Water temperature	°C	27.5 ± 1.25
pH	—	8.5 ± 0.25
Dissolved oxygen	mg L^{-1}	7.1 ± 0.84
Salinity	mg L^{-1}	2.63 ± 0.15
Electro conductivity	µs cm^{-1}	4630±55
Total hardness (as CaCO3)	mg L^{-1}	300 ± 17.5
Alkalinity (as CaCO3)	mg L^{-1}	350 ± 20.3
Calcium	mg L^{-1}	110 ± 11.7

Toxicity stress and behavioral and apparent physiological symptoms

Uncoordinated behavior was observed in fish exposed to different concentrations of Flonicamid. At the initial of exposure, the brownish gray powder of Flonicamid caused the water appearance foamy and cloudy. The fish stopped swimming with a swinging motion in their location in response to sudden changes in the surrounding environment. Then, to avoid the toxicant water, they swam to the water surface with hyper excitement behavior and fast swimming which seems this behavior was because of low oxygen conditions. Also, faster activity of opercula was observed as surfacing and gulping of air. After some time, the fish became slow with erratic swimming. Body pigmentation increased, therefore, the fish coloration became dark especially fins and tail. The darkish color increased gradually and expanded to other parts of the body. The fish exposed to higher concentration of Flonicamid showed a faster color exchange. Eventually, fish with lateral swimming lost their equilibrium and consciousness. Then, they stayed motionless on the bottom of tank and became exhausted and lethargic. Lastly, they died with open mouth and operculum. These alterations occurred so fast in fish exposed to higher Flonicamid concentration (50 and 60 mgL^{-1}) compared to the lower one (30 and 40 mgL^{-1}) which some behaviors were not obvious clearly in higher dose, such as erratic and slow swimming.

Lethal concentration of Flonicamid

Lethal concentration (LC50) is basically for acute toxicity. LC50 is the concentration of a toxicant chemical compound such as pesticide, which kills 50 % of the experimental organism in a specific period of time exposure, usually 96 h. According to Figure 1, by increasing the concentration of Flonicamid, the fish mortality rate has also increased, which indicates a direct relationship between mortality and concentration of the pesticide. LC50 of Flonicamid after 24, 48, 72 and 96 h exposure were determined 47.54, 41.83, 43.51 and 43.17 mgL^{-1}, respectively (Table 3). LC10-99 in each time exposure of Flonicamid is in Table 4.

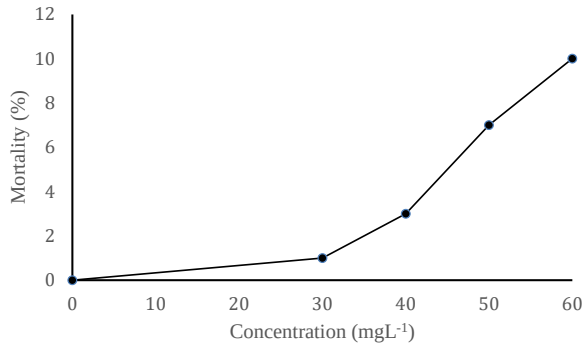


Fig. 1. Percentage mortality of *Cyprinus carpio* after 96 h exposure to different concentrations of Flonicamid

Table 3

Lethal concentration of Flonicamid (mgL⁻¹)

Duration (h)	LC50 values (mg L⁻¹)	95 % confidence limit		Slope ± SE	Intercept ± SE	Sig
		lower	upper			
24	47.54	42.35	52.67	25.512 ± 7.80	–42.78 ± 13.14	0.001
48	41.83	35.05	48.18	14.48 ± 4.38	–23.49 ± 7.18	0.001
72	43.51	38.88	48.09	28.37 ± 8.63	–46.49 ± 14.20	0.001
96	43.17	37.36	48.77	18.29 ± 5.36	–29.91 ± 8.83	0.001

Table 4

Lethal concentrations of Flonicamid (mgL–1) (95 % confidence intervals) depending on exposure time for Cyprinus carpio

Lethal concentration	Exposure time (h)			
	24	48	72	96
LC10	38.99 (27.68—43.38)	29.50 (16.34—35.16)	36.40 (26.71—40.24)	32.74 (21.17—31.70)
LC50	47.54 (42.35—52.67)	41.83 (35.05—48.18)	43.51 (38.88—48.09)	43.17 (37.36—48.77)
LC90	57.97 (52.40—79.09)	59.32 (50.69—97.89)	52 (47.28—68.80)	56.92 (50.01—83.17)
LC99	71.98 (60.94—133.71)	86.85 (65.73—244.75)	63.18 (54.30—109.63)	76.97 (61.73—165.86)

