

Original Research Article

Effect of Amoxicillin Antibiotic on Germination Rate and Active Components of *Triticumaestivum* L. (Wheat) Seeds

Sundus Hameed Ahmed^{1*}, Alyaa Mouhsen Yousif¹, Hashim Kadhim Mohammed¹, Rasha Sattam Hameed¹, Khaled F. M. Salem²

¹Biology Department, College of Science, Mustansiriyah University, Baghdad, Iraq

²Sadat University, Iraq

*Corresponding Author: Sundus Hameed Ahmed

Email: drsundusahmed@uomustansiriyah.edu.iq

Biology Department, College of Science, Mustansiriyah University, Baghdad, Iraq

Article History

Received: 07.07.2024

Accepted: 16.08.2024

Published: 20.08.2024

Abstract: According to the best germination percentage and its rate of *TriticumAestivum* L (Wheat) seeds treated with Amoxicillin 50mg/ml gave the highest germination percentage of 100% and the highest germination rate 0.6, also it gave highest scavenging free radicals of DPPH with a mean percentage of 82.07%. The GC-MAS tests for the control model and wheat plant treated with the antibiotic amoxicillin at concentrations of 25 and 50 mg. It was found that only two materials present in the three models under study: such as Phytol and gamma.-Sitosterol.

Keywords: Amoxicillin, DPPH, GC-MAS, antibiotic.

INTRODUCTION

TriticumAestivum Lis the most widely grown cereal grain in the world and is essential to agriculture [1-4]. It is cultivated in 70% of the world's cultivated regions and provides the primary nutrition for 36% of the world's population [5, 6]. Approximately 55% of the carbs and 21% of the calories consumed globally come from wheat [6, 7]. Being cultivated in a wide range of climatic conditions and surpassing all other grain crops (such as rice, maize, etc.) in terms of productivity and acreage, it is the most significant grain crop on the planet [8, 9]. Among the cereals, wheat is the most significant primarily due to its grains, which contain unique protein properties both chemically and physically. It also has additional beneficial elements, likes (Cu, Mg, Zn, Fe, and P), It is a good source of protein, carbs, and vitamins (riboflavin, thiamine, niacin, and alpha-tocopherol) [10, 11]. Nevertheless, it has been discovered that essential amino acids like lysine and threonine are absent from wheat proteins [12-14].

The introduction of new and improved varieties that can give higher yields and perform better across a range of agro-climatic stresses and circumstances may be a means of improving wheat production and quality [15]. Everyone agrees that one of the most important aspects of plant breeding is the diversity of germplasm in the breeding material [16, 17].

Antibiotics work by either directly eliminating bacteria or preventing their growth in order to treat illnesses in both humans and animals [18]. Animals' stomachs do not absorb most antibiotics well, and up to 90% of them may be eliminated [19]. Some antibiotics are very stable in manure and soil, with residues still detectable a year after application [20]. These antibiotics may be discharged into the environment by grazing animals or dung. Certain antibiotics have the potential to last for several years [21]. For instance, tetracycline and sulfadiazine were found at average soil concentrations of 10–15 µg kg⁻¹ and 32–198 µg kg⁻¹, respectively, in agricultural landscapes with typical land use and manure fertilization [22]. Antibiotics may then be carried from the farmlands to ditches, streams, and rivers by runoff [23], and to groundwater by leaching (24) or may be consumed by organisms directly [25]. The potential negative impacts of antibiotics ingested by agricultural plants on human health have been thoroughly studied [26] but considerably less research has been done on the impact of antibiotics on plants, especially non-crop species. Antibiotics have been shown to significantly impair plant growth and performance [27]; on the other hand, they may potentially stimulate allomet-ric responses.

