ISSN:2148-6905

INTERNATIONAL JOURNAL OF SECONDARY METABOLITE

www.ijate.net/index.php/ijsm

IJSM

VOLUME 3
2016
NO: 2

INTERNATIONAL JOURNAL OF SECONDARY METABOLITE

ISSN:2148-6905

www.ijate.net/index.php/ijsm

IJSM

Prof. Dr. Ramazan MAMMADOV
Editor
International Journal of Secondary Metabolite Chief Editor

Pamukkale Universitiy,
Art & Science Faculty,
Department of Biology,
20070,
Turkey

Phone: +90 258 296 3575
Fax: +90 258 296 3535
e-Mail: ijsm.editor@gmail.com

Support Contact
Prof. Dr. Izzet KARA
Journal Manager & Founding Editor

Phone: +90 258 296 1036
e-Mail: ikara@pau.edu.tr
ISSN:2148-6905

Year: 2016
Volume: 3
No: 2

ISSN: 2148-6905

Table of Contents

ISSN: 2148-6905

INTERNATIONAL JOURNAL OF SECONDARY METABOLITE

Int. J. Sec. Metabolite, Vol. 3, Issue 2 (2016) pp. 49-57

Research Article

ISSN: 2148-6905 online Journal homepage: http://www.ijate.net/index.php/ijsm

The content of phenolic compounds in the seedlings of triticale *in vitro* at infectious stress

Natalya Osokina[1], Elena Kalasnikova[1,*]

[1] Russian State Agrarian University - MTAA, Russian Federation

Received: 02 December 2015 - Revised: 09 February 2016 - Accepted: 19 February 2016

Abstract: The study analyzes the influence of growth regulators on the development of seedlings triticale sufficed *Fusarium*. The triticale is a valuable crop as the grain and green mass is used for feeding of farm animals. For the study the species of Fusarium fungi were used, which are often occurs found on the crops of our region, they are: F. culmorum, F. sporotrichioides, F. oxysporum and F. avenaceum. The research was focused on their correlation with different concentrations of growth regulators dissolved in nutrient medium. These drugs are environmentally friendly, which is very important when using triticale for human nutrition and animal feed. The experiments showed that concentrations in some medications could have an inhibitory effect on the development of the fungi and inhibit the development of *Fusarium*. First culture for triticale it is shown that in seedlings, cultivated in vitro, increases the total content of soluble phenolic compounds in response to infectious stress when used as the drug "Immunocytophite" and arachidonic acid of plant origin. It is established that the phenolic compounds varies depending on the concentration of the drug and the studied accessions.

Key words: Growth Regulators, Arachidonic Acid, Triticale, Fusarium, *In Vitro.*

Содержание фенольных соединений в проростках тритикале *in vitro* при инфекционном стрессе

Abstract: В наши дни для борьбы с грибными болезнями, которые значительно снижают урожайность и качество сельскохозяйственной продукции значимых культур, широко приме няют химические препараты. Альтернативным решением этой проблемы может стать применение регуляторов роста. В работе изучали грибы рода Fusarium: F. culmorum, F. sporotrichioides, F. oxysporum и F. avenaceum. В качестве регуляторов роста применяли препараты Иммуноцитофит, арахидоновая кислота на основе морских водорослей. Установлено, что препараты Иммуноцитофит (в концентрации 7,5 мл/л) и арахидоновая кислота на основе морских водорослей (в концентрации 1мл/л) оказывают ингибирующее действие на развитие поверхностного мицелия всех исследуемых патогенов. В вариантах совместного культивирования патогена и зерновок тритикале действие регуляторов роста усиливается. Впервые для культуры тритикале показано, что в проростках, культивируемых *in vitro*, повышается суммарное содержание растворимых фенольных соединений в ответ на инфекционный стресс при использовании как Иммуноцитофита, так и арахидоновой кислоты растительного происхождения. Установлено, что образование фенольных соединений изменяется в зависимости от концентрации препарата и изучаемого сортообразца.

Ключевые слова: регуляторы роста, иммуноцитофит, арахидоновая кислота, тритикале, фузариоз, *in vitro.*

[*]Corresponding Author E-mail: kalash0407@mail.ru natali5-13@mail.ru

1. Введение

Основной целью селекционных программ в настоящее время является повышение урожайности сельскохозяйственных культур, создание новых сортов и гибридов, обладающих улучшенными качествами продукта, комплексной устойчивостью к болезням, вредителям и стрессовым факторам окружающей среды [1, 2].

Зерновые культуры вносят наибольший вклад в обеспечение населения земного шара продуктами питания. Среди этих культур особое место отводится амфидиплоиду тритикале, который совмещает ценные качества родительских форм – пшеницы и ржи. Тритикале привлекает к себе внимание в связи с тем, что по ряду таких важнейших показателей, как урожайность, питательная ценность продукта и другие, эта культура способна во многих сельскохозяйственных районах мира превосходить обоих родителей. Однако данная культура часто подвергается различным инфекционным заболеваниям, в частности, семена, а также надземная часть растений поражаются грибами рода *Fusarium* L. [3, 4].

Для повышения устойчивости растений к болезням, как правило, в промышленных посевах широко применяют опасные для окружающей среды фунгициды. Одной из альтернативных и эффективных мер по увеличению невосприимчивости зерновых культур к возбудителям болезни с экологической и практической точек зрения, является использование регуляторов роста. Данное направление исследований, прежде всего, позволит снизить уровень фунгицидной нагрузки на окружающую среду. По литературным данным известно, что регуляторы роста в определённых концентрациях оказывают влияние на устойчивость растений к грибным болезням разного вида, в частности фузариозу. Эти вещества способны ингибировать развитие инфекции и ускорять ответную реакцию растения на действие патогена. Ответом на патоген является повышение в их тканях, например, содержания фенольных соединений, которые относятся к защитному механизму растений. Известно, что фенольные соединения являются вторичными метаболитами растения, а также принимают участие во многих биохимических процессах. Эти соединения относятся также и к стрессовым метаболитам, синтез которых резко возрастает при поранении или поражении растения, что предполагает их участие в комплексной защитной реакции [5, 6].

Исходя из выше изложенного, следует заключить, что исследования в этом направлении являются актуальными, а полученные результаты могут иметь как практическое, так и теоретическое значение. Особую актуальность такие исследования представляют для культуры тритикале, так как в доступной для нас литературе работы по применению регуляторов роста при борьбе с фузариозом отсутствуют.

2. Материалы и методы

Для эксперимента было выбрано три сортообразца тритикале (Укро, Дублет, С95) из коллекции кафедры генетики, биотехнологии, селекции и семеноводства Российского государственного аграрного университета-МСХА имени К.А. Тимирязева. Сортообразцы отличались между собой по восприимчивости к грибным болезням и устойчивостью к фузариозу.

В эксперименте были выбраны два регулятора роста, отличающиеся по своему спектру действия: Иммуноцитофит (этиловый эфир арахидоновой кислоты), Арахидоновая кислота (на основе морских водорослей).

Иммуноцитофит – регулятор роста, обладающий ростовой и антистрессовой активностью. Действующее вещество - этиловый эфир арахидоновой кислоты (этиларахидонат).

Арахидоновая кислота растительного происхождения (АК) – омега-6 полиненасыщенная незаменимая жирная кислота, которая является составной частью витамина F. Это вещество природного происхождения, активно участвует в регуляции функционирования клеточных мембран и играет важную роль в метаболических процессах. АК растительного происхождения массово получают их морской одноклеточной водоросли *Porphyridium.*

Исследуемые препараты изучали в следующих концентрациях: Иммуноцитофит - 7,5 мл/л, арахидоновая кислота на основе морских водорослей – 1 мл/л. Регуляторы роста добавляли в состав питательной среды, на которой в дальнейшем осуществляли совместное культивирование патогена и зерновки. В качестве контроля использовали вариант, в котором зерновки культивировали на безгормональной питательной среде и без присутствия патогена.

В качестве стрессового фактора были выбраны разновидности грибов рода *Fusarium* L.: *Fusarium culmorum, Fusarium avenaceum, Fusarium oxysporum* и *Fusarium sporotrichioides*. Размножение чистой культуры патогенов для получения достаточного количества инокулюма проводили на безгормональной агаризованной питательной среде, содержащей ½ концентрации минеральных солей по прописи Мурасига и Скуга (МС ½) [7]. Грибы выращивали в условиях световой комнаты при температуре 25^0C, 16-часовом фотопериоде, при интенсивности света 3000 лк (рис. 1).

Рисунок 1. Размножение чистой культуры гриба *Fusarium* L. в условиях световой комнаты

Совместное культивирование зерновок тритикале с мицелием исследуемых грибов проводили следующим образом: в центр чашки Петри помещали патоген (0,5×0,5 см), вокруг которого на расстоянии 4 см. располагали зерновки, которые предварительно поверхностно стерилизовали раствором сулемы (0,1%) в течение 10 мин., после чего их трижды промывали стерильной дистиллированной водой (рис.2).

В качестве показателя активизирования защитных функций растения при стрессовых условиях изучали изменение содержания фенольных соединений в проростках тритикале. Для этого из полученных проростков был приготовлен растительный экстракт. Сырую массу надземной части (200-400 мг) проростков растирали в 7 мл 96%-ного этанола до однородной массы и оставляли при комнатной

температуре в тёмном месте на сутки. По истечении времени экстракции образец фильтровали через фильтровальную бумагу в пробирки (рис. 3).

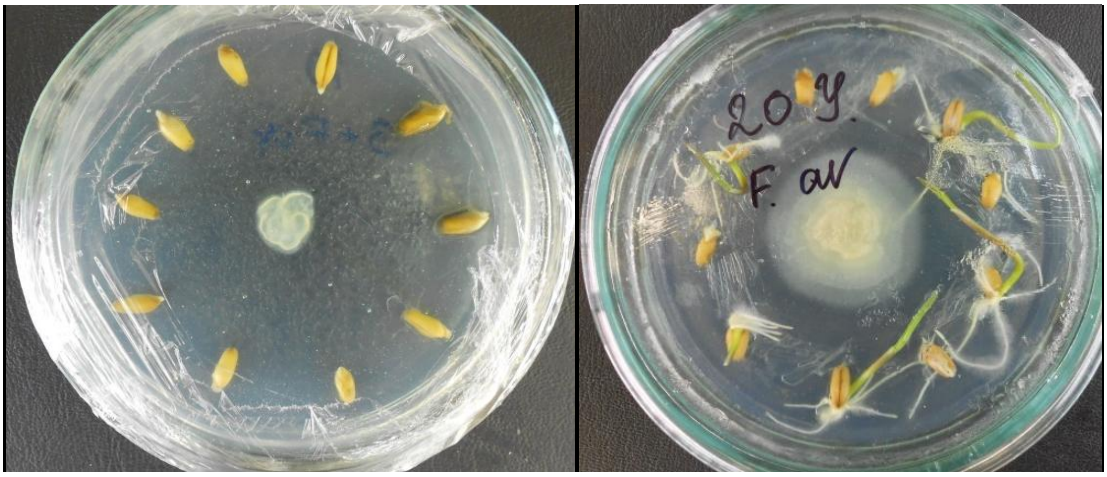

Рисунок 2. Совместное культивирование грибов рода *Fusarium* L. и зерновок тритикале на питательной среде с добавлением регуляторов роста

А Б В Г

Рисунок 3. Получение экстрактов из молодых проростков: А – исходный материал, Б – гомогенизирование растительного материала, В– экстрагирование, Г– готовый экстракт

Определение суммарного содержания фенольных соединений в полученных экстрактах проводили по следующей методике: в каждую пробирку добавляли по 3 мл дистиллированной воды, после чего добавляли 0,5 мл полученного экстракта и 1 мл содового раствора. Полученный раствор интенсивно перемешивали и добавляли 0,5 мл реактива Фолина-Дениса. Перед определением суммарного содержания фенольных соединений раствор перемешивали.

Содержание фенольных соединений в экстрактах определяли спектрофотометрическим методом при длине волны 725 нм. Далее по формуле рассчитывали суммарное содержание фенольных соединений в исходном образце:

$$\frac{\text{Е } 725 * \text{К}(89) * \text{Vобщ.}}{0{,}5 * m} = \text{Сфс}$$

Где Е- показания спектрофотометра

К=89 (const)

Vобщ.- общий объём образца

m- масса растительной навески для экстракта

Эксперименты проводил в трех биологических и 5 аналитических повторностях. На графиках и диаграммах представлены средние арифметические значения определений и их стандартные отклонения.

3. Результаты и обсуждение

В результате проведенных исследований нами были выявлены некоторые особенностей и корреляционные зависимости: 1) во всех вариантах выявлена тенденция увеличения содержания фенольных соединений в проростках, полученных на средах содержащих регуляторы роста и патоген; 2) этот эффект сильнее проявлялся при применении препарата Иммуноцитофит (рис. 4-6). Возможно, это объясняется содержанием в составе Иммуноцитофита помимо основного действующего вещества и вспомогательных веществ, которые способствуют его эффективности.

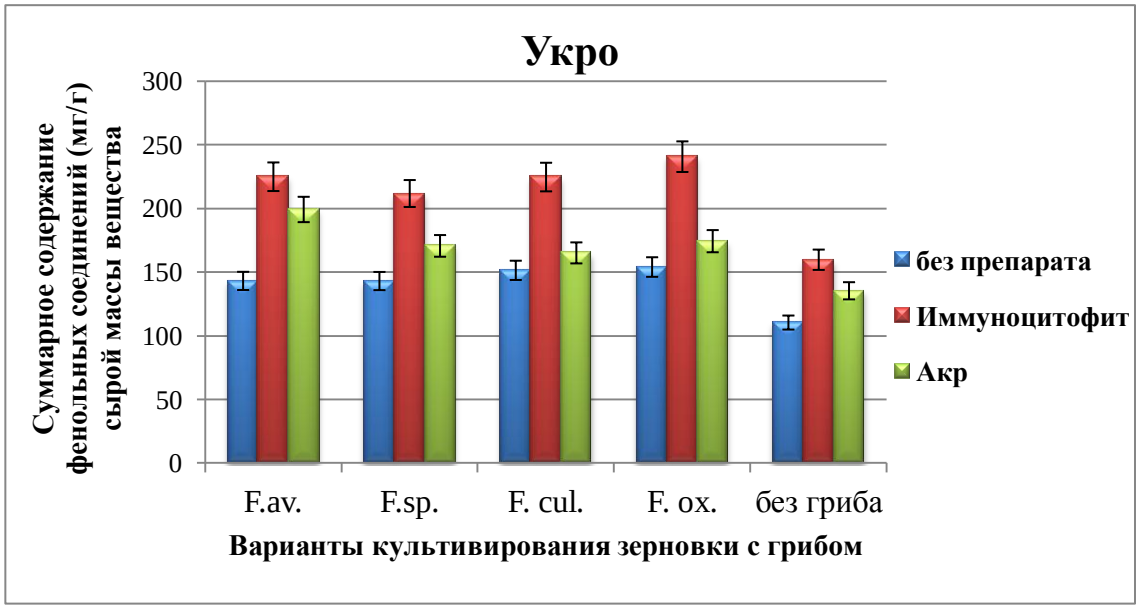

Рисунок 4. Суммарное содержание фенольных соединений в 7-суточных проростках тритикале сорта Укро