Some apparent physiological and behavioral alterations observed in juvenile common carp exposed to Flonicamid and they were obvious approximately 30 min after exposure to the highest concentration of Flonicamid (60 mgL^{-1}). The sequence of intoxication symptoms were initially stopped swimming with a swinging motion due to abrupt stress (it was clear in high Flonicamid concentrations), hyperactivity and irregular swimming, swimming near the water surface for oxygen uptake, erratic swimming, darkening the body color due to accumulation of pigments, lethargy because of energy consumption to against stress and death, ultimately. Some behavior alterations and intoxication symptoms observed in the previous studies. Jonsson, Toledo [8] reported excitation, erratic swimming with increase in respiratory frequency, swimming near the water surface for oxygen uptake, convulsion and hysteria in two species *Hyphessobrycon bifasciatus* and *Brachydanio rerio* exposed to acute toxicity of endosulfan. Joshi, Rege [9] also noticed similar symptoms in *Gambusiaaffinis* exposed to other organochlorine insecticides such as DDT and BHC and a few inorganic salts. The teleosts exposed to pyrethroids and some organophosphates showed behavior alterations and intoxication symptoms [10].

Hyperactivity and irregular movement are the symptoms related to the central nervous symptoms. Although the mecanism of action of Flonicamid is not comletely known in fish, this pesticide may interfere withthe connection of some neurotransmitters in specific receptors like their function in insects [8]. Alterations in the levels of intra- and extracellular sodium and potassium may be another reason to the hyperactivity and irregular movement in fish exposed to the pesticides [11]. The various behavioral changes such as abnormal swimming, jerks of body, loss of balance and anorexia are some indications observed in *Oreochromis niloticus* and *Chrysichthys auratus* exposed to atrazine, *Channa punctatus* exposed to mecuric chloride and malathion and *Heteropneutes fossilis* exposed to Malathion [12—14]. Fast swimming and jumping, faster opercula activity, erratic swimming, vigorous jerks of the body, mucus elevation, increased body pigmentation, loss of balance and consciousness, rolling movement, became exhausted and lethargic were the intoxication symptoms reported in *Channa punctatus* exposed to carbosulfan, glyphosate and atrazine [6].

Dark coloration due to pesticides exposure is a symptom which reported in previous studies like *Cyprinus carpio* exposet to indoxacarb, *Salmo salar* and *Oncorhynchus mykiss* exposed to emamectin benzoate [15, 16]. Dark coloration of fish exposed to Flonicamid might be as a result of interference of pesticide in melanophore aggregation due to cortisol hormone elevation in the stressful condition. According to Nunes, Gaio, Carvalho, Guilhermino [17] different factors may affect dark pigmentation in fish for example, capture, environmental stress, and as a defense mechanism when they feel threatened. Increasing the intensity of fish pigmentation associates with stress responsiveness, since fish subjected to stressful conditions [18]. Common carp to 2,4-Dichlorophenoxyacetic acid [19] showed light coloration. Light coloration may due to mucus hypersecretion.

Acute toxicity data has been used to determine water quality guidelines. The results of the LC50 (median lethal concentration) of the present study at 96 h were 43.17 mgL^{-1} for Flonicamid. Therefore, it seems Flonicamid may be the moderate toxic substance for juvenile common carp. Toxicity of the pesticides was both time and concentration dependent, thus accounting for differences in LC10-99 values attained at different times and concentrations of exposure. There is no report on Flonicamid LC50 in any fish species. Therefore, may be further investigations show different data even in the common carp. Because toxicity of chemicals to aquatic organisms has been shown to be affected by age, size, health of the species and also, physiological parameters like temperature, pH, dissolved oxygen and turbidity of water and concentration and formulation of chemical [20].

Conclusions

The result of the present study showed that Flonicamid as a new pesticide has adverse effects on behavioral and some apparent physiological parameters of common carp. Initially swinging motion, hyperexcitement, erratic swimming, dark coloration, loss of equilibrium and lethargy are the intoxication symptoms due to Flonicamid exposure. The symptoms are related to the time and concentration of Flonicamid exposure as other pesticides. Calculating the lethal concentration of Flonicamid in different times and concentrations indicate that pesticide toxicity depends on different parameters.