Copyright © 2024 The Author(s): This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC BY-NC 4.0) which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

Citation: Sundus Hameed Ahmed, Alyaa Mouhsen Yousif, Hashim Kadhim Mohammed, Rasha Sattam Hameed, Khaled F. M. Salem (2024). Effect of Amoxicillin Antibiotic on Germination Rate and Active Components of *Triticumaestivum* L. (Wheat) Seeds. *South Asian Res J App Med Sci*, 6(4), 98-105. 98

The study aims to investigate the following: the impact of the antibiotic amoxicillin on wheat seed germination; the active ingredients in wheat models treated with the antibiotic and their comparison to the control; and the antibacterial and antioxidant efficacy of germinated seed extracts.

MATERIALS AND METHODS

The purpose of the study was to ascertain how the antibiotic amoxicillin affected the germination of seeds. Seed germination prior to germination, the seeds were steeped in 70% ethanol for around two minutes. They were then rinsed numerous times in sterile water to remove any last traces of alcohol (ethanol), and finally submerged in sterile water together with amoxicillin (25–50 mg/ml).

The germination rate was calculated according to:

$$\text{Percentage of Germination rate (GP\%)} = \frac{GN}{SN} \times 100 \dots\dots 1$$

Where GN denotes the number of wheat seeds that germinated and SN is the number of tested wheat seeds. The equation that follows: Ten seeds each were allowed to germinate for five days at 25 °C in a dark atmosphere on a Petri plate. The length of the shoot and roots was then measured in order to assess the germination rate.

Extract Preparing

Following a 4-day soak in 400 mL of water (v/v) at room temperature (28 C), all of the germinated seeds from the treated plant and the control were dried in an oven at 40 C and ground into powder using a grinder. Cheesecloth and Whatman filter paper were used to filter out the dry leaves. A rotary evaporator was then used to further concentrate the filtrate. Glass Petri dishes were used to hold all of the extracts. After being dried, the plant extracts were redissolved in water to create a solution that had 3 mg/mL of extract, which was subsequently utilized for tests.

Activity of Antioxidants (DPPH test)

All extracts were evaluated for their antioxidant activity (free radical scavenging activity) using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) test. First, 5 mL of a 0.004% (w/v) DPPH in methanol solution was mixed with 50 µL of 3.0 mg/mL extracts (70% methanol as blank). Using the dark box, this was incubated in the dark for 30 minutes. Every sample that was computed using the following formula underwent the same process:

RESULT AND DISCUSSION

Table 1A presents the percentage of germination, was calculated according to Equation 1 as well as the actual measurements Based on Table 1A, it is noticed that the best solutions for wheat seed germination are Amoxicillin 50mg/ml. According to the best germination percentage and its rate, both of them had the highest germination percentage of 100% and the highest germination rate of 0.6.

Table 1A: Germination percentage and germination rate

No.	Treatment (GP %) GR	Treatment (GP %)	GR
1	Control	84.32	0.4
2	Amoxicilline 50mg/ml	100	0.6
3	Amoxicilline 25mg/ml	58.54	0.34

Figure 1, indicates a significant difference of mean percentage scavenging between all the tested extracts (Treated wheat plant with 50mg/ml, treated wheat plant with 25mg/ml, 3: Control, 4: Ascorbic Acid. The results showed that extract of treated wheat plant with 500mg/ml exhibited the greatest free radical scavenging activity among the plant extracts with a mean percentage of 82.07%. On the other hand, the scavenging activity of treated wheat plant with 250mg/ml and not treated wheat plant extracts (control) was 52.31%, control 72,18% and Ascorbic Acid 92.86% (standard), respectively. It should be emphasized that toxic effect of the antioxidant and antimicrobial agent on the host cells must be considered, as a substance may exhibit antioxidant and antimicrobial activity by virtue of its toxic effect on the cells [22].