F. av. – Fusarium avenaceum

F.sp. – Fusarium sporotrichioides

F. cul. – Fusarium culmorum

F. ox. – Fusarium oxysporum

Акр - арахидоновая кислота растительного происхождения

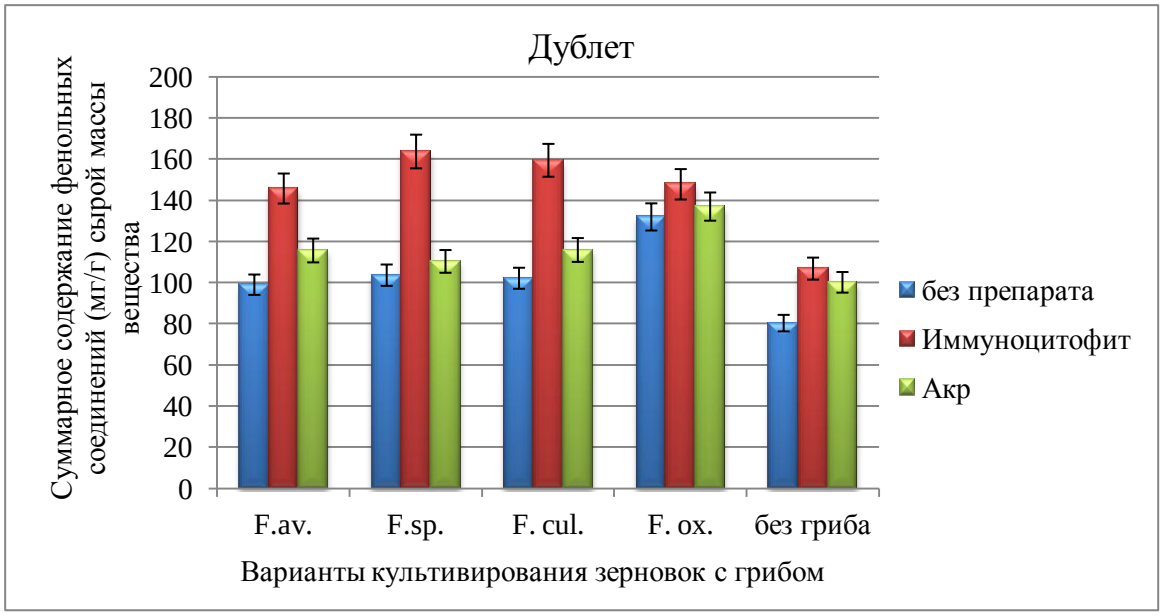

Рисунок 5. Суммарное содержание фенольных соединений в 7-суточных проростках тритикале сорта Дублет

F. av. – Fusarium avenaceum
F.sp. – Fusarium sporotrichioides
F. cul. – Fusarium culmorum
F. ox. – Fusarium oxysporum
Акр - арахидоновая кислота растительного происхождения

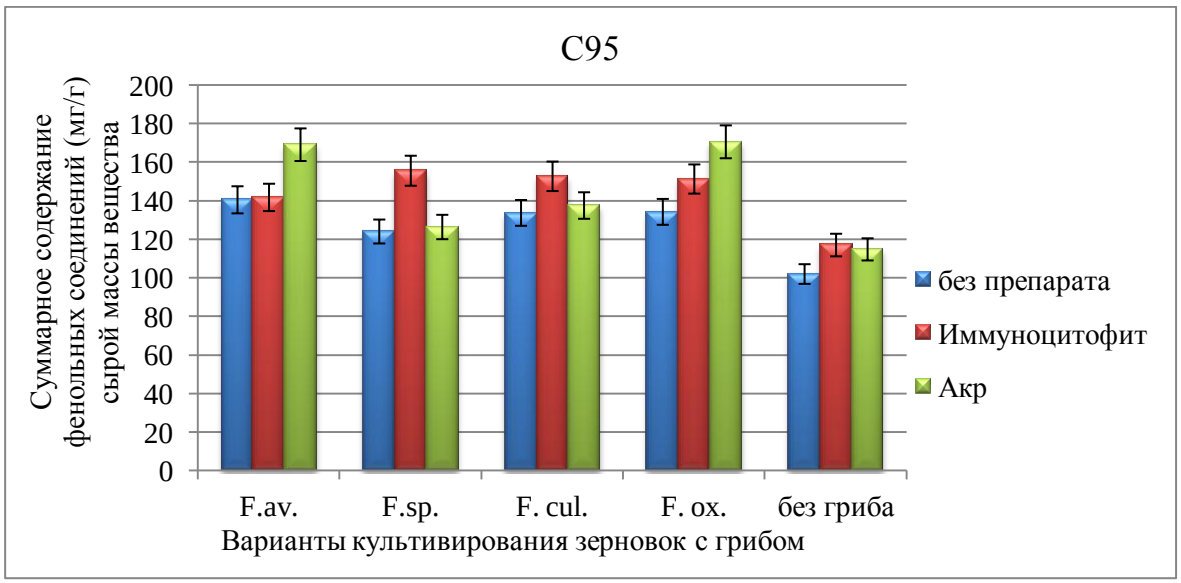

Рисунок 6. Суммарное содержание фенольных соединений в 7-суточных проростках тритикале сортообразца С95

F. av. – Fusarium avenaceum
F.sp. – Fusarium sporotrichioides
F. cul. – Fusarium culmorum
F. ox. – Fusarium oxysporum
Акр - арахидоновая кислота растительного происхождения

Согласно литературным данным, полученным на зерновых культурах, устойчивость растений к патогенам часто коррелирует с содержанием в них фенольных соединений [8]. Для наших исследований были выбраны сортообразцы тритикале, характеризующиеся хорошей устойчивостью к фузариозу – Укро, Дублет и С95. Однако, между собой они отличаются по невосприимчивости к данному заболеванию и располагаются в следующем порядке: Укро - невосприимчивый, С95 – средневосприимчивый и Дублет – восприимчивый. В наших исследованиях было установлено, что проростки тритикале сорта Укро во всех вариантах характеризовались более высоким уровнем накопления фенольных соединений, по сравнению с остальными сортообразцами. Меньше всего фенольных соединений было обнаружено у проростков сорта Дублет, который из представленных образцов характеризовался как самый восприимчивый к фузариозу (рис. 7). Таким образом, полученные в ходе биохимического анализа данные, подтверждают теорию о том, что в устойчивых к болезням сортах растений суммарное содержание фенольных соединений превышает те же показатели восприимчивых сортов.

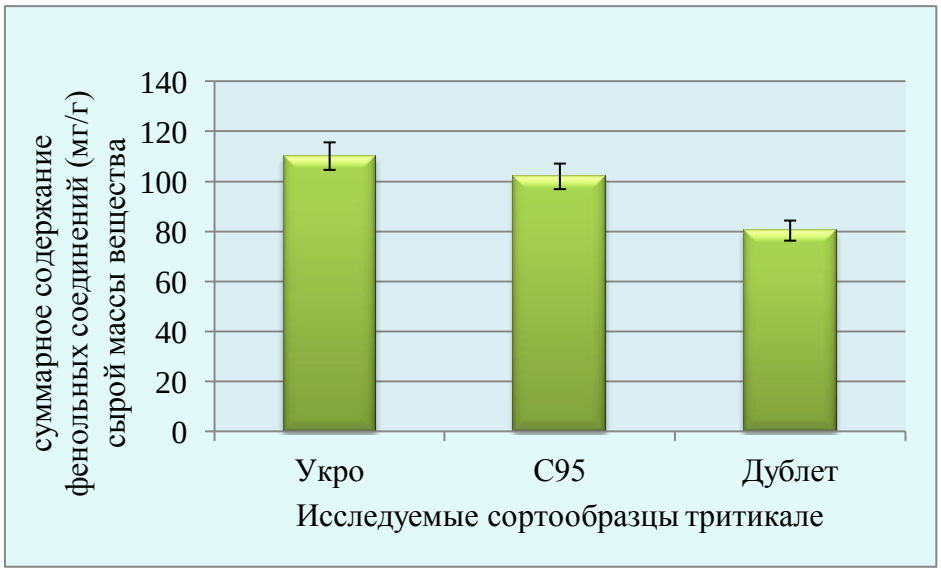

Рисунок 7. Средний показатель содержания фенольных соединений в проростках разных сортов тритикале, характеризующихся различной устойчивостью к инфекции

Исходя из литературных данных и полученным нами результатам, можно предположить, что при попытке поражения патогеном клетки здорового растения, метаболит гриба диффундирует к тканям проростка тритикале и имеет место их взаимодействие. В результате этого взаимодействия происходит образование нового вещества, вторичного метаболита растения, которое диффундирует обратно к грибу и, являясь для него токсичным, подавляет его рост. Иными словами, восприятие растением биотического или абиотического стресса способствует возникновению специального эндогенного сигнала, который приводит в действие защитные механизмы участков растения, в том числе и не контактирующих с патогеном. Они выражаются в интенсификации синтеза фенольных соединений, которые, как известно, участвуют в защите клеток от проникновения патогенов, а также действия их метаболитов.

Представленные в ходе эксперимента данные подтверждают теорию о том, что увеличение содержания фенольных соединений в проростках тритикале является ответом на инфекционный стресс. Действие регуляторов роста, вероятно, направлено

на ускорение активации защитных механизмов растения, что способствует повышению их невосприимчивости к инфекции. Они также способствуют быстрой адаптации растения к стрессовым факторам и возвращению физиологического равновесия. Исследуемые регуляторы роста, помимо ростостимулирующей активности, в определенной концентрации обладают явными иммунными свойствами, что даёт возможность говорить о полифункциональности данных препаратов.

4. Библиографический список

[1]. Калашникова, Е.А., Клеточная селекция растений на устойчивость к грибным болезням. Автореферат диссертации, – 38 с. (2003).

[2]. Калашникова, Е.А., Клеточная инженерия растений. Учебное пособие/Е.А. Калашникова. – М.:РГАУ-МСХА, – 318 с. (2012).

[3]. Бадина, Г.В. Основы агрономии Г.В., Бадина – Л.:ВО Агропромиздат, – 134 с. (1990).

[4]. Батуро, С.А., Поражение снежной плесенью и зимостойкость озимого тритикале в Беларуси С.А. Батуро, С.И. Гриб // Земледелие и селекция в Беларуси: Сб. науч. трудов ИзиС. – Жодино, – Вып.39. – С. 234-237 (2003).

[5]. Загоскина, Н.В., Фенольные соединения: фундаментальные и прикладные аспекты / Н.В. Загоскина, Е.Б. Бурлакова. – М.: Научный мир, – 400 с. (2010).

[6]. Запрометов, М.Н., Фенольные соединения и их роль в жизни растения: 56-е 311 Тимирязевское чтение / М.Н. Запрометов. – М.: Наука, – 45 с. (1996).

[7]. Murashige, T., A revised medium for rapid growth and bio assays with tobacco tissue cultures / T. Murashige, F. Skoog // Physiol. Plant. vol. 15. – p. 473-497 (1962).

[8]. Волынец, А.П., Фенольные соединения в жизнедеятельности растений/А.П. Волынец. – Минск: Беларус. навука, – 283 с. (2013).

Summary

Introduction

The genus *Fusarium* comprises many plant-pathogenic species, causing diseases in the most important agricultural crops, and also can be harmful for humans and animals since many of species produce biologically active secondary metabolites (e.g., phytotoxins and mycotoxins) with an extraordinary chemical diversity. The economic importance of Fusarium species is high due to both their impact on crop yields and accumulation of mycotoxins in the colonized crops, which can make food commodities unacceptable for marketing or consumption. Research on *Fusarium*, carried out globally, concentrates the efforts of thousands of scientists and experts in different fields of science: mycology, plant pathology, genetics, agronomy, ecology, chemistry, biochemistry, and toxicology. Despite the efforts of the scientific community, many problems in this area are still not solved. Nowadays, to combat fungal diseases of important crops chemicals are widely used, which is harmful to human and animal health. In certain concentrations plant growth regulators are capable of inhibiting the development of fungi and can be considered as an alternative solution. The paper analyzes the influence of growth regulators on the development of *Fusarium* triticale. Triticale is a crop characterized by high yield, high protein and essential amino acids content that defines its food and fodder importance. Grain triticale is used in baking and confectionery 35 industries, as well as for alcohol and industrial starch production. Also triticale is a valuable crop as the grain and green mass is used for feeding of farm animals. For the study the species of

Fusarium fungi were used, which are most often found on the crops of our region, they are: *F. culmorum*, *F. sporotrichioides*, *F. oxysporum* and *F. avenaceum*. These drugs are environmentally friendly, which is very important when using triticale for human nutrition and animal feed.

The Aim of Research

The research was focused on their correlation with different concentrations of growth regulators dissolved in nutrient medium.

Results and Discussion

In the experiment we have chosen two growth regulator, which differ in their spectrum of action: immunocytophite (ethyl ester of arachidonic acid), Arachidonic acid (from marine algae). For experiment were chosen three triticale accessions (Ukro, Doublet, S95). The accessions differed in susceptibility to fungal diseases and resistance to Fusarium. Joint cultivation of triticale grains with mycelium of examined fungi was carried out as follows: in the center of the Petri dishes were placed pathogen (0,5×0,5 cm), around which at a distance of 4 cm had a weevil. The total content of phenolic compounds was determined in 7-day-old triticale seedlings. Experiments conducted in three 5 biological and analytical replicates. The graphs and charts, presents the arithmetic means of definitions and their standard deviations.

Conclusion

The experiments showed that concentrations in some medications can have an inhibitory effect on the development of the fungi and inhibit the development of *Fusarium*. First culture for triticale it is shown that in seedlings, cultivated in vitro, increases the total content of soluble phenolic compounds in response to infectious stress when used as the Immunocytophite and arachidonic acid of plant origin. It is established that the phenolic compounds varies depending on the concentration of the drug and the studied accessions.

INTERNATIONAL JOURNAL OF SECONDARY METABOLITE

Int. J. Sec. Metabolite, Vol. 3, Issue 2 (2016) pp. 58-65

Research Article

ISSN: 2148-6905 online Journal homepage: http://www.ijate.net/index.php/ijsm

The Effectiveness of Vitamin and Mineral Feed Additives in the Form of a Bolus for Dairy Cows

Dzmitry V. Voronov[1], Leu B. Zavodnik[1,*]

[1]Grodno State Agricultural University, Volkovich St. 1, 230023, Grodno, Belarus

Received: 02 December 2015 - Revised: 07 February 2016 - Accepted: 19 February 2016

Abstract: The article deals with the results of the study of efficiency of feed additives in the form of a bolus ("Megabolus"). We established decrease in pathology of the reproductive system in comparison with the control by 9.8%, artificial insemination index in cows of the experimental group (EG) decreased by 11.1%. Components of the "Megabolus" increased of amount of albumin in the EG of cows up to 32.2±0.9 g/l; in the control up to - 30.2 g/l. Indicators of blood in the EG confirm the efficiency of boluses: increase the absorption of manganese is up to 25.6%, carotene - 16.2%.

Key words: dairy cows, feed additive, bolus, reproductive function, blood

ЭФФЕКТИВНОСТЬ ВИТАМИННО-МИНЕРАЛЬНОЙ КОРМОВОЙ ДОБАВКИ В ФОРМЕ БОЛЮСА ДЛЯ ДОЙНЫХ КОРОВ

Резюме: В статье представлены результаты исследования эффективности кормовой добавки в форме болюса («Мегаболюс») для дойных коров. Нами было установлено снижение болезней репродуктивной системы в сравнении с контрольной группой на 9,8%, индекса искусственного осеменения – на 11,1%. Благодаря компонентам «Мегаболюса» увеличилось количество альбуминов в крови у экспериментальной группы животных на 32,2±0,9 г/л; у контрольной – на 30,2 г/л. Показатели крови подтверждают эффективность болюсов: абсорбция марганца увеличилась на 25,6%, каротина – на 16,2%.

Ключевые слова: дойная корова, кормовая добавка, болюс, репродуктивная система, кровь

1. Введение

Обеспечение крупного рогатого скота достаточным количеством микроэлементов и витаминов является значимым для профилактики болезней на протяжении всего цикла эксплуатации животного. Коровы не получают необходимого количества микроэлементов и витаминов. Это связано с дефицитом биологически активных веществ в кормах [2] и наличием биогеохимических провинций [1; 9]. Все это приводит к снижению резистентности животных и частой заболеваемости в послеродовой период

*Corresponding Author E-mail: dvvoronov@tut.by leuzavodnik@ya.ru

(задержкой последа, эндометритами, маститами и болезнями обмена веществ). Это ведет к значительным потерям молочной продуктивности и снижению товарности молока, так как в период лечения дойных коров молоко не может быть использовано для пищевых целей. Стоит отметить, что у коров с витаминно-минеральной недостаточностью повышается риск рождения неполноценного, с низкой резистентностью телят [8; 10].

Дефицит микроэлементов и витаминов можно ликвидировать разными способами: путем введения их с водой, кормом, инъекционно [7]. Установлено, что при парэнтеральном введение витаминов и части минералов большая их часть теряется, не усваиваясь, так как не способна «включаться» в обменные процессы [6]. При скармливании микродоз с кормом поступление минералов и витаминов будет зависеть от интенсивности кормления, равномерности размешивания и т.п. Однако в сухостойный и послеотельный периоды аппетит у коровы снижен или может вообще отсутствовать, а потому премиксы и брикеты-лизунцы здесь могут оказаться малоэффективными [10; 11]. Альтернативным способом ликвидации гиповитаминозов и микроэлементозов является дача витаминно-минеральных добавок в форме болюса [3; 11].