References

1. Cho SR, Koo HN, Yoon C, Kim GH. Sublethal effects of Flonicamid and thiamethoxam on green peach aphid, Myzus persicae and feeding behavior analysis. *Journal of the Korean Society for Applied Biological Chemistry*. 2011; 54(6):889—898. doi: 10.1007/BF03253177
2. Morita M, Ueda T, Yoneda T, Koyanagi T, Haga T. Flonicamid, a novel insecticide with a rapid inhibitory effect on aphid feeding. *Pest Management Science: formerly Pesticide Science*. 2007; 63(10):969—973. doi: 10.1002/ps.1423
3. Pazhanisamy K, Indra N. Toxic effects of arsenic on protein content in the fish, Labeo rohita (Hamilton). *Nature, Environment and Pollution Technology*. 2007; 6(1):113—116.
4. Mirghaed AT, Ghelichpour M. Determination of lethal concentrations of indoxacarb in fingerling Cyprinus carpio at two temperatures. *International journal of aquatic biology*. 2015; 3(5):339—345. doi: 10.22034/ijab.v3i5.113
5. Sandahl JF, Baldwin DH, Jenkins JJ, Scholz NL. Comparative thresholds for acetylcholinesterase inhibition and behavioral impairment in coho salmon exposed to chlorpyrifos. *Environmental Toxicology and Chemistry: An International Journal*. 2005; 24(1):136—145. doi: 10.1897/04-195R.1
6. Nwani CD, Nagpure NS, Kumar R, Kushwaha B, Kumar P, Lakra WS. Lethal concentration and toxicity stress of Carbosulfan, Glyphosate and Atrazine to freshwater air breathing fish Channa punctatus (Bloch). *International Aquatic Research*. 2010; 2:105—111.
7. Mohapatra B, Rengarajan K. Manual on bioassays in the laboratory and their techniques. *CMFRI special publication*. 1995; 64:1—75.
8. Jonsson CM, Toledo MCF. Acute toxicity of endosulfan to the fish Hyphessobrycon bifasciatus and Brachydanio rerio. *Archives of Environmental Contamination and Toxicology*. 1993; 24(2):151—155. Available from: doi: 10.1007/BF01141341
9. Joshi A, Rege M. Acute toxicity of some pesticides and a few inorganic salts to the mosquito fish Gambusia affinis (Baird & Girard). *Indian Journal of Experimental Biology*. 1980; 18(4):435—437.
10. Naqvi SM, Hawkins R. Toxicity of selected insecticides (Thiodan®, Security®, Spartan®, and Sevin®) to mosquitofish, Gambusia alfinis. *Bulletin of Environmental Contamination and Toxicology*. 1988; 40(5):779—784. doi: 10.1007/BF01697530
11. Swarup PA, Rao DM, Murty A. Toxicity of endosulfan to the freshwater fish Cirrhinus mrigala. *Bulletin of Environmental Contamination and Toxicology*. 1981; 27(1):850—855. doi: 10.1007/BF01611106
12. Hussein S, El-Nasser M, Ahmed S. Comparative studies on the effects of herbicide atrazine on freshwater fish Oreochromis niloticus and Chrysichthyes auratus at Assiut, Egypt. *Bulletin of Environmental Contamination and Toxicology*. 1996; 57(3):503—510. doi: 10.1007/s001289900218
13. Pandey S, Kumar R, Sharma S, Nagpure N, Srivastava SK, Verma M. Acute toxicity bioassays of mercuric chloride and malathion on air-breathing fish Channa punctatus (Bloch). *Ecotoxicology and Environmental Safety*. 2005; 61(1):114—120. doi: 10.1016/j.ecoenv.2004.08.004
14. Chandra S. Toxic effect of Malathion on acetylcholinesterase activity of liver, brain and gills of freshwater catfish Heteropneutes fossilis. *Environ. Conserv*. 2008; 9:45—52.
15. Taheri Mirghaed A, Ghelichpour M, Mirzargar SS, Joshaghani H, Ebrahimzadeh Mousavi H. Toxic effects of indoxacarb on gill and kidney histopathology and biochemical indicators in common carp (Cyprinus carpio). *Aquaculture Research*. 2018; 49(4):1616—1627. doi: 10.1111/are.13617
16. Roy W, Sutherland I, Rodger H, Varma K. Tolerance of Atlantic salmon, Salmo salar L., and rainbow trout, Oncorhynchus mykiss (Walbaum), to emamectin benzoate, a new orally administered treatment for sea lice. *Aquaculture*. 2000; 184(1-2):19—29. doi: 10.1016/S0044-8486(99)00307-5
17. Nunes B, Gaio A, Carvalho F, Guilhermino L. Behaviour and biomarkers of oxidative stress in Gambusia holbrooki after acute exposure to widely used pharmaceuticals and a detergent.