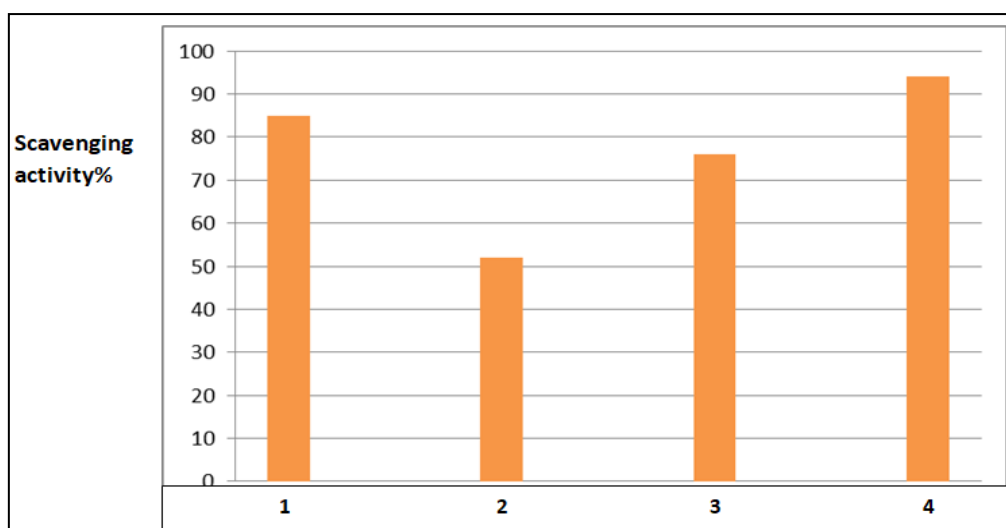


Figure 1: Scavenging effect (%) of plant extracts and known, 1: treated wheat plant with 50mg/ml, 2: treated wheat plant with 25mg/ml, 3: Control, 4: Ascorbic Acid

To evaluate the scavenging effect of the extracts in this study, DPPH reduction was investigated against positive controls (Ascorbic acid). The more antioxidants occurred in the extract, the more DPPH reduction will occur. High reduction of DPPH is related to the high scavenging activity performed by particular sample treated with 50mg/ml amoxicillin compared with other treatment [20]. Tables (1 and 2) showed the results of the GC-MAS tests for the control model of the wheat plant treated with the antibiotic amoxicillin at concentrations of 25 and 50 mg. It was found that new medicinal substances appeared in treated plant differed from the active ingredients originally present in the control wheat plant, the highest peaks appeared in GC-MAS tests as shown: Phytol, 35.40% (anticancer, anti-inflammatory, antimicrobial, diuretic), Methyl 9,12,15-octadecatrienoate, 15.36% (Anti-microbial), Methyl palmitate 9.32% (antioxidant, hypocholesterolemic, antiandrogenic, Hemolytic [21].