В отечественной литературе мало данных, показывающих эффективность применения витаминно-минеральных болюсов длительного срока рассасывания. Учитывая влияние полноценного кормления на здоровье коров, наличие у них дефицита микроэлементов и витаминов возникла необходимость проведения исследований.

Цель работы – определить влияние кормовой добавки (КД) в форме болюса на гемато-биохимические показатели и репродуктивную функцию у коров.

2. Материал и Методы

Опыты по оценке эффективности использования КД в форме болюса «Мегаболюс» проводили в условиях «Ачукевичи» Сельскохозяйственный производственный кооператив «Принеманский» Новогрудского района Гродненской области (Республика Беларусь).

Схема опыта

Период проведения опытов с апреля 2014 по июнь 2014. Содержание − пастбищное. Оценивали эффективность КД «Мегаболюс» для дойных коров. Формировали две группы: контрольную и опытную. Коровы 3-5 лактации с продуктивностью более 5000 кг молока в год. По 15 голов в каждой группе. Дозировка КД в форме болюсов: 2 штуки на голову. В контрольной группе использовали витаминно-минеральную подкормку (1 %-й премикс, введенный в полнорационный комбикорм). Все группы животных содержались в однотипных условиях; кормление одинаковым рационом.

КД «Мегаболюс» в виде болюса весит 107 грамм, из них активные компоненты составляют 78,6 граммов. Предназначен для дойных коров. Период рассасывания до 200-240 дней. Задается болюс с помощью аппликатора (болюсодавателя), в количестве 2 штуки на одно животное. Согласно данным изготовителя, состав КД «Мегаболюс» (Agrimin Ltd, Великобритания) и премикса (ОАО «Лида хлебопродукт», Беларусь) в таблице 1.

Для того чтобы болюс мог длительное время находится в сетке, используются специальные ингредиенты, которые позволяют поддерживать массу болюса постоянной, меняя его плотность. После попадания болюса в сетку его плотность

составляет 2,8-2,9 г/см3, в последствии, когда часть массы болюса теряется при рассасывании и он становится меньшего размера его плотность меняется и становится 5 г/см3. Весь болюс покрыт смолоподобной оболочкой, за исключение одной торцевой стороны, через которую происходит рассасывание.

Таблица 1. Активные компоненты витаминно-минеральной подкормки в опыте

Активные компоненты	Содержание в одном болюсе, г, не менее	Суточная доза при рассасывании 2 штук болюсов, г, не менее	Премикс для контрольных коров (норма ввода в комбикорм 1 %), г (МЕ*)/тонне	Суточная доза при потреблении премикса, г/гол, не менее
Цинк	13,55	0,1129	24000	1,2
Медь	16,5	0,1375	1400	0,07
Марганец	8,88	0,0740	11000	0,55
Селен	0,245	0,0020	8	0,0004
Кобальт	0,240	0,0020	440	0,022
Йод	0,252	0,0021	500	0,025
Витамин А	475 тыс. МЕ	4,0 тыс. МЕ	5200*	0,26
Витамин Д	93 тыс. МЕ	0,8 тыс. МЕ	600*	0,03
Витамин Е	0,925	7,7	3000	0,15

Кровь для исследования брали до опыта и через 1,5 месяца. Исследования крови проводили в научно-исследовательской лаборатории УО ГрГАУ, аккредитованной в органах БелГосСтандарта (требования международного стандарта ИСО/МЭК 17025).

3. Результаты Исследований и Обсуждение

Согласно данным, полученным от ветеринарной службы хозяйства, заболеваемость среди новотельных коров достигает 54 % (в зависимости от периода года). Во время проведения опыта, заболеваемость составляла 48,2 % (таблица 2).

В рамках исследования оценивали заболеваемость. В понятие «заболеваемость» включали регистрируемые болезни органов половой системы (воспалительного и невоспалительного характера). Использование добавки «Мегаболюса» не оказало существенного влияния на снижение количества патологии половой системы в сравнении с контролем. Разница в конце опыта в сравнении с контрольными коровами – 9,8 %. Период от отела до оплодотворения также не имел существенных различий. Однако индекс осеменения был ниже на 11,1 % у коров опытной группы. Это свидетельствует о преимуществе по данному показателю группы, которым применяли КД «Мегаболюс».

Таблица 2. Влияние на репродуктивную функцию

Показатель	Заболеваемость органов половой системы, % (начало/конец опыта)	Период от отела до оплодотворения, дней	Индекс осеменения, ед.
Опыт	48,4/22,9*	66,2±5,2	1,6±0,08
Контроль	48,0/25,4*	67,5±1,5	1,8±0,01

В целом, данные таблицы 2 указывают на более эффективное влияние КД «Мегаболюс» на некоторые показатели репродуктивной функции у коров. Стоит отметить, что существенного преимущества у КД в форме болюса нет. Возможно,

положительный эффект связан с ежедневным, регулярным и ровным поступление компонентов «Мегаболюса» в организм коров (например, витамина А). Также усвоение витаминов и микроэлементов не зависело от поедаемости корма и качества смешивания.

Результаты общего клинического анализа крови (ОКА), полученной от коров подопытной и контрольной групп после завершения опыта представлены в таблице 3. Животные как контрольной, так и подопытной групп испытывают значительную физиологическую нагрузку на организм. В целом, достоверных различий между опытной и контрольной групп не выявлено, но обращает на себя внимание увеличение лейкоцитов у животных обеих групп. Согласно литературным данным, лейкоцитоз у коров может быть предопределен введением вакцины, а также нагрузкой на иммунную систему при интенсивной эксплуатации животных.

Таблица 3. Результаты ОКА

Показатели	Контроль	Опыт	Норма
Эритроциты, $\times 10^{12}$/л	5,85±0,1	5,98±0,2	5,0-7,5
Лейкоциты, $\times 10^{9}$/л	22,6±0,2	27,5±1,1	4,5-12,0
Тромбоциты, $\times 10^{9}$/л	204±10,5	254±20,1	250-450
Гемоглобин, г/л	103,5±5,2	98,3±8,5	90-120
Гематокрит, %	27,0±1,1	26,7±2,1	35-46
РЭрО*, %	15,55±0,1	17,4±0,1	11,5-14,5
ЦП*, ед.	1,25±0,05	1,15±0,05	0,85-1,15
СГЭ*, пг	17,8±0,6	16,5±0,5	13-17

Примечание: * РЭрО – распределение эритроцитов по объему; ЦП – цветовой показатель; СГЭ – содержание гемоглобина в эритроците.

На более активный гемопоэз в подопытной группе может указывать показатель количество тромбоцитов и РЭрО, которые составили 254х10^{9}/л и 17,4 %, а в контроле – 204х10^{9}/л и 15,55 % соответственно. При этом, у коров контрольной группы наблюдали увеличение ЦП (1,25±0,08 ед.) и СГЭ (17,83±1,17 пг). Эти показатели были выше, чем аналогичные у животных подопытной группы, а также не соответствовали физиологически допустимым нормам.

Выше указанные изменения характерны для скрытой анемии, при которой эритроциты не приобретают типичный размер вследствие дефицита витаминов и микроэлементов (меди, марганца) [4]. Остальные показатели не имели значительных отличий.

В таблице 4 представлены результаты биохимического исследования крови от молочных коров МТК «Ачукевичи», которым задавали КД «Мегаболюс».

Согласно представленным данным, у животных произошло существенное изменение параметров белкового обмена (таблица 4). Количество общего белка увеличилось на 25,7 %. При этом повысилось количество альбуминовой фракции (32,2±0,9 против показателя до опыта, равного 22,49±0,8). Процент альбуминовой фракции также вырос. Такое изменение описываемых показателей указывает на увеличение потребления белковой части корма. Это характерно для животных на раздое из-за концентратного типа кормления [3; 4]. Обычно это приводит к повреждению гепатоцитов, вследствие развития кетоза.

Таблица 4. Показатели белкового обмена

Показатель	*Опыт*		Контроль	
	до опыта	через 1,5 есяца	до опыта	через 1,5 месяца
Белок, г/л	64,13±1,5	86,40±3,7	73,03±7,0	78,6±4,7
Альбумины, г/л	22,49±0,8	32,20±0,9	19,88±2,0	30,2±1,5
Альбумин, %	35,23±1,9	37,42±0,9	27,93±3,4	39,1±3,9
Глобулин, г/л	41,65±2,0	54,2±2,9	53,15±7,5	48,4±5,9
А/Г, ед.	0,55±0,01	0,6±0,01	0,40±0,1	0,7±0,1

Компоненты КД «Мегаболюс» оказали гепатопротекторный эффект, защитив печень. Это подтверждает тот факт, что увеличение процента альбуминов указывает на синтетическую способность печени, что является положительным стороной при описании здоровья коров. На увеличение альбуминовой фракции также указывает А/Г-соотношение. Оно увеличилось с 0,5 до 0,6 единиц.

В контрольной группе показатели белкового обмена к конце отчетного периода изменились. Регистрировали увеличение общего количества белка на 4,5 %. Однако наибольшее изменение было связано с альбуминовой фракцией: она увеличилась до 30,2 % (до опыта 19,8 %). О значении данной фракции с точки зрения клинической биохимии и диагностики говорилось выше. Стоит добавить, что А/Г-соотношение увеличилось до 0,7.

Изменились показатели минерального обмена (таблица 5). Через 1,5 месяца произошло увеличение количества кальция при неизменившемся существенно уровне фосфора. Это привело к изменению Ca/P-соотношения (1,45 в начале опыта и 2,08 – в конце). Это объясняется особенностями кормления животных в данный период лактации.

Увеличение количества марганца напрямую подтверждает способность болюсов «Мегаболюс» обеспечивать организм коровы минералами. В частности, в состав данной добавки входит соль марганца, а у животных через 1,5 месяца регистрируется существенный рост его уровня. Увеличение составило 49,7 %. В контроле – 24,1 %.

Таблица 5. Показатели минерального обмена

Показатель	Опыт		Контроль	
	до опыта	через 1,5 месяца	до опыта	через 1,5 месяца
Ca, ммоль/л	1,83±0,1	2,49±0,1	1,64±0,2	2,3±0,02
P, ммоль/л	1,28±0,01	1,22±0,1	1,14±0,01	1,3±0,2
Ca/P, ед.	1,45±0,1	2,08±0,2	1,4±0,1	1,8±0,2
Марганец*, кмоль/л	2,01±0,2	4,01±0,2	2,2±0,1	2,9±0,3

Примечание: * – в цельной крови

Показатель уровня глюкозы низкий, как в начале опыта, так и через 1,5 месяца (таблица 6). На фоне высокой концентрации белка, это может служить основой для развития кетоза. У данных животных, в целом, можно констатировать предкетозное состояние [7]. Это подтверждает наличие опасности для здоровья коров из-за риска накопления кетоновых тел, с дальнейшим повреждением печени и др. внутренних органов.

Гепатоспецифические ферменты указывают на функциональное состояние печени, а также целостность структуры гепатоцитов [4; 5]. Как правило при гепатите,

гепатодистрофии их количество постепенно увеличивается. В данном случае, наблюдается снижение выше перечисленных ферментов. Количество АлАТ снизилось на 8,6 %, АсАТ – на 12,5 %, ГГТ – на 24,5 % в сравнении с периодом до опыта. У контрольных животных гепатоспецифические ферменты за весь период опыта не имели существенных колебаний (от 3 (АлАТ) до 15 % (ГГТ)). У коров подопытной группы увеличилось количество каротина на 40,7 %, а в контрольной группе – 24,5 %.

Таблица 6. Биохимические показатели крови

Показатель	Опыт		Контроль	
	до опыта	через 1,5 месяца	до опыта	через 1,5 месяца
Глюкоза, моль/л	1,63±0,1	1,86±0,2	1,35±0,2	1,3±0,2
моль-рин, моль/л	2,15±0,3	4,65±2,8	1,69±0,2	2,7±2,3
АлАТ*, Ед/л	30,26±2,3	27,65±2,8	27,18±1,9	26,3±2,3
АсАТ*, Ед/л	65,13±5,1	57,0±5,3	57,6±1,8	48,7±2,8
Коэфф. Де-Ритиса, ед	1,97±0,3	2,11±0,2	2,12±0,2	1,9±0,2
Билирубин, мкмоль/л	5,40±1,3	8,08±0,9	6,41±0,9	7,6±2,1
ГГТ*, Ед/л	17,67±1,9	13,33±2,8	12,0±1,1	13,0±2,3
Мочевина, моль/л	3,07±0,8	5,68±0,8	3,4±1,0	5,0±0,3
Каротин, мкмоль/л	25,2±2,5	42,5±1,5	24,6±2,0	32,6±1,5

Примечание: АлАТ – аланинаминотрансфераза; АсАТ – аспартатаминотрансфераза; ГГТ – гамма-глутамилтрансфераза.

Увеличение количества билирубина и мочевины, как правило, происходит при усилении белкового обмена [5]. Эти показатели не превышают предельные границы физиологической нормы.

4. Заключение

Несмотря на более низкую суточную порцию витаминов и микроэлементов у коров, которым применяли болюсы, эффективность оказалась достаточной для улучшения показателей крови репродуктивной функции. КД «Мегаболюс» положительно влияет на репродуктивную функцию у коров. Гемато-биохимические показатели крови у подопытной группы подтверждают способность болюсов влиять на обмен веществ положительно. Установлено наличие ряда факторов: оптимизация рациона по микроэлементам и витаминам и гепатопротекторное влияние компонентов добавки. КД «Мегаболюс» активизирует антитоксическую функцию организма и повышают иммунную реактивность животных.

Следовательно, несмотря на длительный период рассасывания, КД в форме болюса способна снабжать организм дойных коров микроэлементами и витаминами на высоком уровне при пастбищном условии содержания. Применение болюсов не имеет существенных недостатков в сравнении с премиксом, который вводят в комбикорм.

5. Использованная Литература

[1]. Воробьев, Д.В., (2011). Физиологический статус и его коррекция у жвачных, всеядных животных и птиц в биогеохимических условиях региона Н. Волги: монография, Санкт-Петербург, 180.

[2]. Воронов, Д.В., (2011). Новый способ профилактики дефицита микроэлементов и витаминов у высокопродуктивных коров в период. *Наше сельское хозяйство,* 8, 2-4.

[3]. Воронов, Д.В., Бобер, Ю.Н., & Корочкина, Е.А., (2014). Эффективность профилактики гипокальциемии у коров с использованием кальциболюса и мела кормового. *Иппология и ветеринария,* 12, 51-56.

[4]. Джексон, М.Л., (2009). Ветеринарная клиническая патология. Введение в курс, Москва, 384.

[5]. Камышников, В.С., (2003). Клинико-биохимическая лабораторная диагностика: справочник: В 2 т., Минск, Том 1.

[6]. Кучинский, М.П., Карпуть И.М., & Курдеко А.П., (2006). Биоэлементозы животных. *Эпизоотология, иммунобиология, фармакология и санитария: международный научно-теоретический журнал,* 1, 11-15.

[7]. Кучинский, М.П., (2003). Современные проблемы минерального питания сельскохозяйственных животных и пути их решения. *Современные вопросы патологии сельскохозяйственных животных: Материалы международной научно-практической конференции.* 22-24.

[8]. Шимкус, А., (2007). Органический селен в рационе телят. *Материалы конференции "Современные технологии сельскохозяйственного производства": X международная научно-практическая конференция,* 182.

[9]. Grodzinska, K., (2003). Trace element contamination in industrial regions of Poland studied by moss monitoring. *Environ. Monit. Assess.* (87), 255-270.

[10]. Noordhuizen, J.P.T.M., (2012). Dairy herd health and management. *Context Products Ltd. Packington, UK*, 465.

[11]. Casalone, M., (2008). Mineral metabolism during late pregnancy and calcium status after parturition in dairy cows. *Poster at the 25th World Buiatrics Congress,* 6, 338.