Ecotoxicology and Environmental Safety. 2008; 71(2):341—354. doi: 10.1016/j.ecoenv.2007.12.006
18. Backström T, Brännäs E, Nilsson J, Magnhagen C. Behaviour, physiology and carotenoid pigmentation in Arctic *charr Salvelinus alpinus*. *Journal of Fish Biology*. 2014; 84(1):1—9. doi: 10.1111/jfb.12240
19. Sarikaya R, Yılmaz M. Investigation of acute toxicity and the effect of 2, 4-D (2, 4-dichlorophenoxyacetic acid) herbicide on the behavior of the common carp (Cyprinus carpio L., 1758; Pisces, Cyprinidae). *Chemosphere*. 2003; 52(1):195—201. doi: 10.1016/S0045-6535(03)00106-1
20. Farah MA, Ateeq B, Ali MN, Sabir R, Ahmad W. Studies on lethal concentrations and toxicity stress of some xenobiotics on aquatic organisms. *Chemosphere*. 2004; 55(2):257—265. doi: 10.1016/j.chemosphere.2003.10.063

About authors:
Ghelichpour Melika — Department of Aquatic Animal Health, Faculty of Veterinary Medicine, University of Tehran, 16th Azar St., Enghelab Sq., Tehran, Iran, 14174; Center for International Scientific Studies & Collaboration (CISSC), Ministry of Science Research Technology, Tehran, Iran, 15875-7788; e-mail: m.ghelichpour@ut.ac.ir; SCOPUS ID: 39961248800; ORCID: 0000-0002-8338-4235

Mirghaed Ali Taheri — Department of Aquatic Animal Health, Faculty of Veterinary Medicine, University of Tehran, 16th Azar St., Enghelab Sq., Tehran, Iran, 14174; Center for International Scientific Studies & Collaboration (CISSC), Ministry of Science Research Technology, Tehran, Iran, 15875-7788; SCOPUS ID: 56158853500

Jimenez Amalia Perez — Department of Zoology, University of Granada, Campus of Fuentenueva, Granada, Spain; Acera de San Ildefonso, Granada, Spain, 18071; Interdisciplinary Center for Marine and Environmental Research (CIIMAR), University of Porto, Porto, Portugal, 4099-002; SCOPUS ID: 7202356622

Научная статья

DOI 10.22363/2312-797X-2019-14-3-279-288

Определение средней летальной концентрации и симптомов интоксикации нового пиридинкарбоксамидного пестицида флоникамида на карпа *Cyprinus carpio*

М. Геличпур*[1, 2], А.Т. Миргаед[1, 2], А.П. Хименес[3, 4]

[1]Тегеранский университет, *Тегеран, Иран*

[2]Центр международных научных исследований и сотрудничества (CISSC), *Тегеран, Иран*

[3]Университет Гранады, *Гранада, Испания,*

[4]Университет Порту, *Порту, Португалия*

**m.ghelichpour@ut.ac.ir*

Аннотация. Настоящее исследование было проведено для оценки летальной токсичности и определения симптомов интоксикации флоникамида по отношению к молодому карпу (*Cyprinus carpio*). Средняя летальная концентрация за 96 ч отмечается при 43,17 мг/л с помощью пробит-анализа в полустатической системе. Средняя летальная концентрация через 24, 48 и 72 ч составили 47,54, 41,83

и 43,51 мг/л соответственно. Среди поведенческих изменений преобладали гипервозбуждение, неустойчивое плавание, темная окраска, потеря равновесия и летаргия, которые наблюдались с различной интенсивностью. Следовательно, уровень смертности, стрессовые признаки и поведенческие изменения, наблюдаемые в ответ на прием флоникамида, зависят от дозы и времени воздействия препарата.