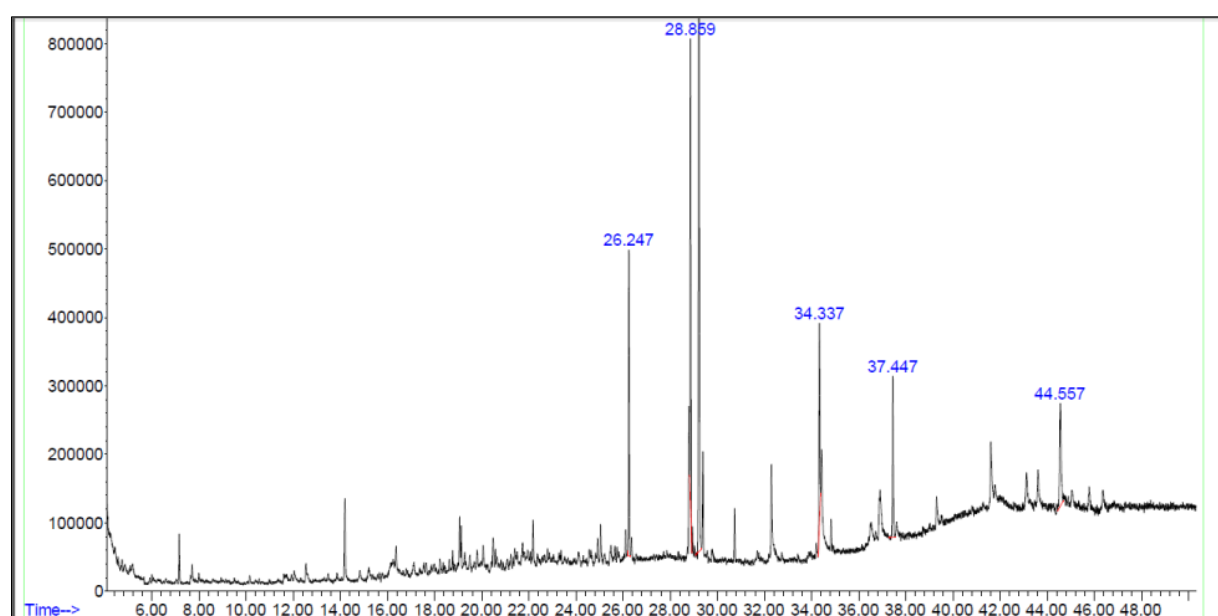
Seed extract treated with amoxicillin 50mg/ml, Phytol, 30.185% (anticancer, anti-inflammatory, antimicrobial, diuretic), Methyl linolenate, 14.76% Antiinflammatory, Hypocholesterolemic, Cancer preventive Hepatoprotective, Nematicide, Insectifuge Antihistaminic, AntieczemicAntiacne [22], 5-Alpha reductase inhibitor Antiandrogenic, AntiarthriticAnticoronary, (9-Octadecenamamide, 9.10% (Moisturiser for skin, nails, and hair Hepatoprotective, antihistaminic, hypocholesterolemic, anti-eczemic [23] (Amoxicilene) seed extract treated with amoxicilene 25mg/ml. Phytol 32.87%, 9-Octadecenamamide, (Z)-, 17.77% (Anti inflammatory, Antiandrogenic, Cancer preventive, Dermatitogenic, Hypocholesterolemic [24], 5- Alpha reductase inhibitor, Anemiagenic, Insectifuge).

Methyl9,12,15-octadecatrienoate), 11.34% (Antiinflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge Antihistaminic, Antiarthritic, Anticoronary, AntieczemicAntiacne [25], 5-Alpha reductase inhibitor Antiandrogenic, It was found that there are two materials present in the three models under study: such as Phytol and gamma.-Sitosterol [26].

Table 1: GC- Mass of control germinated seed extract

No	RT (min)	Area %	Name	Quality	Bioactivity
1	14.189	4.51	2-Propenoic acid, 2-methyl-, 1,2-ethanediyl ester	40	Antimicrobial and antioxidant properties Mujeeb <i>et al.</i> , (2014)
2	26.247	9.32	Methyl palmitate	98	Antioxidant, Hypocholesterolemic, Antiandrogenic, HemolyticGc15
3	28.8	4.22	9,12-Octadecadienoic acid (Z, Z)-, methyl ester	99	Moisturiser for skin, nails, and hair3
4	28.857	15.36	Methyl 9,12,15-octadecatrienoate	98	Antibacterial, anticancer, anticandidal, anti-inflammatory, hypocholesterolemic, hepatoprotective,3
5	28.94	0.80	Methyl trans-8-octadecenoate	96	Anti-microbial 5
6	29.023	0.38	Dihydro-.beta.-agarofuran	43	

No	RT (min)	Area %	Name	Quality	Bioactivity
7	29.215	35.40	Phytol	93	Anticancer, anti-inflammatory, antimicrobial, diuretic effects Bharathy <i>et al.</i> , (2012) 2 Antimicrobial, Anti-inflammatory, Anticancer, Diuretic, Antifungal against <i>S. typhi</i> , resistant gonorrhoea, joint dislocation, headache, hernia, stimulant and antimalarial 4
8	29.387	3.21	Octadecanoic acid, methyl ester	97	antimicrobial activity Kannan & Kannan (2019) 2Hepatoprotective, antihistaminicGc19
9	34.331	9.79	alpha.-Monopalmitin	43	
10	34.419	4.76	1-Heneicosyl formate	81	
11	37.444	5.60	Diisooctyl phthalate	16	Antimicrobial, Antifouling4Anti-carcinogenic, Used in skin treatments and Cosmetic products 5
12	44.558	6.66	gamma.-Sitosterol	91	Anticancer, antimicrobial, antidiabetic, antifertility, antioxidant 5