Summary

Introduction

The article deals with the results of the study of blood indicators and reproductive function in cows under the use of feed additives in the form of a bolus. Providing cattle with sufficient amount of minerals and vitamins is important for the prevention of diseases in the post-partum period. Cows with lack of necessary amount of microelements and vitamins, have reduced resistance, morbidity in post-partum period increases. There are effects of such a mineral-vitamin deficiency; they delay in subinvolution processes in the genital organs, lack or weak estrus, poor insemination.

The Aim of Research

To determine the influence of the feed additive (CD) in the form of a bolus on the blood biochemical indices and reproductive performance of cows.

Results and Discussion

To provide cows with vitamins and minerals they use feed additives with basic feed. Their use does not always have high efficiency that’s why learning of new ways of vitamin and mineral providing with bolus using is the actual direction of scientific research. The studies were conducted on a farm in Belarus. Period of the experiments is from April 2014 until June 2014. We evaluated the efficacy of the feed additive (FA) "Megabolus" (2 boluses per cow). The

FA "Megabolus" total weighs 107 grams, of which the active components comprise 78.6 grams. It is designed for dairy cows. Total period of sucking up is up to 200-240 days. We gave bolus via the applicator in an amount of 2 pieces per animal. The FA "Megabolus» is made by Agrimin Ltd, UK; and premix made by "Lidahleboproduct", Belarus). We used premix for the cattle for the control group. During the study, two groups were formed: control and experimental. There were 15 animals in each group. Cows were of 3-5 period of lactation; their productivity was of more than 5,000 kg of milk per year. In the control group we used 1% premix. We established decrease in pathology of the reproductive system in comparison with the control by 9.8%, artificial insemination index in cows of the experimental group decreased by 11.1 %. Components of the feed additive "Megabolus" had hepatoprotective effect: increase of amount of albumin fraction in the experimental group of cows up to 32.2±0.9 g/l; in the control up to - 30.2 g/l. Indicators of blood in the control group confirm the ability of boluses to enter the blood effectively (the absorption of manganese is up to 25.6%, carotene - 16.2%).

Conclusion

Although a lower daily dose of vitamins and minerals in cows, which are used boluses, efficiency was sufficient to improve blood parameters of reproductive function. FA "Megabolus" has a positive effect on the reproductive function in cows. Hemato-biochemical parameters of blood in the experimental group confirm the ability of boluses to influence metabolism positively. The presence of a number of factors: optimizing the diet of micro-elements and vitamins hepatoprotective effect of components and additives. FA "Megabolus" activates the antitoxic function and boost the immune reactivity of the animals.

Therefore, despite the long period of absorption, the FA “Megabolus” in the form of a bolus is able to supply the body of dairy cows with trace elements and vitamins at a high level when pasture conditions. Application bolus doesn’t have significant disadvantages compared to the premix which is administered in the feed.

INTERNATIONAL JOURNAL OF SECONDARY METABOLITE

Int. J. Sec. Metabolite, Vol. 3, Issue 2 (2016) pp. 66-73

Research Article

ISSN: 2148-6905 online Journal homepage: http://www.ijate.net/index.php/ijsm

Antibacterial and insecticidal activity of volatile compounds of three algae species of Oman Sea

Ardalan Pasdaran[1, 2*], Azadeh Hamedi[2, 3] and Nazim Mamedov[4]

[1] *Department of Pharmacognosy, School of Pharmacy, Research and Development Center of Plants and Medicinal Chemistry, Guilan University of Medical Sciences, Rasht, Iran,*

[2] *Medicinal Plants Processing Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*

[3] *Department of Pharmacognosy, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran*

[4] *Medicinal Plants Program, Stockbridge School of Agriculture University of Massachusetts at Amherst, USA*

Received: 23 February 2016 - Revised: 03 April 2016 - Accepted: 18 April 2016

Abstract: Many of the volatile oils showed important biological and pharmacological activities, these compounds as part of the traditional medicine in many cultures used as long time. Potencies of them caused these natural products gained many scientific researches in felid of natural products. The volatile oils of *Actinotrichia fragilis* (Forsskål) Børgesen, *Liagora ceranoides* J.V.Lamouroux and *Colpomenia sinuosa* (Mertens ex Roth) Derbes and. Solier were extracted by hydrodistillation. These volatile oils were analyzed by GC-MS and GC-FID techniques and tested for their toxicity against *Oryzeaphilus mercator* and *Tribolium castaneum*, antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* using by the disc diffusion method also free-radical-scavenging properties. The identified constituents of these volatile oils represented 92.7%, 99.9% and 93.8% of the total volatile oils, respectively, of *A. fragilis*, *L. ceranoides* and *C. sinuosa*. Ethyl cinnamate and Tetradecane were the main compounds in *L. ceranoides*, 1-dodecanol and caryophyllene oxide in *A. fragilis* whilst hexadecane and 7-pentadecanone were the principal components of *C. sinuosa* volatile oil. All three volatile oils showed 55-90% mortality of *O. mercator* and 60-80% mortality of *T. castaneum* at a dose of 12 µL/L air after 48h of exposure. Based on the observed contact toxicity of the essential oils of these species, it is fair to state that these volatile oils may have some potential as an insecticide against the crop pests, *O. mercator* and *T. castaneum*. Also antibacterial activity of *L. ceranoides* volatile oil against *Pseudomonas aeruginosa* and *Staphylococcus aureus* is significant.

Keywords: antibacterial activity; contact toxicity; *Actinotrichia fragilis; Liagora ceranoides; Colpomenia sinuosa*

1. Introduction

Secondary metabolites isolated from different alga are playing an important role as lead components, natural medicine or nutriceuticals in drug discovery researches and pharmaceutical industries [1-3]. Recently, due to the resistance of different pathogenic bacteria and pest to antibiotics and insecticidal agents [4-6], finding new active components against these health and environmental problems is one of the major areas of medical and agricultural researches. Peculiarity of the marine environment and high biological activity of marine natural products make algal metabolites fascinating source for finding new antimicrobial and insecticidal compound [6, 7]. The aim of this study is to investigate volatile

*Corresponding Author Tel.: +989127948891; Fax: +981313263670. E-mail address: Ardalan_pasdaran@gums.ac.ir (Ardalan Pasdaran) or mamedov@cas.umass.edu (Nazim Mamedov)

components, insecticidal and antibacterial properties of three algae from Oman Sea (*Actinotrichia fragilis* (Forsskål) Børgesen, *Liagora ceranoides* J.V.Lamouroux and *Colpomenia sinuosa* (Mertens ex Roth) Derbes and. Solier). Red alga *A. fragilis* (Galaxauraceae, Nemaliales), is a small (c. 1±5–5 cm high) calcified, dichotomously divided multiaxial species, with an Indo-Pacific tropical distribution [8]. Some studies can be found on the biology and reproductive system of this alga [8-10]. Red alga, *L. ceranoides* J.V.Lamouroux is (Liagoraceae, Nemaliales) was previously reported to exhibit antioxidant properties [11]. Distribution and biological aspects of this alga were previously reported in literature [12-15]. Brown alga, *C. sinuosa* (Mertens ex Roth) Derbes and. Solier, which is known as the oyster thief or sinuous ballweed is found throughout South Africa and is widespread around Australia and some other tropical areas. Its distribution and history of life was investigated in some previous studies [16-18]. Methanol extract and body mass powder of this alga exhibited antibacterial effect against *Staphylococcus aureus* [6]. Despite some reports on biology and distribution of these three algae, there metabolite or biological activates have not been studied to date.

2. Experimental

2.1. Plant Materials

The *A. fragilis, L. ceranoides* J and *C. sinuosa* were collected from Chabahar coast wild populations growing in the Sistan and Baluchestan province, Iran. Voucher specimens (2558, 2559 and 2560, respectively), were deposited in the herbarium of pharmacognosy department, pharmacy faculty, Guilan University of Medical Sciences, Rasht, Iran.

2.2. Extraction of the Essential Oils

The air-dried ground of *A. fragilis, L. ceranoides and C. sinuosa* (500 g each) were subjected to hydrodistillation for 3h using a Clevenger type apparatus, yielding respectively, 1.3%, 0.5% and 0.8% v/w yellowish essential oils with distinct fragrance. The volatile oil samples were dried over anhydrous sodium sulphate (Na_2SO_4) and stored at 4°C in the dark until analyzed.

2.3. Analysis of the Essential Oils

The volatile oils were analyzed by Shimadzu GC-MS-QP5050A fitted with a fused methyl silicon DB-5 column (60 m × 0.25 mm i.d., 0.25 μm film thickness). Helium was used as carrier gas at a flow rate of 1.3 mL/min. The column temperature was kept at 50°C for 3 min, increased to 300°C at a rate of 5°C/min, and finally kept at 300°C for 5 min. The injector temperature was 270^{o}C and split ratio was adjusted at 1:33. The injection volume was 1 μL. The mass spectral (MS) data were obtained at the following conditions: ionization potential 70 eV; ion source temperature 200^{o}C; quadrupole temperature 100^{o}C; solvent delay 2 min; resolution 2000 amu/s and scan range 30-600 amu; EM voltage 3000 volts. Identification of compounds was based on direct comparison of the Kovats indices and MS data with those for standard compounds or by comparison of their relative Kovats indices to series of *n*-alkanes, and computer matching with the NIST NBS54K Library, by comparison with references. For quantization (area %), the GC analyses were also performed on an Agilent 6890 series apparatus fitted with a FID detector. The FID detector temperature was 300ºC. To obtain the same elution order as with GC-MS, simultaneous auto-injection was performed on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

2.4. Contact Toxicity Assay

The contact toxicity of the volatile oils of these algae was determined by previously insect toxicity assay model [19, 20]. All insect species were watched in controlled laboratory

conditions for about three weeks (25-29 ° C and relative humidity of 80%). The adult insect samples were collected of 1-3 week old and of mixed sex. All essential oils were applied with an automatic pipette on a paper strip (6 cm × 3 cm). The amounts of essential oils applied were 12, 24, 36 and 48 µL, corresponding to 3, 6, 9 and 12 µL/L air. Each dose was applied with automatic pipette as 100 µL acetone solution and acetone was used as a control. After evaporation of the acetone, twenty adults of *O. mercator* and *T. castaneum* were placed in Petri dishes (9 cm) (at 27±2°C, 12% moisture and 12h photoperiod). The experimental design was completely randomized, with three replicates. Mortality of the samples was evaluated after 12, 24, and 48h of exposure. Responses to treated sample versus control were converted to "percentage of mortality" [21].

2.5. Antibacterial Assay

Antibacterial activity assays of the volatile oils were carried out by the disk diffusion method. Bacteria were purchased in lyophilized form from the Institute of Pasture, Iran. Suspensions (100 µL) of the bacteria were adjusted to 10 cfu/mL final cell concentration after this 50 µL of bacterial suspension was poured by 25 mL of sterile normal saline into Petri dish flasks (spread by a sterile swab). Amounts of 60, 90, and 120 mg of the volatile oils were dissolved in 1 mL of methanol. Sterilized disks (5 mm) were impregnated with 10 µL of these volatile oil solutions, corresponding to 600, 900, and 1200 µg/disk, respectively, placed on the inoculated agar. Penicillin (1 mg of penicillin was added into 1 mL of sterilized and distilled water, and then the sterilized disk was soaked with 10 µL of this solution) was used as a positive control, corresponding to 10 µg/disk. At the end of 6 days, inhibition zones were measured in diameter (millimeter) around the disks. All of the tests were made in triplicate [22, 23].

Data Analysis

Statistical analysis of the data was done using SPSS 10.0 software package. The results were showed significant difference at $p < 0.05$ levels.

3. Results and Discussion

This study investigated volatile constituents of *A. fragilis*, *L. ceranoides* and *C. sinuosa* using GC-MS apparatus and the identified components are shown in Table 1. The identified constituents of these volatile oils represented 92.7%, 99.9% and 93.8% of the total volatile oils, respectively. Different aliphatic alcohols and long chain hydrocarbon were the major components of the extracted essential oil. We could not find any report on volatile components of the selected alga to compare the result but these components were previously from other alga [24-26].

In this study, *O.mercator* and *T. castaneum* were selected as model insects to evaluate insecticidal activities of the extracted volatile components. The insect, *O. mercator* (merchant grain beetle, Coleoptera) feeds from food stuff with high oil content such as oatmeal, bran, brown rice and processed foods, cereals, dried fruit, nuts and seeds. They can spread as a chronic pest and contaminate and damage food quality. The *T. castaneum* (red flour beetle, Tenebrionidae), is another pest of stored products, such as food grains. This beetle is used as a model organism for ethological and food safety research. Both selected insects are worldwide most common pests and food contaminants. They also can cause allergic responses in human. These two insect can cause serious financial damages to food industry and routinely used in insecticides development researches.

Table 1. Major constituents of the volatile oils of *A. fragilis, L. ceranoides and C. sinuosa*

				(%)	
	Chemical compounds	Chemical formula	Kovats' Indices	*A. fragilis*	*L. ceranoides*	*C. sinuosa*
1	2-iodo-3-methyl-Butane	$C_5H_{11}I$	983	-	-	1.1
2	2-nonanone	$C_9 H_{18} O$	1096	1.1	-	-
3	Alpha-terpinolene	$C_{10} H_{16}$	1207	2.0	-	-
4	Citronellal	$C_{10} H_{18} O$	1161	1.9	-	-
5	2-Undecanone	$C_{11}H_{22}O$	1291	2.3	-	1.4
6	Tetradecane	$C_{11}H_{12}O_2$	1399	-	24.4	-
7	1-Dodecene	$C_{12} H_{24}$	1193	3.1	-	-
8	1-Dodecanol	$C_{12}H_{26}O$	1474	39.6	-	2.3
9	2-Tridecene	$C_{13}H_{26}$	1315	-	-	2.8
10	Tridecane	$C_{13}H_{28}$	1299	0.9	-	4.8
11	Beta- Ionone	$C_{13} H_{20}O$	1485	0.8	-	-
12	Neryl acetone	$C_{13} H_{20}O$	1428	-	-	1.5
13	Edulan I	$C_{13} H_{20}O$	1469	-	-	0.9
14	Ethyl cinnamate	$C_{13} H_{26}O$	1374	-	33.8	-
15	Pseudoionone	$C_{13} H_{26}O$	1469	-	20.7	0.8
16	1-Tridecanol	$C_{13}H_{28}O$	1572	0.6	-	3.6
17	1-Tetradecene	$C_{14}H_{28}$	1393	2.8	-	1.2
18	1-Tetradecanol	$C_{14}H_{30}O$	1680	1.7	-	3.4
19	Tridecanoic acid methyl ester	$C_{14}H_{28}O_2$	1625	-	21.0	-
20	Germacrene D	$C_{15} H_{24}$	1598	0.5	-	-
21	Beta-elemene	$C_{15} H_{24}$	1384	1.6		-
22	1-Pentadecene	$C_{15} H_{30}$	1492	1.0	-	-
23	Pentadecane	$C_{15} H_{32}$	1510	-	-	2.5
24	Caryophyllene oxide	$C_{15} H_{24}O$	1590	16.7	-	-
25	7-Pentadecanone	$C_{15}H_{30}O$	1699	-	-	35.8
26	(8S,14) Cedran-diol	$C_{15}H_{26}O_2$	1876	2.0	-	-
27	Hexadecane	$C_{16}H_{34}$	1589	3.8	-	28.5
28	1-Hexadecanal	$C_{16}H_{32}O$	1819	1.3	-	-
29	1-Hexadecanol	$C_{16} H_{34}O$	1880	1.5	-	2.1
30	1-Heptadecanol	$C_{17}H_{36}O$	1958	5.6	-	-
31	8-Heptadecene, 1-chloro	$C_{17}H_{33}$ CL	1992	1.4	-	1.1
	Grouped components					
	Terpenoids compounds			21.6	-	0.9
	Alcoholic hydrocarbons and derivatives			52.3	-	11.4
	Aldehydes and ketones hydrocarbons			6.6	20.7	39.5
	Long chain hydrocarbons			11.6	24.4	39.8
	Others			6	54.8	2.2
	Total identified			**92.7**	**99.9**	**93.8**

The compounds have been sorted according to their Kovats retention indices on a DB-5 capillary column