Ключевые слова: флоникамид, летальная концентрация, *Cyprinus carpio*, симптомы интоксикации, поведение

БЛАГОДАРНОСТИ
Данное исследование было проведено при поддержке Центра международных научных исследований и сотрудничества (CISSC) Министерства науки, исследований и технологий Ирана.

История статьи:
Поступила в редакцию: 1 июля 2019 г. Принята к публикации: 1 августа 2019 г.

Для цитирования:
Ghelichpour M., Mirghaed A.T., Jimenez A.P. LC50 Determination and intoxication symptoms of a new pyridine carboxamide pesticide Flonicamid on common carp *Cyprinus Carpio* // Вестник Российского университета дружбы народов. Серия: Агрономия и животноводство. 2019. Т. 14. № 3. С. 279—288. doi: 10.22363/2312-797X-2019-14-3-279-288

Библиографический список

1. *Cho S.R., Koo H.N., Yoon C., Kim G.H.* Sublethal effects of Flonicamid and thiamethoxam on green peach aphid, Myzus persicae and feeding behavior analysis // Journal of the Korean Society for Applied Biological Chemistry. 2011. Vol. 54. № 6. P. 889—898. doi: 10.1007/BF03253177
2. *Morita M., Ueda T., Yoneda T., Koyanagi T., Haga T.* Flonicamid, a novel insecticide with a rapid inhibitory effect on aphid feeding // Pest Management Science: formerly Pesticide Science. 2007. Vol. 63. № 10. P. 969—973. doi: 10.1002/ps.1423
3. *Pazhanisamy K., Indra N.* Toxic effects of arsenic on protein content in the fish, Labeo rohita (Hamilton) // Nature, Environment and Pollution Technology. Vol. 2007. Vol. 6. № 1. P. 113—116.
4. *Mirghaed A.T., Ghelichpour M.* Determination of lethal concentrations of indoxacarb in fingerling Cyprinus carpio at two temperatures // International journal of aquatic biology. 2015. Vol. 3. № 5. P. 339—345. doi: 10.22034/ijab.v3i5.113
5. *Sandahl J.F., Baldwin D.H., Jenkins J.J., Scholz N.L.* Comparative thresholds for acetylcholinesterase inhibition and behavioral impairment in coho salmon exposed to chlorpyrifos // Environmental Toxicology and Chemistry: An International Journal. 2005. Vol. 24. № 1. P. 136—145. doi: 10.1897/04-195R.1
6. *Nwani C.D., Nagpure N.S., Kumar R., Kushwaha B., Kumar P., Lakra W.S.* Lethal concentration and toxicity stress of Carbosulfan, Glyphosate and Atrazine to freshwater air breathing fish Channa punctatus (Bloch) // International Aquatic Research. 2010. № 2. P. 105—111.
7. *Mohapatra B., Rengarajan K.* Manual on bioassays in the laboratory and their techniques // CMFRI special publication. 1995. № 64. P. 1—75.
8. *Jonsson C.M., Toledo M.C.F.* Acute toxicity of endosulfan to the fish Hyphessobrycon bifasciatus and Brachydanio rerio // Archives of Environmental Contamination and Toxicology. 1993. Vol. 24. № 2. P. 151—155. doi: 10.1007/BF01141341
9. *Joshi A., Rege M.* Acute toxicity of some pesticides and a few inorganic salts to the mosquito fish Gambusia affinis (Baird & Girard) // Indian Journal of Experimental Biology. 1980. Vol. 18. № 4. P. 435—437.
10. *Naqvi S.M., Hawkins R.* Toxicity of selected insecticides (Thiodan®, Security®, Spartan®, and Sevin®) to mosquitofish, Gambusia alfinis // Bulletin of Environmental Contamination and Toxicology. 1988. Vol. 40. № 5. P. 779—784. doi: 10.1007/BF01697530