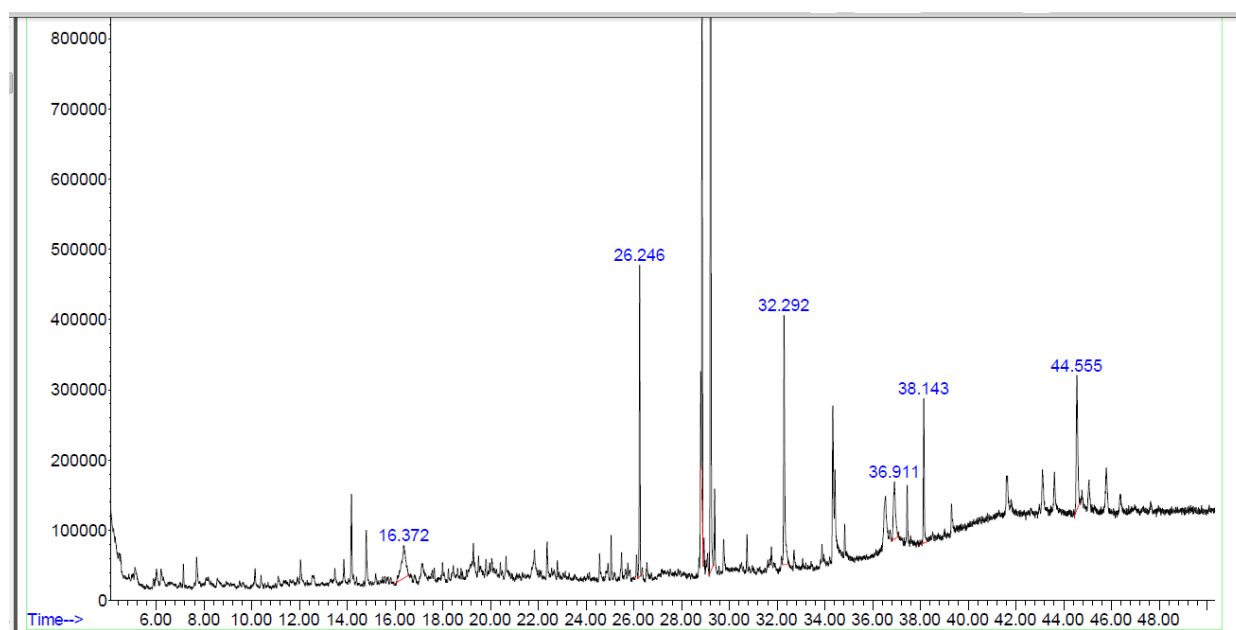


GC-Mass for Control germinated seed extract

Table 2: GC- Mass of control germinated seed extract T 50 mg/ml

No	RT (min)	Area %	Name	Quality	
1	14.173	2.59	2-Methoxy-4-vinylphenol	96	Antimicrobial, antioxidant, anti-inflammatory, analgesic, anti-germination
2	14.796	2.54	4-Methylproline methyl ester	64	
3	16.373	4.63	D-Fructose, 1-O-methyl-	22	
4	26.247	7.78	Methyl palmitate	98	Antioxidant, Hypocholesterolemic, Antiandrogenic, Hemolytic
5	28.8	4.98	9,12-Octadecadienoic acid (Z, Z)-, methyl ester	99	Moisturiser for skin, nails, and hair Hepatoprotective, antihistaminic, hypocholesterolemic, anti-eczemic
6	28.857	14.76	Methyl linolenate	99	Cosmetic, Coloring agent. Antiinflammatory, Hypocholesterolemic, Cancer preventive HepatoprotectiveNematicide, Insectifuge Antihistaminic, AntieczemicAntiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge
7	28.935	0.73	Methyl oleate	87	Antiinflammatory, Antiandrogenic Cancer preventive, Dermatitogenic