Although, all three volatile oils showed 55-90% mortality of *O. mercator* and 60-80% mortality of *T. castaneum* at a dose of 12 µL/L air after 48h of exposure (Table 2), among the selected algae, *A. fragilis* volatile oil had the best insecticidal activity with mortality rate of 80-90% for both *T. castaneum* and *O. Mercator*. Interestingly, this oil was consisted of 49% aliphatic alcohols particularly 1-didacanol. Different aliphatic alcohols (C2 to C18) have been reported to exhibit insecticidal properties against different insects such as *Pediculus*

humanus capitis [1] *Rhodnius prolixus*, *Triatoma infestans* [2] and Aedes mosquitoes [3]. They proposed to have ovicide and larvicide effect against the mosquitoes *Aedes aegypti* Linneo and *Aedes scutellaris* Walker. The highest activity was reported for the 1-dodecanol and the lowest for 1-octanol. In addition, co-exposure of head lice to 1-dodecanol and d-phenothrin lotions, was made it more susceptible to insecticidal activity of pyrethroid [27]. The insecticidal activity of 1-dodecanol is suggested to be a result of interruption in the cuticular tanning process and thus interruption in the development of the physiological properties. [28]

Table 2. The toxicity of the volatile oils of *A. fragilis, L. ceranoides and C. sinuosa* against *O. Mercator* and *T. castaneum*

Essential oils	Dose (µL/L air)	% Mortality[a]					
		O. mercator			*T. castaneum*		
		Exposure time (h)			Exposure time (h)		
		12	24	48	12	24	48
A. fragilis	3	15.7± 3.3*	25.3± 3.3*	45.3± 3.3*	0.0± 0.0*	0.0± 0.0*	3.3± 3.3*
	6	30.0± 3.3*	40.0± 3.3*	50.7± 0.0*	0.0± 0.0*	15.7± 3.3*	20.3± 3.3*
	9	45.0± 3.3*	51.7± 0.0*	61.0± 3.3*	21.0± 3.3*	30.0± 0.0*	50.0± 0.0*
	12	50.3± 0.0*	86.3± 0.0*	90.0± 0.0*	45.3± 3.3*	51.3± 3.3*	80.0± 3.3*
L. ceranoide	3	0.0± 0.0*	0.0± 0.0*	0.0± 0.0*	0.0± 0.0*	0.0± 0.0*	0.0± 3.3*
	6	0.0± 0.0*	0.0± 3.3*	3.3± 3.3*	0.0± 0.0*	0.0± 0.0*	3.3± 0.0*
	9	3.3± 3.3*	18.7± 3.3*	23.3± 5.8*	0.0± 0.0*	3.3± 3.3*	15.3± 3.3*
	12	25.0± 5.8*	35.0± 5.8*	55.0± 0.0*	18.5± 0.0*	28.7± 0.0*	45.0± 3.3*
C. sinuosa	3	0.0± 0.0*	0.0± 0.0*	3.3± 3.3*	0.0± 0.0*	0.0± 0.0*	3.3± 0.0*
	6	6.7± 0.0*	25.0± 0.0*	35.0± 0.0*	0.0± 0.0*	6.0± 0.0*	18.0± 0.0*
	9	20.0± 0.0*	36.7± 3.3*	45.0± 3.3*	20.7± 0.0*	21.3± 3.3*	35.7± 0.0*
	12	35.0± 0.0*	45.7± 0.0*	55.0± 0.0*	30.3± 0.0*	35.3± 3.3*	60.0± 0.0*

[a] Mean±S.E. of three replicates, each set-up with 20 adults.
* There were no significant differences among treatments.

We could not find any report on insecticidal activity of *A. fragilis* volatile oil or 1-dodecanol on *T. castaneum* and *O. mercator* thus it is not possible to compare the efficacy or the applied doses. We believe that this is the first report on insecticidal effects of *A. fragilis* volatile oil or 1-dodecanol on these two crop pest. On the other hand the highest applied lethal dose was 12 (µL/L) is not a high dose and did not need topical exposure which can be considered as a bonus for its application as in insecticide. Despite the non-polar narcotic toxicity of 1-dodecanol to aquatic organisms of about 1 mg/l, the aliphatic alcohols especially 1-dodecanol are readily degradable and do not give rise to environmental concerns. 1-Dodecanol also considered non-toxic to human health, and is a permitted as a food additive.

The algae *C. sinuosa* volatile oil also showed some degree of insecticidal activity with the mortality rate of 55-60% within 48h. The major constituents of *C. sinuosa* essential oil were 7-pentadecanone and hexadecane. We could not find any reports on insecticidal activity for these two components but Long chain aliphatic methyl ketone series of C_{11}-C_{15} particularly 2-pentadecanone was reported to exhibit insect repellency [29]. Although the algae *C. sinuosa* volatile oil contained 2.3% of 1-dodecanol, but the observed insecticidal activity could not be just related to 1-dodecanol and it may worth the potential insecticidal activity of 7-pentadecanone in future works.

Despite the considerable amount of ethyl cinnamate (33.8%) in *L. ceranoides* volatile oil and the previous reports on potent insecticidal activity of ethyl cinnamate [30] against *S.*

littoralis (LD_{50} = 0.37 µg/larva), this oil did not showed a significant insecticidal properties especially at low doses or in shorter exposure time (12-24h). The observed different results might be due to the differences in selected insects' type which was applied in these two studies.

Antibacterial activities of these volatile oils were investigated against *E. coli*, *P. aeruginosa* and *S. aureus*. The selected bacteria are among the most common causes of infectious diseases. As it seen in Table 3, only *A. fragilis* showed significant antimicrobial properties. The three investigated algae showed some degrees of inhibition on microbial growth. But among them, *L. ceranoides* volatile oil had the highest antimicrobial activity (Table 3). As it was mentioned above, ethyl cinnamate is the major volatile constituents of this alga and may have an important role in the observed result. Several reports have demonstrated that essential oils containing cinnamate derivatives as one of the major constituents, exhibit antibacterial activity [31-34]. Also, Stefanović et al. reported antimicrobial activity of different synthetic derivatives of cinnamate particularly against *S. aureus* agent with minimum inhibitory concentration of 62.5 µg/ml [35].

Table 3. Antibacterial activities of the volatile oils of *A. fragilis, L. ceranoides and C. sinuosa*

Bacterial strain	*L. ceranoides* 600-1200 µg/disk	*A. fragilis* 600-1200 µg/disk	*C. sinuosa* 600-1200 µg/disk	Penicillin 10 µg/disk
E. coli	10-12	6-8	- [b]	-
P. aeruginosa	15-18	10-12	7-11	42
S. aureus	17-19	8-11	9-12	25

[a] Inhibition zones are given as minimum and maximum inhibition zones in diameter (mm) around the disks impregnated at 600, 900, and 1200 µg/disk doses. [b] Not active.

The result of present study is consistence with the previous reports. All three tested bacterium in this study are Gram-negative bacteria and has been developing resistance to common antibiotics. The outer membrane of these bacteria inhibits permeation of antibacterial compounds. Although the activity of tested essential oils is lower than the pure penicillin. But, considering the results of previous studies about antibacterial activity of naturally occurring or synthesized cinnamates, it can be concluded that essential oil of *L. ceranoides* and ethyl cinnamate indicate significant activity and may possess potential application as antibacterial agents [35-37]. The observed activity of A. *fragilis* might be due to the high concentration of long chain aliphatic alcohol including 1- dodecanol and 1-tridecanol. These two components have been previously reported to exhibit bactericidal effects [38]. Despite the previous reports on antibacterial effects of methanolic extract of *C. sinuosa* [6], we did not observe a significant antimicrobial effects. This might be due to application of volatile components in the present study instead of methanolic extract which can contain volatile and non-volatile metabolites of this alga.

4. References

[1]. Bugni, T.S., et al., *Marine natural product libraries for high-throughput screening and rapid drug discovery.* Journal of natural products, 2008. **71**(6): p. 1095-1098.

[2]. Rengasamy, K.R., et al., *Advances in algal drug research with emphasis on enzyme inhibitors.* Biotechnology advances, 2014. **32**(8): p. 1364-1381.

[3]. Smit, A.J., *Medicinal and pharmaceutical uses of seaweed natural products: a review.* Journal of applied phycology, 2004. **16**(4): p. 245-262.

[4]. Xu, L., et al., *Antibacterial and antifungal compounds from marine fungi.* Marine drugs, 2015. **13**(6): p. 3479-3513.

[5]. Soko, W., M.J. Chimbari, and S. Mukaratirwa, *Insecticide resistance in malaria-transmitting mosquitoes in Zimbabwe: a review.* Infectious diseases of poverty, 2015. **4**(1): p. 1-12.

[6]. Salvador Soler, N., et al., *Antimicrobial activity of Iberian macroalgae.* Scientia Marina, 2007, vol. 71, num. 1, p. 101-113, 2007.

[7]. Salem, W., H. Galal, and F. Nasr El-deen, *Protective strategies induced by marine algae extracts against bean leaf spot disease.* Assiut University Journal of Botany, Assiut Univ., 2011.

[8]. Wang, W.-L. and Y.-M. Chiang, *The reproductive development of the red alga Actinotrichia fragilis (Galaxauraceae, Nemaliales).* European Journal of Phycology, 2001. **36**(04): p. 377-383.

[9]. Wiriyadamrikul, J., K. Lewmanomont, and S.M. Boo, *Molecular diversity and morphology of the genus Actinotrichia (Galaxauraceae, Rhodophyta) from the western Pacific, with a new record of A. robusta in the Andaman Sea.* Algae, 2013. **28**(1): p. 53-62.

[10]. Titlyanov, E.A., et al., *Inventory change (1990s–2010s) in the marine flora of Sanya Bay (Hainan Island, China).* Journal of the Marine Biological Association of the United Kingdom, 2015. **95**(03): p. 461-470.

[11]. Zubia, M., D. Robledo, and Y. Freile-Pelegrin, *Antioxidant activities in tropical marine macroalgae from the Yucatan Peninsula, Mexico.* Journal of applied phycology, 2007. **19**(5): p. 449-458.

[12]. Tsiamis, K., et al., *Marine benthic algal flora of Ascension Island, South Atlantic.* Journal of the Marine Biological Association of the United Kingdom, 2015: p. 1-8.

[13]. Huisman, J.M., *The type and Australian species of the red algal genera Liagora and Ganonema (Liagoraceae, Nemaliales).* Australian Systematic Botany, 2002. **15**(6): p. 773-838.

[14]. Titlyanov, E. and T. Titlyanova, *Changes in the species composition of benthic macroalgal communities of the upper subtidal zone on a coral reef in Sanya Bay (Hainan Island, China) during 2009–2012.* Russian Journal of Marine Biology, 2013. **39**(6): p. 413-419.

[15]. Titlyanov, E.A., et al., *Influence of winter and spring/summer algal communities on the growth and physiology of adjacent scleractinian corals.* Botanica Marina, 2006. **49**(3): p. 200-207.

[16]. Arévalo, R., S. Pinedo, and E. Ballesteros, *Changes in the composition and structure of Mediterranean rocky-shore communities following a gradient of nutrient enrichment: descriptive study and test of proposed methods to assess water quality regarding macroalgae.* Marine Pollution Bulletin, 2007. **55**(1): p. 104-113.

[17]. Kogame, K., *Life histories of Colpomenia sinuosa and Hydroclathrus clathratus (Scytosiphonaceae, Phaeophyceae) in culture.* Phycological Research, 1997. **45**(4): p. 227-231.

18]. Toste, M.F., et al., *Life history of Colpomenia sinuosa (Scytosiphonaceae, Phaeophyceae) in the Azores.* Journal of phycology, 2003. **39**: p. 1268-1274.

[19]. Pasdaran, A., et al., *GC-MS Analysis, Free-Radical-Scavenging and Insecticidal Activities of Essential Oil of Scrophularia oxysepala Boiss.* Pharmaceutical Sciences, 2013. **19**(1): p. 1.

[20]. Pasdaran, A., et al., *Phytochemical and Bioactivity Evaluation of Scrophularia amplexicaulis Benth*, 2016. p. 519-525.

[21]. Isman, M.B., P. Proksch, and J.Y. Yan, *Insecticidal chromenes from the Asteraceae: structure-activity relations.* Entomologia experimentalis et applicata, 1987. **43**(1): p. 87-93.

[22]. Kordali, S., et al., *Determination of the chemical composition and antioxidant activity of the essential oil of Artemisia dracunculus and of the antifungal and antibacterial activities of Turkish Artemisia absinthium, A. dracunculus, Artemisia santonicum, and Artemisia spicigera essential oils.* Journal of agricultural and food chemistry, 2005. **53**(24): p. 9452-9458.

[23]. Hamedi, A., K. Zomorodian, and F. Safari, *Antimicrobial activity of four medicinal plants widely used in Persian folk medicine.* Research Journal of Pharmacognosy, 2015. **2**(1): p. 25-33.

[24]. Karabay-Yavasoglu, N.U., et al., *Antimicrobial activity of volatile components and various extracts of the red alga Jania rubens.* Phytotherapy research, 2007. **21**(2): p. 153-156.

[25]. Dembitsky, V. and M. Srebnik, *Use of serially coupled capillary columns with different polarity of stationary phases for the separation of the natural complex volatile mixture of the marine red alga Corallina elongata.* Biochemistry (Moscow), 2002. **67**(9): p. 1068-1074.

[26]. Payo, D.A., et al., *Variability of non-polar secondary metabolites in the red alga Portieria.* Marine drugs, 2011. **9**(11): p. 2438-2468.

[27]. Cueto, G.M., et al., *Toxic effect of aliphatic alcohols against susceptible and permethrin-resistant Pediculus humanus capitis (Anoplura: Pediculidae).* Journal of medical entomology, 2002. **39**(3): p. 457-460.

[28]. Cueto, G.M., E. Zerba, and M.I. Picollo, *Biological effect of 1-dodecanol in teneral and post-teneral Rhodnius prolixus and Triatoma infestans (Hemiptera: Reduviidae).* Memorias do Instituto Oswaldo Cruz, 2005. **100**(1): p. 59-61.

[29]. Innocent, E., N. Gikonyo, and M. Nkunya, *Repellency property of long chain aliphatic methyl ketones against Anopheles gambiae ss.* Tanzania journal of health research, 2008. **10**(1): p. 50-54.

[30]. Abdelgaleil, S.A., et al., *Bioactivity of two major constituents isolated from the essential oil of Artemisia judaica L.* Bioresource technology, 2008. **99**(13): p. 5947-5950.

[31]. El-Baroty, G., et al., *Characterization of antioxidant and antimicrobial compounds of cinnamon and ginger essential oils.* African Journal of Biochemistry Research, 2010. **4**(6): p. 167-174.

[32]. Gilles, M., et al., *Chemical composition and antimicrobial properties of essential oils of three Australian Eucalyptus species.* Food Chemistry, 2010. **119**(2): p. 731-737.

[33]. Tonari, K., K. Mitsui, and K. Yonemoto, *Structure and Antibacterial Activity of Cinnamic Acid Related Compounds.* Journal of Oleo Science, 2002. **51**(4): p. 271-273.

[34]. Peretto, G., et al., *Increasing strawberry shelf-life with carvacrol and methyl cinnamate antimicrobial vapors released from edible films.* Postharvest Biology and Technology, 2014. **89**: p. 11-18.

[35]. Stefanović, O.D., I.D. Radojević, and L.R. Čomić, *Synthetic cinnamates as potential antimicrobial agents.* Hem. ind. **69**(1): p. 37-42.

[36]. Jantan, I.b., et al., *Correlation Between Chemical Composition and Antifungal Activity of the Essential Oils of Eight Cinnamomum. Species.* Pharmaceutical Biology, 2008. **46**(6): p. 406-412.

[37]. Venkateswarlu, S., et al., *Antioxidant and antimicrobial activity evaluation of polyhydroxycinnamic acid ester derivatives.* INDIAN JOURNAL OF CHEMISTRY SECTION B, 2006. **45**(1): p. 252.

[38]. Togashi, N., et al., *Antibacterial activity of long-chain fatty alcohols against Staphylococcus aureus.* Molecules, 2007. **12**(2): p. 139-148.