11. *Swarup P.A., Rao D.M., Murty A.* Toxicity of endosulfan to the freshwater fish Cirrhinus mrigala // Bulletin of Environmental Contamination and Toxicology. 1981. Vol. 27. № 1. P. 850—855. doi: 10.1007/BF01611106
12. *Hussein S., El-Nasser M., Ahmed S.* Comparative studies on the effects of herbicide atrazine on freshwater fish Oreochromis niloticus and Chrysichthyes auratus at Assiut, Egypt // Bulletin of Environmental Contamination and Toxicology. 1996. Vol. 57. № 3. P. 503—510. doi: 10.1007/s001289900218
13. *Pandey S., Kumar R., Sharma S., Nagpure N., Srivastava S.K., Verma M.* Acute toxicity bioassays of mercuric chloride and malathion on air-breathing fish Channa punctatus (Bloch) // Ecotoxicology and Environmental Safety. 2005. Vol. 61. № 1. P. 114—120. doi: 10.1016/j.ecoenv.2004.08.004
14. *Chandra S.* Toxic effect of Malathion on acetylcholinesterase activity of liver, brain and gills of freshwater catfish Heteropneutes fossilis // Environ. Conserv. 2008. № 9. P. 45—52.
15. *Taheri Mirghaed A., Ghelichpour M., Mirzargar S.S., Joshaghani H., Ebrahimzadeh Mousavi H.* Toxic effects of indoxacarb on gill and kidney histopathology and biochemical indicators in common carp (Cyprinus carpio) // Aquaculture Research. 2018. Vol. 49. № 4. P. 1616—1627. doi: 10.1111/are.13617
16. *Roy W., Sutherland I., Rodger H., Varma K.* Tolerance of Atlantic salmon, Salmo salar L., and rainbow trout, Oncorhynchus mykiss (Walbaum), to emamectin benzoate, a new orally administered treatment for sea lice // Aquaculture. 2000. Vol. 184. № 1-2. P. 19—29. doi: 10.1016/S0044-8486(99)00307-5
17. *Nunes B., Gaio A., Carvalho F., Guilhermino L.* Behaviour and biomarkers of oxidative stress in Gambusia holbrooki after acute exposure to widely used pharmaceuticals and a detergent // Ecotoxicology and Environmental Safety. 2008. Vol. 71. № 2. P. 341—354. doi: 10.1016/j.ecoenv.2007.12.006
18. *Backström T., Brännäs E., Nilsson J., Magnhagen C.* Behaviour, physiology and carotenoid pigmentation in Arctic *charr Salvelinus alpinus* // Journal of Fish Biology. 2014. Vol. 84. № 1. P. 1—9. doi: 10.1111/jfb.12240
19. *Sarikaya R., Yılmaz M.* Investigation of acute toxicity and the effect of 2, 4-D (2, 4-dichlorophenoxyacetic acid) herbicide on the behavior of the common carp (Cyprinus carpio L., 1758; Pisces, Cyprinidae) // Chemosphere. 2003. Vol. 52. № 1. P. 195—201. doi: 10.1016/S0045-6535(03)00106-1
20. *Farah M.A., Ateeq B., Ali M.N., Sabir R., Ahmad W.* Studies on lethal concentrations and toxicity stress of some xenobiotics on aquatic organisms // Chemosphere. 2004. Vol. 55. № 2. P. 257—265. doi: 10.1016/j.chemosphere.2003.10.063

Об авторах:

Геличпур Мелика — кафедра здоровья водных животных, факультет ветеринарной медицины, Тегеранский университет, Иран, 14174, Тегеран, Енгхелаб пл., ул. Азар, 16; Центр международных научных исследований и сотрудничества (CISSC), Министерство науки, технологии исследований, Иран, 15875-7788, Тегеран; электронная почта: m.ghelichpour@ut.ac.ir; Scopus ID: 39961248800; ORCID: 0000-0002-8338-4235

Миргаед Али Тахери — кафедра здоровья водных животных, факультет ветеринарной медицины, Тегеранский университет, Иран, 14174, Тегеран, Енгхелаб пл., ул. Азар, 16; Центр международных научных исследований и сотрудничества (CISSC), Министерство науки, технологии исследований, Иран, 15875-7788, Тегеран; Scopus ID: 56158853500

Хименес Амалия Перес — факультет зоологии, Университет Гранады, кампус Фуэнтенуэва, Испания, 18071; Гранада, Ацера де Сан Илдефонсо; Междисциплинарный центр морских и экологических исследований (CIIMAR), Университет Порту, Португалия, 4099-002; Порту; Scopus ID: 7202356622

ДЛЯ ЗАМЕТОК

ДЛЯ ЗАМЕТОК