No	RT (min)	Area %	Name	Quality	
					Hypocholesterolemic, 5-Alpha reductase inhibitor, Anemiagenic Insectifuge, Flavor
8	29.034	0.25	trans-.delta.(sup 9)-Octadecenoic acid	91	Emulsifying agent Anti-cancer
9	29.091	0.45	2,5-Diaminobenzophenone	42	
10	29.215	30.18	Phytol	90	anticancer, anti-inflammatory, antimicrobial, diuretic effects
11	29.381	2.24	Octadecanoic acid, methyl ester	98	Anti-microbial 5 Anti-cancer5 Cosmetic, Flavour, Hypocholesterolemic, Lubricant, Perfumery, Propepic, Suppository. Gc25
12	32.292	9.10	9-Octadecenamide, (Z)-	87	Anti inflammatory, Antiandrogenic, Cancer preventive, Dermatitigenic, Hypocholesterolemic, 5-Alpha reductaseinhibitor, Anemiagenic, Insectifuge 9
13	36.91	4.14	1,3-dimethyl-4-azaphenanthrene	38	
14	38.145	4.31	Bis(2-ethylhexyl) sebacate	50	
15	41.606	2.47	Cyclotrisiloxane, hexamethyl-	74	No activity reported 11
16	43.11	2.33	Cyclotrisiloxane, hexamethyl-	72	
17	44.553	6.53	gamma.-Sitosterol	91	Anticancer, antimicrobial, antidiabetic, antifertility, antioxidant 5