INTERNATIONAL JOURNAL OF SECONDARY METABOLITE

Int. J. Sec. Metabolite, Vol. 3, Issue 2 (2016) pp. 74-81

Research Article

ISSN: 2148-6905 online Journal homepage: http://www.ijate.net/index.php/ijsm

Phenolic Contents and Antioxidant Properties of *Echinops ritro* L. and *E. tournefortii* Jaup. Et. Spach Extract

Çiğdem Aydın [1, 2*], Gülten Taşdelen Özcan[1], Murat Turan[1], Ramazan Mammadov[1]

[1]Department of Biology, Faculty of Science and Arts, Pamukkale University, 20100 Denizli, Turkey

Received: 01 February 2016 - Revised: 30 March 2016 - Accepted: 26 April 2016

Abstract: Aim of the study was to evaluate antioxidant activity and total phenolic content of *Echinops ritro* L. and *E. tournefortii* (Asteraceae). The dried leaves and seeds of *E. ritro* and *E. tournefortii* were extracted separately with ethanol, methanol, chloroform and dH_2O. Total phenolic content was measured by Folin-Ciocalteu method. Antioxidant activities of the extracts were determined by two test systems namely, radical scavenging on DPPH and β-carotene bleaching methods. dH_2O extracts has the highest phenolic content (92.24 GAE mg/100g). The results were compared to those of BHT as synthetic antioxidant. dH_2O extracts were found to be rich as a source of phenolics. According to the results of antioxidant activity, dH_2O extracts exhibited higher antioxidant activity than all types of solvent. The strongest antioxidant properties were obtained by dH_2O extract. Radical scavenging activities (%) were found to be in the following order: Chloroform<Ethanol<Methanol<dH_2O<BHT. These results of the present study demonstrated that the extracts of *E. ritro* and *E. tournefortii* may be used as a source of natural antioxidant in the food and pharmaceutical industries.

Keywords: *Echinops ritro*, *E. tournefortii*, antioxidant activity, phenolic content

1. Introduction

Plants are good source of biologically active secondary metabolites which have many therapeutic potential in many diseases and even in free radical associated disorders [1]. Among secondary metabolites synthesized, plant polyphenols are the aromatic hydroxylated compounds which have the most potent and therapeutically useful bioactive substances. Promising radical scavenging ability of the phenolic compounds produced in higher plants is studied extensively [2]. Oxidation stress is one of the major concerns of health in modern era and antioxidants have been reported to prevent oxidative damage caused by free radical, via interfering with the oxidation process by reacting with free radicals, by chelating with catalytic metals, and also by acting as oxygen scavengers [3]. In the presence of antioxidants, the oxidative rates decrease due to an increased activation energy for reaction, thus increasing the "lifetime" of the substrate, serving as a parameter for the evaluation of the antioxidant activity [4]. Although several synthetic antioxidants, such as butylated hydroxyanisole and butylated hydroxytoluene, are available because of their toxicity problems; there is an upsurge of interest in the therapeutic potentials of plants as antioxidants. In addition to the natural antioxidants like vegetables, fruits, spices and tea, scientific evaluation of plant's properties

*Corresponding Author Tel.: +90 505 5712960. E-mail: cdem.86@hotmail.com

through potent pharmacological activities, toxicity profiling and economic viability are needed for growing recognition for medicinal plants and herbal products as novel antioxidants in recent decades. Therefore, significant consideration has been directed toward the detection of antioxidant properties in plant species.

The genus *Echinops* L. (Asteraceae) consists of approximately 120 species [5], distributed in Africa and the Mediterranean basin [6]. In Turkey, the genus comprises 19 species, including 2 subspecies and 3 varieties [7,8]. *Echinops* plant was reported to possess variety of compounds belonging to various classes like: alkaloids, flavonoids, terpenoids, lipids, steroids and polyacetylenes [9]. Echinopsine was quinoline alkaloid isolated in 1900 by M. Greshoff from seeds of the blue globe thistle, *E. ritro* and its presence was also demonstrated in 14 other species of *Echinops* like *E. latifolius*, *E. setifer* [10]. *Echinops* species have been used as traditional medicine for treatment of migraine, diuretic, heart diseases, urinary infection, as well as worm and hemorrhoid in Ethiopia [11]. In the present study, total phenolic of the ethanol, methanol, chloroform and dH_2O extracts prepared from *E. ritro* and *E. tournefortii* the were determined as mg/g GAE. These extracts were tested for their antioxidant activity by using two methods namely β- carotene-linoleic acid test system and DPPH free radical scavenging assay. This study examined the antioxidant activities of these species for the first time.

2. Material and Methods

2.1. Plant materials

Echinops ritro and *E. tournefortii* was collected from Denizli Kınıklı field during the period of investigation and in July 2015. The voucher specimen of *E. ritro* was confirmed and deposited in Herbarium at the Department of Biology. The collected plant material was air-dried in darkness at room temperature (20^{o}C). Dried plant parts were cut up and stored in tight-seal dark containers until needed.

2.2. Preparation of plant extracts

Echinops ritro and *E. tournefortii* species cut into small pieces with a blender. Extractions were prepared using different solvents (methanol, ethanol, chloroform and dH_2O). For extractions 10 g of the plant and 100 mL of solvent (Merck) were used for each sample. The mixture was extracted after being heated in a shaker water bath at 55ºC for 6 h. The extract obtained was filtered through filter paper (Whatman No: 1), and the solvents were evaporated in a rotary evaporator (IKA, RV 10 basic V-C, Germany) at 48 – 49°C. The water in each extract was frozen in Freeze-drying (Thermo, savant) machine and then drawn out (stored at -20^{o}C).

2.3. Determination of total phenolic content

The total phenolic content of extracts was determined using to the Folin-Ciocalteu method [12]. Briefly, 0.75 mL of Folin-Ciocalteu reagent (1:9; Folin-Ciocalteu reagent: distilled water) and 100 mL of sample (5 mg/mL) were put into a test tube. The mixture was mixed and allowed to stand for 5 min at room temperature. The mixture was allowed to stand at room temperature for 5 min. 0.75 mL of 6 % (w/v) Na_2CO_3 was added to the mixture and then mixed gently. The mixture was homogenized and allowed to stand at room temperature for 90 min. Total polyphenol content was determined using a spectrophotometer at 750 nm. The standard calibration (0.01-0.05 mg/mL) curve was plotted using gallic acid. The total phenolic content was expressed as gallic acid equivalents (GAE) in mg/g plant extract.

2.4. Determination of total antioxidant activity

The antioxidant activity of the crude extracts was evaluated using the β-carotene-linoleic acid test system with slight modifications [13]. β-Carotene (0.2 mg) in 1mL of chloroform was added to 20 μL of linoleic acid, and 200 mg of Tween-20 emulsifier mixture. The mixture was then evaporated at 40^{o}C for 10 min by means of a rotary evaporator to remove chloroform. After evaporation of chloroform, 100 mL of distilled water saturated with oxygen, 4.8 mL of this emulsion was placed into test tubes which had 0.2 mg of the sample and 0.2 of the extract in them. For control, 0.2 mL of solvent (methanol, ethanol, chloroform and dH_2O) was placed in test tubes instead of the extract. As soon as the emulsion was added into the test tubes, initial absorbance was measured with a spectrophotometer (Shimadzu UV-1601, Japanese) to be at 470 nm. The measurement was carried out at 0.5 h intervals for 2 h. All samples were assayed in triplicate. BHT was used as standards. The antioxidant activity was measured in terms of successful bleaching of β-carotene by using the following equation. The measurements were made using the equation below:

$$AA: [\ 1- (A0-At\ /\ A0o - Ato) \times 100$$

Where AA is the total antioxidant activity, A0 is the initial absorbance of the sample, At is the initial absorbance of the control, A0o is the sample's absorbance after 120 min, and Ato is the control's absorbance after 120 min.

2.5. Determination of DPPH free radical scavenging activity

Free radical scavenging activity of the extracts was determined using the free radical DPPH [14]. 4 ml of the DPPH's 0.004% metanolic solution was mixed with 1 ml (0.2 - 1.0 mg/mL) of the extracts, and their absorbances were measured to be at 517 nm after incubation for 30 min at room temperature the absorbance value of the samples were evaluated against empty control group (where all determinants except the test compound were present). Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Every test was treated three times and the averages as determined. Free radical scavenging activity was measured using the equation below:

$$\text{Scavenging activity} = [(A_0 - A_1 / A_0) \times 1\ 00\ )]$$

where A0 is the absorbance of the control (blank, without extract) and A1 is the absorbance in the presence of the extract.

3. Results and Discussion

Polyphenols are known for their antioxidant activity as radical scavengers and possible beneficial roles in human health, such as reducing the risk of cancer, cardiovascular disease, other pathologies [15]. Plants containing high phenolic compounds can be a good source of antioxidants. For this reason, this information has led to the determination of the total phenolic content of the sample under study. The amounts of total phenolic contents ranged from 31.54-92.24 (GAE mg/100g) for extracts respectively (Table 1.). In our investigation, the dH_2O extract of *E. ritro* (92.24 GAE mg/100g) exhibited the highest total phenol content.

Table 1. Total phenolic content of extracts

Extract	Total phenols (GAE mg/100 g)	
	E. ritro	*E. tournefortii*
Ethanol	58.21	49.32
Methanol	83.45	76.81
Chloroform	47.34	31.54
dH_2O	92.24	83.48

According to β-carotene-linoleic acid bleaching assay, β-carotene undergoes rapid discoloration in the absence of an antioxidant, which results in a reduction in absorbance of the test solution with reaction time. This is due to the oxidation of linoleic acid that generates free radicals that attack the highly unsaturated β-carotene molecules in an effort to reacquire a hydrogen atom. When this reaction occurs the β-carotene molecule loses its conjugation and, as a consequence, the characteristic orange color disappears. The presence of antioxidant avoids the destruction of the β-carotene conjugate system and the orange color is maintained

Inhibition of linoleic acid was affected by different solvents [16]. BHT showed maximum value of inhibition (94.07 %). The antioxidant activity efficiency were also calculated and given in Fig. 1 and Fig 2. As it can be seen from this figure, the highest antioxidant activity efficiency is determined in dH_2O extract of *E.ritro* (76.79 %) and the least efficiency in chloroform extract of *E. tournefortii* (13.30 %).

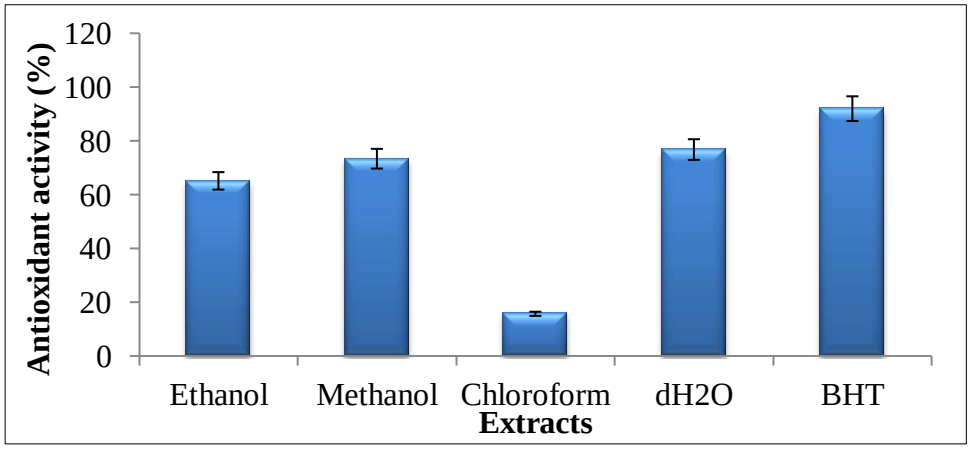

Fig. 1. Antioxidant activities efficiency in the extracts of *E.ritro* and BHT

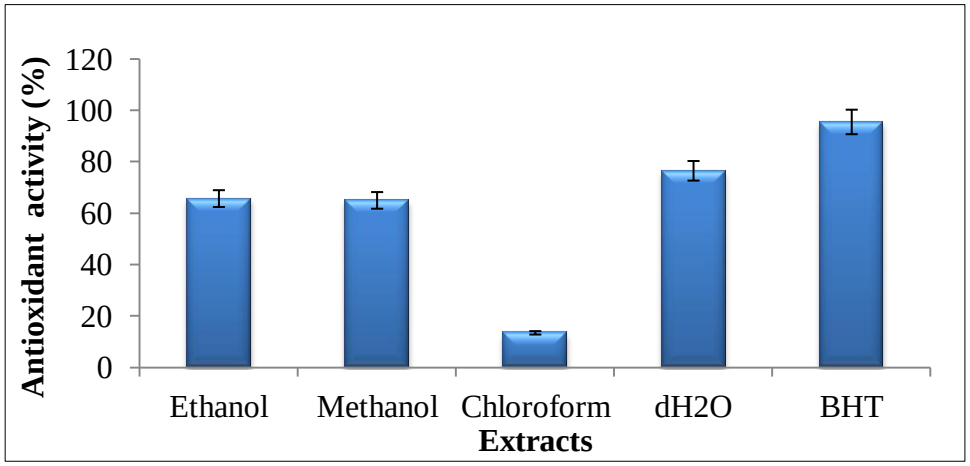

Fig. 2. Antioxidant activities efficiency in the extracts of *E. tournefortii* and BHT

DPPH radical scavenging activity assay is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of many plant extracts or compounds [17]. DPPH is a stable free radical which exhibits a deep purple color with maximum absorption at 517 nm. Antioxidant molecules react with the free radical by hydrogen or electron donation, resulting in discoloration of DPPH because of their conversion into yellow colored diphenylpicryl hydrazine [18]. As shown in Fig.3 and 4, the

DPPH radical scavenging activities of four extracts of *E. ritro* and *E. tournefortii* were concentration-dependent. DPPH radical scavenging activities (%) were found to be in the following order: Chloroform<Ethanol <Methanol <dH_2O < BHT (Fig.3 and 4).

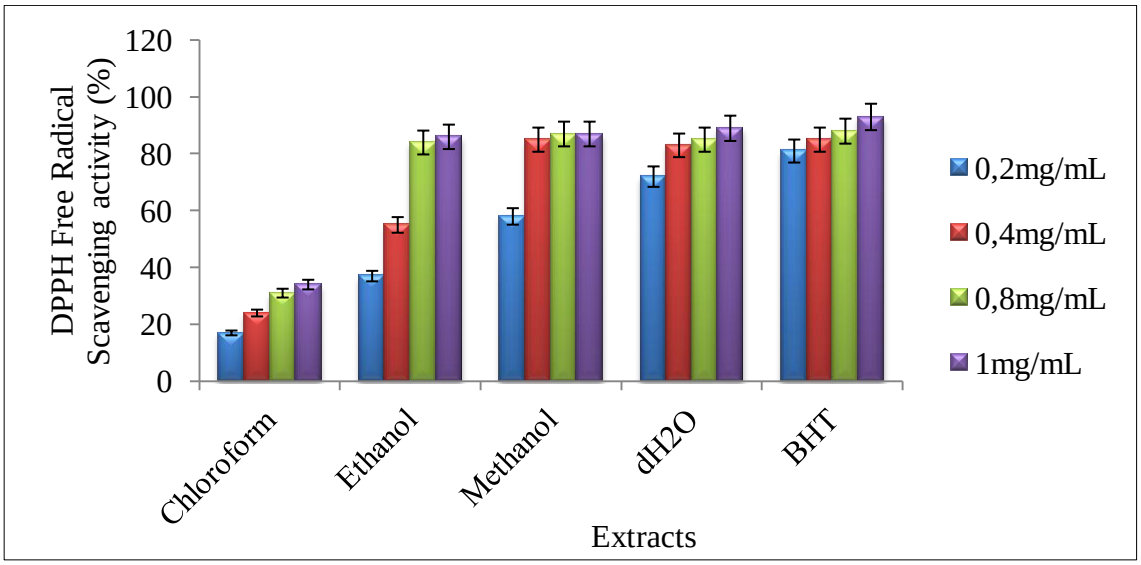

Fig. 3. The DPPH radical scavenging activities of *E. ritro* extracts and BHT

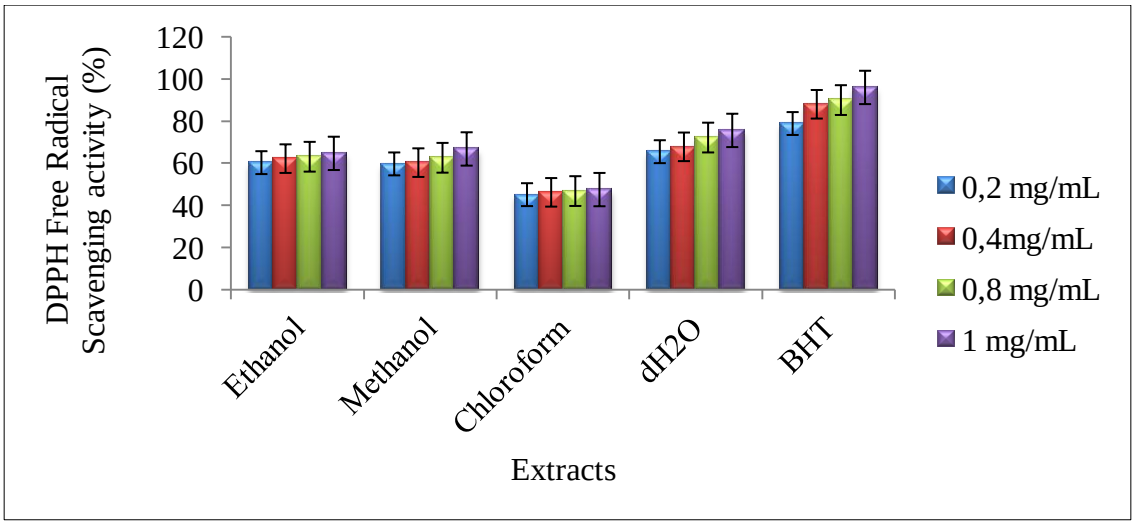

Fig. 4. The DPPH radical scavenging activities of *E. tournefortii* extracts and BHT

Fokialakis et al. (2006) reported that the root extracts of *E. ritro* were evaluated for their antifungal activity using direct-bioautography assays with three *Colletotrichum* species that cause strawberry anthracnose. Among the bioactive extracts, the dichloromethane extract of the radix of *E. ritro* was the most potent [19]. It was indicated that aerial parts of *E. ritro* L. and *E.spinosissimus* from the Greek island of Crete could be extracted, and the extracts obtained have been investigated for in-vitro anti-protozoal activity. The activity against chloroquinesensitive (D6) and resistant (W2) strains of *Plasmodium falciparum* and *Leishmania donovani promastigotes* was determined as well as the cytotoxicity on a mammalian kidney fibroblast (Vero) cell line was tested. Dichloromethane of aerial part

extract *of E. ritro* and *E. spinosissimus* had moderate activity against *L. donovani* with no significant anti-malarial activity or cytotoxicity [20]. Apigenin-glucopyranoside 1, Apigenin-glucoside 2, methoxycarbonylindole 3 and beta-sitositerol 4 were isolated from of *E. orientalis* dried leaves and seeds. Isolated compounds and extracts were applied to antioxidant activity tests. While seeds and leaves extracts have high DPPH and moderate ABTS radical scavenging activities, the isolated flavones exhibited high cation radical scavenging activities [21].

The antioxidant activities of methanolic extracts of the *E. kotschyi* were determined via 2,2- diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, and also screened for cytotoxic activity against three human cancerous cell lines (MOLT-4, K562 and MCF7) using the MTT assay (3-[4,5-dimethylthiazol-2-yl]- 2,5 diphenyl tetrazolium bromide) assay. The methanolic extract of *E. Kotschyi* exhibited potent cytotoxic activity against MOLT-4 and K562 cell lines among all extracts tested in this study [22]. On the other hand, the ethanolic extract of *E. spinosus* has efficient action on muscular fibers; anti-inflammatory activity; The ethanolic extract of *E. Spinosus* (100 mg/kg, intraperitoneal) exhibited a very good anti-inflammatory activity against carrageenan-induced paw edema in mice and rats, and it selectively inhibited prostaglandin E2 (PGE2) -induced inflammation [23].

In the present study, the antioxidant capacities and phenolic content of various extracts (methanol, dH_2O, ethanol and chloroform) from *E. ritro* were determined. The dH_2O extract had higher antioxidant capacity and free radical scavenging activity than other extract at the same concentrations. Results of the study indicated that dH_2O extract derived from *E. ritro* possessed remarkable antioxidant activities. Because the synthetic antioxidants (BHA and BHT) have some toxic effects, such as the promotion of carcinogenesis, *E. ritro* can be considered as a source of both natural antioxidants and lauric acid in the food industry and pharmacological applications.

4. Conclusion

According present the study, it may be concluded that the extracts of *Echinops ritro* and *E. tournefortii* demonstrated *in vitro* antioxidant activities. Higher levels of total phenolics of plant are probably responsible from the biological activities observed. This finding candidates the plant as a good case for more in-depth studies and we wish our future research lead to the identification of biologically active molecules present in its extracts

5. References

[1]. Lee, Y.M., Kim, H., Hong, E.K., Kang, BH., Kim, S.J. (2000). Water extract of 1:1 mixture of *Phellodendron cortex* and *Aralia cortex* has inhibitory effects on oxidative stress in kidney of diabetic rats. *J. Ethnopharmacol*., *73,* 429-36.

[2]. Pai, S.R., Nimbalkar, M.S., Pawar, N.V., Patil, R.P., Dixit, GB. (2010). Seasonal discrepancy in phenolic content and antioxidant properties from bark of *Nothopodytes nimmoniana* (Grah.) Mabb. *Int J Pharm Biosci*., 1, 1-17.

[3]. Buyukokurouglu, ME., Gulcin, I., Oktay, M., Kufrevioglu, O.I. (2001). *In vitro* antioxidant properties of dantrolene sodium. *Pharmacol Res. 44,* 491.

[4]. Carvalho, R.N., Moura, L.S, Rosa, P.T.V, Meireles, M.A.A. (2005). Supercritical fluid extraction from rosemary (Rosmarinus officinalis): kinetic data, extract's global yield, composition, and antioxidant activity. *J. Supercrit Fluids*, *35,* 197-204.

[5]. Susanna, A., Garcia-Jacas, N. The tribe Cardueae In: Kadereit J & Kubitzki K (eds.). Compositae. *The Families and Genera of Vascular Plants*. Heidelberg: Springer-Verlag, 2007, pp. 135-158.

[6]. Garnatje, T., Valles, J., Garcia, S., Hidalgo, O., Sanz, M., Canela, MA., Siljak-Yakovlev, S. (2004). Genome size in *Echinops* L. and related genera (Asteraceae, Cardueae): karyological, ecological and phylogenetic implications. *Biol Cell. 96*, 117-124.

[7]. Özhatay, N., Kültür, Ş., Aslan, S. (2009). Check-list of additional taxa to the Supplement Flora of Turkey IV. *Turk J Bot. 33*, 191-226.

[8]. Vural, C., Şapcı, H. (2012). Five new records of the genus *Echinops* (Asteraceae) from Turkey. *Turk. J. Bot. 36*: 151-160.

[9]. Shukla, Y.N. (2003). Chemical, botanical and pharmacological studies on the genus *Echinops* : A review. *J. Med Arom Pl Sci.*, *25*, 720-32.

[10]. Suarez, C., Barrera, C., Caballero, A. (2011). Quinolone alkaloids and friedelanetype triterpenes isolated from leaves and wood of *Esenbeckia alata* kunt Rutaceae. *Quim. Nova, 34(6)*, 984-86.

[11]. Desta, B. (1993). Ethiopian Traditional Herbal Drugs Antimicrobial activity of 63 medicinal-plants. *J. Ethnopharmacol., 39*, 129-139.

[12]. Slinkard, K., Singleton, V.L. (1977). Total Phenol Analysis: Automation and Comparison with Manual Methods. *American J. Enology and Viticulture*., *28(1)*, 49-55.

[13]. Amin, I., Tan, S.H. (2002). Antioxidant activity of selected commercial seaweeds. *Malaysian Journal of Nutrition., 8*, 167-177.

[14]. Wu, C., Chen, F., Wang, X., Kim, H., He, G., Haley-Zitlin, V., Huang, G. (2006). Antioxidant constituents in feverfew (*Tanacetum parthenium*) extract and their chromatographic quantification. *Food Chem*., *96*, 220–227.

[15]. Yesıloglu, Y., Sıt, L., Kılıc, I. (2013). I*n vitro* Antioxidant Activity and Total Phenolic Content of Various Extracts of *Satureja hortensis* L. Collected from Turkey. *Asian Journal of Chemistry*, *25(15)*, 8311-8316.

[16]. Khadri, A., Serralheiro, M.L.M., Nogueira, J.M.F., Neffati, M., Smiti, S., Araújo, M.E.M. (2008). Antioxidant and antiacetylcholinesterase activities of essential oils from *Cymbopogon schoenanthus* L. Spreng. Determination of chemical composition by GC-mass spectrometry and ^{13}C NMR. *Food Chemistry*, *109(3*), 630–637.

[17]. Kamatou, G.P.P., Viljoen, A.M., Steenkamp, P. (2010). Antioxidant, anti-inflammatory activities and HPLC analysis of South African *Salvia* species. *Food Chem., 119*, 684-688.

[18]. Krimat, S., Dob, T., Toumi, M., Kesouri, A., Noasri, A. (2015). Assessment of phytochemicals, antioxidant, antimicrobial and cytotoxic properties of *Salvia chudaei* Batt. et Trab. endemic medicinal plant from Algeria. *J. Mater. Environ. Sci., 6(1*), 70-78.

[19]. Fokialakis, N., Cantrell, CL., Duke, SO., Skaltsounis, AL., Wedge, DE. Antifungal activity of thiophenes from *Echinops ritro*. J. *Agric. Food Chem.* 2006, *54(5)*, 1651-5.

[20]. Fokialakis, N., Kalpoutzakis, E., Tekwani, B.L., Khan, S.I., Kobaisy, M., Skaltsounis, A.L., Duke, S.O. (2007). Evaluation of the antimalarial and antileishmanial activity of plants from the Greek island of Crete. *J. Nat. Med., 61*, 38–45.

[21]. Erenler, R., Yilmaz, S., Aksit, H., Sen, O., Genc, N. Elmastas, M., Demirtas, I. (2014). Antioxidant Activities of Chemical Constituents Isolated from *Echinops orientalis* Trauv. *Rec. Nat. Prod., 8(1*), 32-36.

[22]. Afshaki, S., Jafari, A., Javidnia, K., Firuzi, O. (2012). Antioxidant and cytotoxic activities of four plant extracts from Dena region of Iran. *Research in Pharmaceutical Sciences., 7(5),* 853-9.

[23]. Rimbau, V., Cerdan, C., Vila, R., Iglesias, J. (1999). Antiinflammatory activity of Some extracts from plants used in the traditional medicine of North-African Countries. *Phytother Res., 13(2*), 128-32.

INTERNATIONAL JOURNAL OF SECONDARY METABOLITE

Int. J. Sec. Metabolite, Vol. 3, Issue 2 (2016) pp. 82-87

Research Article

ISSN: 2148-6905 online Journal homepage: http://www.ijate.net/index.php/ijsm

Assessment of Fruit and Some Biochemical Characteristics of Almond Genotypes Selected from Natural Populations of Kayseri Province

Aydın Uzun[1,*], Erhan Akcalı [1]

[1]Department of Horticulture, Erciyes University, Turkey

Received: 26 May 2016 - Revised: 10 June 2016 - Accepted: 19 June 2016

Abstract: Almond (*Prunus amygdalus* L.) can grow under dry climate and harsh soil conditions. The fruits are drupe. Seed-propagated almond populations exist in various parts of Turkey. Several studies have been performed to select the promising genotypes among these populations with regard to fruit quality, yield, late foliations and etc. characteristics. Rich almond populations are shown around the foothills of Erciyes Mountain in Kayseri province of Central Anatolia. In this study, some fruit and biochemical characteristics of 34 almond genotypes selected as promising genotypes with regard to late foliation and yield were determined. Significant variations ($P < 0.05$) were observed in investigated traits of the genotypes. Of selected genotypes, fruit weights varied between 1.5±0.4- 7.6±0.5 g, fruit lengths between 40.7±0.7 - 19.9±2.9 mm, fruit heights between 17.4±0.8 – 10.3±2.1 mm and fruit widths between 27.6±0.7 – 11.8±0.7 mm. With regard to fruit shape of genotypes, 13 were identified as long oval, 12 as elliptical, 5 hearth-shaped and 4 as round. Considering the biochemical characteristics, crude oil contents varied between 54.9 - 42.1% and protein contents varied between 24.6 – 17.7%.

Keywords: diversity, fruit breeding, genetic resources, *Prunus amygdalus*

1. Introduction

Almond culture was said to be initiated four thousand years ago in Iran, Turkey, Syria and Palestine regions and spread throughout the world from these regions [1]. Almond is native to Western and Central Asia [2]. It is quite resistant to droughts and can grow in poor soils and under various ecological conditions [3-5]. As compared to native countries, productions have grown quite more rapidly in the USA and Spain [4, 5]. The reasons for such slow growths in productions of native countries may be related to early-blooming of almond species and thus much more prone nature to spring late freezes, large portions of production sites are composed of wild populations, yields are not consistent and socio-economic states of the countries may influence the productions [5, 6].

The world almond production is around 1.9 million tons and the USA, Spain and Australia are the leading producers. With 85% increase during the last decade, Turkish almond production reached to 75 thousand tons [7]. Although almond is cultured in several regions of Turkey, Mersin (9400 tons), Antalya (5700 tons), Muğla (5700 tons), Çanakkale (5300 tons) and Denizli (4600 tons) are the prominent provinces [8].

[*]Corresponding Author Tel.: +90 3524371790, Fax: +90 3524376290 E-mail: uzun38s@yahoo.com

Selection breeding is the oldest breeding method and breeder selects proper plants from natural populations without creating a genetic variation and using the natural variation [9]. There are rich almond genetic resources in various parts of Anatolia. Seed-propagated trees with different genetic characteristics play a significant role in this richness. There is a need to assess the current gene sources and to get promising genotypes with regard to fruit quality attributes and yields. Almond species are quite prone to late spring freezes and thus identification of late-blooming genotypes will only be possible with selections to be made from these large populations. Since the almond is the first blooming tree in spring, culture is quite restricted in regions with late-spring freeze risks. Therefore, development of late-blooming cultivars has become the primary target of almond breeding programs [6, 10]. The cultivars of Avalon, Solana, Sonora, Price Texas, Ne Plus Ultra, Peerles, Rosetta and Thomson used in leading almond producer country, the USA, were obtained through selections as chance seedlings [1, 11]. The almond trees in current local populations are lost either with natural means or with anthropogenic reasons. Therefore, these populations should urgently be searched through for promising genotypes. In this way, it will be possible to identify chance seedlings with desired fruit and tree characteristics among the genetic resources spread over regions with different climates and ecological conditions [1].

Seed-propagated almond trees are quite common over the rough terrains around the northern foothills of Erciyes Mountain of Kayseri Province. In this study, naturally-growing almond populations of the region were investigated and fruit quality attributes, protein and crude oil contents of promising genotypes were identified.

2. Material and Methods

Almond tree populations located around Alidağı mountain, north of Erciyes mountain and south of Kayseri city center, (Haymana Bağları, Hisarcık Valley, Talas Tablakaya, Beğendik Bağları, Sakar Çiftliği, mountainous districts around Kayseri Organized Industrial Region and Yılanlıdağ regions) were used as the plant material of the present study. A total of 480 almond trees were evaluated and 34 of them were selected with regard to blooming, yield and fruit characteristics. Yield levels were assessed through a scale (1: low yield; 2: medium yield; 3: high yield). Fruit weight, fruit length, fruit width, fruit height, kernel taste, double kernel and fruit shape of selected genotypes were analyzed. Among the biochemical characteristics, crude oil and protein contents were determined in accordance with previous study [12]. For investigated fruit characters, data were analyzed using JMP trial version (SAS Institute Inc.) and means were separated and grouped using Tukey's test ($P < 0.05$). Differences among selected genotypes were put forth and ultimately superior ones were identified.

3. Results and Discussion

Variations were observed among genotypes with regard to investigated traits. Of the selected almond genotypes, 19 were identified as high yield, 11 as medium yield and 4 as low yield. Fruit weights varied between 1.5 – 7.6 g and significant variations were observed in fruit weights of the genotypes. Genotype 34 (7.6±0.5 g) and genotype 33 (6.9 ±0.5 g) had the highest fruit weights (Figure 1). It was reported in a study carried out in Kahramanmaraş province, fruit weights of selected genotypes as between 1.31 - 7.58 g [13]. Yıldırım, (2007) in a study carried out in Isparta province, reported fruit weights of promising genotypes as between 3.51 - 5.43 g [14]. Şimsek and Osmanoğlu (2010) in a selection study carried out in Mardin-Derik, reported fruit weights of investigated genotypes as between 1.75 - 4.77 g [15]. Gülsoy and Balta (2014) in a selection work carried out in Aydın province, reported almond fruit weights as between 2.44 - 7.57 g [9]. Current findings on fruit weights were generally complying with those earlier findings.

Significant differences ($P < 0.05$) were also observed in fruit dimensions of the genotypes. Fruit lengths varied between 40.7 - 19.9 mm, fruit heights between 17.4 – 10.3 mm and fruit widths between 27.6 – 11.8 mm (Table 1). In an earlier study, fruit lengths of almond genotypes were reported as between 24.00-42.88 mm, fruit heights between 16.56-29.50 mm and fruit widths between 10.60-19.18 mm [1]. On the other hand, Şimsek and Osmanoğlu (2010) reported these attributes respectively as between 27.81-35.69 mm, 17.11-24.90 and 11.84-16.77 mm [15]. Gülsoy and Balta (2014) reported fruit lengths as between 29.06-39.15 [9].

Figure 1. Fruit and kernel images of the genotypes 34 (above) and 33 (below) with the greatest fruit weights

With regard to fruit shape of the genotypes, 13 were identified as long oval, 12 were elliptical, 5 were hearth-shaped and 4 were round shaped. With regard to kernel color, 4 had light, 8 medium and 22 had dark color kernels. On the other hand in a previous study, kernel color was mostly reported as medium. In that study, 12 genotypes had very light, 55 light, 62 medium and 28 genotypes had dark color kernels [1]. Again, Şimsek and Osmanoğlu (2010) indicated kernel color of 13 selected almond genotypes as medium [15]. Considering the taste of kernels, 9 had bitter taste and 25 had sweet taste. Aslantaş (1993) found 114 of 120 genotypes as sweet taste [12]. On the other hand, in a study carried out in Tunceli province, indicated taste of 82 genotypes as sweet, 8 genotypes as medium and 67 genotypes as bitter [1]. Double kernel was not seen in almond genotypes of the present study. Although double kernel ratios vary with cultivars, it is not desired since it reduces commercial value [9].

Table 1. Some fruit and biochemical characteristics of almond genotypes studied

G. No	F.W. (g)	F.L. (mm)	F.H. (mm)	F.W. (mm)	F.S.	Seed color	O.C. (%)	P.L. (%)
1	3.4 ± 0.5 l-n	37.5 ± 1.9 bc	12.6 ± 0.9 m-o	18.4 ± 0.6 kl	LO	Dark	47.8	18.3
2	2.3 ± 0.5 op	25.7 ± 1.5 p	10.7 ± 0.7 p	18.4 ± 1.4 kl	E	Dark	50.0	19.3
3	3.5 ± 0.3 l-n	29.6 ± 0.5 l-o	12.8 ± 0.6 l-o	20.4 ± 0.8 f-j	LO	Dark	47.1	17.7
4	4.9 ± 0.3 f-ı	32.8 ± 0.5 e-k	14.5 ± 0.7 f-l	22.3 ± 0.4 b-d	LO	Dark	52.4	18.9
5	3.7 ± 0.6 j-n	31.5 ± 2.0 h-l	13.7 ± 0.9 ı-m	20.9 ± 1.0 d-ı	E	Medium	52.0	19.0
6	3.0 ± 0.1 no	31.9 ± 0.2 g-l	11.6 ± 0.4 n-p	17.8 ± 0.2 l	H	Dark	50.0	19.3
7	3.3 ± 0.2 mn	26.9 ± 1.7 op	14.9 ± 0.4 d-k	18.8 ± 0.4 j-l	E	Medium	47.8	19.6
8	1.9 ± 0.4 p	26.0 ± 0.8 p	11.1 ± 1.2 op	15.1 ± 0.4 m	LO	Dark	49.4	18.1
9	3.2 ± 0.5 mn	27.9 ± 1.9 n-p	13.5 ± 1.2 j-m	19.2 ± 1.5 ı-l	LO	Dark	47.3	18.9
10	3.9 ± 0.8 j-m	32.3 ± 1.7 f-l	14.1 ± 0.9 h-m	19.9 ± 1.2 g-k	E	Light	47.4	18.4
11	3.3 ± 0.3 l-n	30.9 ± 0.8 ı-m	14.0 ± 0.7 ı-m	21.5 ± 1.0 c-g	LO	Dark	47.3	18.5
12	4.4 ± 0.2 h-j	35.2 ± 1.0 c-f	13.8 ± 0.4 ı-m	22.2 ± 0.6 b-f	E	Light	52.5	18.3
13	4.5 ± 0.8 g-j	33.6 ± 3.4 d-ı	17.4 ± 0.8 a	21.4 ± 4.0 c-h	E	Dark	48.1	19.9
14	3.6 ± 0.3 j-n	30.1 ± 0.4 k-n	12.8 ± 0.7 l-o	20.4 ± 0.7 e-j	LO	Dark	45.6	19.1
15	4.2 ± 0.2 ı-l	30.5 ± 1.2 j-n	16.9 ± 0.4 a-c	23.4 ± 0.4 b	H	Dark	46.6	19.1
16	2.0 ± 0.4 p	26.1 ± 0.7 p	11.1 ± 1.9 op	15.4 ± 0.5 m	LO	Dark	49.6	18.2
17	5.2 ± 0.6 e-h	33.1 ± 1.2 e-j	14.7 ± 1.0 m-o	22.3 ± 0.5 b-e	E	Dark	51.0	18.0
18	4.4 ± 0.5 h-k	35.2 ± 1.9 c-e	15.2 ± 0.8 d-j	20.8 ± 0.7 d-ı	LO	Dark	53.5	18.3
19	3.1 ± 0.4 m-o	28.4 ± 1.4 m-p	13.2 ± 1.1 k-n	19.6 ± 0.6 h-l	R	Dark	47.3	24.6
20	3.1 ± 0.3 m-o	28.4 ± 2.8 m-p	14.5 ± 1.6 g-l	19.3 ± 0.7 ı-l	LO	Medium	42.1	18.3
21	3.6 ± 0.6 k-n	34.8 ± 1.5 c-g	14.5 ± 1.4 f-l	21.7 ± 0.6 b-g	H	Medium	53.2	18.0
22	1.5 ± 0.4 p	19.9 ± 2.9 q	10.3 ± 2.1 p	11.8 ± 0.7 n	E	Dark	45.9	20.0
23	3.5 ± 0.1 k-n	31.1 ± 0.9 h-m	11.4 ± 0.7 n-p	20.4 ± 0.5 f-j	E	Medium	53.5	18.6
24	5.8 ± 0.8 c-e	38.8 ± 0.6 ab	15.4 ± 0.8 b-ı	27.6 ± 0.7 a	LO	Dark	52.8	19.3
25	3.7 ± 0.6 j-n	31.1 ± 1.5 h-m	14.6 ± 1.1 e-k	21.2 ± 1.0 c-h	E	Dark	49.7	18.0
26	6.8 ± 0.4 b	40.7 ± 0.7 a	15.9 ± 1.0 a-g	27.1 ± 0.6 a	LO	Dark	48.7	21.4
27	4.8 ± 0.5 f-ı	33.9 ± 3.2 d-h	15.2 ± 1.9 c-j	21.4 ± 1.1 c-h	H	Dark	46.9	21.6
28	5.6 ± 0.2 d-f	34.6 ± 0.7 c-g	16.5 ± 0.6 a-d	23 ± 0.3 bc	H	Light	52.0	21.3
29	5.3 ± 0.7 e-g	30.9 ± 1.7 ı-m	16.0 ± 1.0 a-g	22.4 ± 0.7 b-d	R	Medium	53.2	20.2
30	5.0 ± 0.5 e-ı	30.0 ± 2.3 k-n	15.8 ± 0.9 a-h	22.6 ± 0.7 b-d	R	Dark	47.7	21.2
31	6.3 ± 0.8 b-d	37.2 ± 2.2 bc	16.4 ± 1.8 a-e	25.9 ± 1.0 a	R	Light	54.9	20.2
32	6.5 ± 0.4 bc	36.4 ± 1.5 b-d	16.3 ± 0.8 a-e	26.0 ± 0.6 a	E	Medium	53.2	18.7
33	6.9 ± 0.5 ab	38.4 ± 1.9 ab	16.2 ± 1.0 a-f	26.8 ± 0.5 a	LO	Medium	50.6	19.8
34	7.6 ± 0.5 a	37.1 ± 1.3 bc	17.1 ± 1.0 ab	27.4 ± 0.5 a	E	Dark	48.1	18.5

G. No: Genotype No; FW: Fruit weight; FL: Fruit length; FH: Fruit height; FW: Fruit weight; FS: Fruit shape; LO: Long-oval; R: Round; E: Elliptic; OC: Oil content; PL: Protein level

Significant differences were observed in chemical composition of selected genotypes. Crude oil contents varied between 42.1 (Genotype 14) – 54.9% (Genotype 31) and protein contents varied between 17.7 (Genotype 3) – 24.6% (Genotype 19). The crude oil and protein of almond are quite significant for human health. In a previous study, it was noticed that edible seeds and nuts had high contents of lipids, proteins, dietary fiber and ash (minerals) and they had a good essential amino acids profile, usually with a slight lysine deficiency [16].

The genotypes with high crude oil and protein contents identified in these studies may constitute a significant source for human nutrition. Current findings on crude oil and protein contents were generally complying with the findings of previous studies on almonds. I was reported average protein content of almonds as 19% and crude oil content as 54% [17]; Esteban et al. (1985) in a study carried out in Spain, reported average crude oil content as 66.40% and protein content as 15.80% [18]. Abdallah et al. (1998) reported crude oil contents of 21 almond cultivar and genotypes as between 36-53% [19]. Kodad et al. (2008) reported crude oil contents of 8 standard almond cultivars and 47 promising genotypes as between 48-67% [20]. On the other hand, it was determined crude oil contents of different almond genotypes as between 48.7–64.5% and protein contents as between 14.1-35.1% [21]. Considering the statistical evaluation of different fruit and biochemical characteristics together, it was observed that 7 out of 34 genotypes were prominent. These promising genotypes were identified as the genotypes 12, 13, 14, 17, 18, 26 and 28.

The genotypes identified in this study carried out in Kayseri province may be used in further breeding works and they may then be included among current standard varieties. These genotypes should be propagated through vegetative ways to assess the performance of these genotypes under different ecologies. Present outcomes may provide significant contribution in identification of quality genotypes among natural almond population of Turkey and prevention of extinction of genetic resources of the country.

Acknowledgements

The authors thank to Scientific Research Projects Unit of Erciyes University for funding and supporting the study with the project number of FBY-2014-4902.

4. References

[1]. Ağlar, E. (2005). Pertek (Tunceli) Yöresi Bademlerinin (*Prunus amygdalus* L.) Seleksiyonu. *Yüksek lisans tezi* (basılmamıs), Y.Y.Ü Üniversitesi Fen Bilimleri Enstitüsü, Bahçe Bitkileri Anabilim Dalı, Van. (in Turkish).

[2]. Küden, A. B., Küden, A., Kaska, N., (1994). Adaptations of Some selected Almonds to Mediterranean Region of Turkey. *Acta Horticulturae*, 373: 83-90.

[3]. Özbek, S., 1978). *Özel Meyvecilik*, Çukurova Üniversitesi Ziraat Fakültesi Yayınları: 128, Adana (in Turkish).

[4]. Çelik, M., Çelik, H. ve Yanmaz, R., (1995). *Bahçe Bitkilerinin Ekolojik İstekleri* (Genel Bahçe Bitkileri, Bölüm 4). Ankara Ün. Ziraat Fakültesi Eğitim, Araştırma ve Geliştirme Vakfı Yay. No:4, s: 65-106, Ankara (in Turkish).

[5]. Aslantaş, R. ve Güleryüz, M. (1999). *Almond selection in microclimate areas of northeast Anatolia XI: Grempa meeting on Pistacios and Almonds*, Univ. of Harran, Faulty of Agric.-Pistacio Research and Application Center 1-4 September 1999, Şanlıurfa (Turkey).

[6]. Gülcan, R. (1976). *Seçilmiş Badem Tipleri Üzerinde Fizyolojik ve Morfolojik Araştırmalar*. Ege Üniv. Ziraat Fak. Yayınları No.310. Bornova-İzmir, s: 72, (in Turkish).

[7]. FAO, (2012). http://faostat.fao.org/site/567/default.aspx#ancor (Accessed on: 20.12.2015).

[8]. TUIK, (2014). www.tuik.gov.tr (Accessed at 16 June 2015).

[9]. Gülsoy, E, Balta, F. (2014). Aydın ili Yenipazar, Bozdoğan ve Karacasu ilçeleri badem

(*Prunus amygdalus* Batch) seleksiyonu: Pomolojik özellikler. *Akademik Ziraat Dergisi*, 3(2):61-68. (in Turkish).

[10]. Alkan, G., Seferoğlu, H.G. (2014). Bazı Badem Çeşitlerinin Aydın Ekolojisindeki Fenolojik ve Morfolojik Özellikleri. *Meyve Bilimi*, 1 (2): 38-44.

[11]. Kester, D.E., Asay, R.N., Micke, W.C., 1984. Solano, Sonora and Padre Almonds. *HortScience*, 19 (1): 138-139.

[12]. Aslantaş, R. (1993. Erzincan İli Kemaliye İlçesinde Doğal Olarak Yetişen Bademlerin (*Amygdalus communis* L.) Seleksiyon Yoluyla Islahı Üzerinde Bir Araştırma. *Yüksek Lisans Tezi*, Atatürk Üniversitesi Fen Bilimleri Enstitüsü, Erzurum, s. 122. (in Turkish).

[13]. Beyhan, Ö, Şimşek, M. (2007). Kahramanmaraş Merkez İlçe Bademlerinin (*Prunus amygdalus* L.) Seleksiyon Yoluyla Islahı Üzerine Bir Araştırma. *Bahçe* 36 (1-2): 11-18. (in Turkish).

[14]. Yıldırım, A.N. (2007). Isparta Yöresi Bademlerinin (*P.amygdalus* L.) Seleksiyonu, *Doktora Tezi*. Adnan Menderes Üniversitesi Fen Bilimleri Enstitüsü Bahçe Bitkileri Anabilim Dalı, Aydın (in Turkish).

[15]. Şimsek, M, ve Osmanoğlu A., (2010). Derik (Mardin) İlçesinde Doğal Olarak Yetişen Bademlerin (*Prunus amygdalus* L.) Seleksiyonu, *Y.Y.Üniversitesi Tar. Bil. Derg*., 20(3), 171-182, Van (in Turkish).

[16]. Freitas, J.B., Fernandes, D.C., Czeder, L.P., Lima, J.C.R., Sousa, A.G.O., Naves, M.M.V. 2012. Edible Seeds and Nuts Grown in Brazil as Sources of Protein for Human Nutrition. *Food and Nutrition Sciences*, 3: 857-862.

[17]. Kester, D. E., Asay, R., (1975). Almonds. Advences in Fruit Breeding (ed. J. Janick, J. N. Moore) Purdue Universty Press; Westlafayette, Indiana, p: 623.

[18]. Esteban, R.M., Lopez-Andreum, F.S. and Carpena, O., (1985). Protein Extractabilty of Almond (*Prunus amygdalus* B.) Seed. *J. Sci. Food Agric*. 36: 485-490.

[19]. Abdallah, A., Ahumada, M.H., Gradziel, T.M. (1998). Oil content and Fatty Acid Composition of Almond Kernels from Different Genotypes and California Production Regions. *J. Amer. Soc. Hort. Sci*. 123: 1029-1033.

[20]. Kodad, O., Company, S.I. (2008). Variability of Oil Content and of Major Fatty Acid Composition in Almond (*Prunus amygdalus* Batsch) and Its Relationship with Kernel Quality. *J. Agric. Food Chem*. 56: 4096-4101.

[21]. Kodad, O., Estopanan, G., Juan, T., Company, S.I. (2013). Protein Content and Oil Composition of Almond from Moroccan Seedlings: Genetic Diversity, Oil Quality and Geographical Origin. *Journal of the American Oil Chemists' Society*, 90: 243-252.