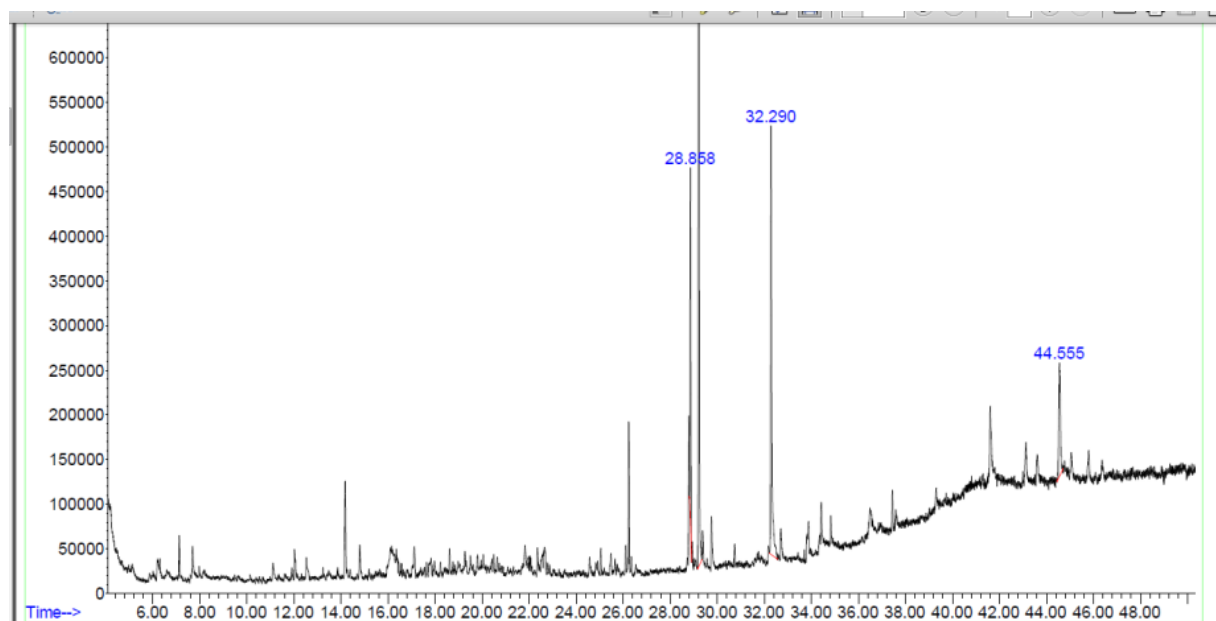


GC- Mass of control germinated seed extract T 50 mg/ml

Table 3: GC- Mass of control germinated seed extract T25

No	RT (min)	Area %	Name	Quality	
1	14.189	5.61	2-Propenoic acid, 2-methyl-, 1,2-ethanediyl ester	47	Antibacterial, flavor 7
2	25.054	1.02	.beta.-Citronellol	55	
3	25.48	0.91	Cyclotetradecane	98	
4	26.237	5.28	Pentadecanoic acid, 14-methyl-, methyl ester	98	
5	28.8	4.04	Methyl linoleate	99	Antiinflammatory, Hypocholesterolemic, Cancerpreventive Hepatoprotective Nematicide, InsectifugeAntihistaminic, AntieczemicAntiacne, 5-Alphareductase inhibitorAntiandrogenic, Antiarthritic,

No	RT (min)	Area %	Name	Quality	
					Anticoronary, InsectifugeGc13
6	28.858	11.34	Methyl 9,12,15-octadecatrienoate	99	Antiinflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge Antihistaminic, Antiarthritic, Anticoronary, AntieczemicAntiacne, 5-Alpha reductase inhibitor Antiandrogenic 17
7	28.941	0.52	trans-.delta.(sup 9)-Octadecenoic acid	90	Emulsifying agent, 1Anti-cancer5
8	29.216	32.87	Phytol	90	anticancer, anti-inflammatory, antimicrobial, diuretic effects Bharathy <i>et al.</i> , (2012) 2 Antimicrobial, Anti-inflammatory, Anticancer, Diuretic, Antifungal against <i>S. typhi</i> , <i>resistant gonorrhoea</i> , <i>joint</i> dislocation, headache, hernia, stimulant and antimalarial 4can be used as a precursor for the manufacture of synthetic forms of vitamin E and vitamin K1gc 12
9	29.76	2.33	Hexadecanamide	94	Antiinflammatory, Antialopepic, Antifibrinolytic, Lubricant, Antiandrogenic, flavour, Hemolytic, Antioxidant, Hypocholesterolemic, Nematicide, Pesticide,5-Alpha-reductase inhibitor. Ge 25
10	32.292	17.77	9-Octadecenamide, (Z)-	96	Anti inflammatory, Antiandrogenic, Cancer preventive, Dermatitigenic, hypocholesterolemic, 5-Alpha reductaseinhibitor, Anemiagenic, Insectifuge 9
11	34.425	4.56	Cyclohexane, 1-(1,5-dimethylhexyl)-4-(4-methylpentyl)-	45	
12	37.44	1.66	N-ethyl-1,3-dithioisindoline	53	
13	41.606	5.42	Cyclotrisiloxane, hexamethyl-	64	No activity reported 11
14	44.553	6.66	.gamma.-Sitosterol	91	Anticancer, antimicrobial, antidiabetic, antifertility, antioxidant 5



GC- Mass of control germinated seed extract T25

Table 4 showed comparison between control and treated seed with 50mg/ml and 25mg/ml, we found that only two materials present in the three models under study: such as Phytol and gamma.-Sitosterol

Table 4: Comparison between active component of treated Wheat plant samples

No	Control	T.Amox.50/ml	T.Amox.25/ml
1	2-Propenoic acid, 2-methyl-, 1,2-ethanediyl ester	2-Methoxy-4-vinylphenol	2-Propenoic acid, 2-methyl-, 1,2-ethanediyl ester
2	Methyl palmitate	4-Methylproline methyl ester	.beta.-Citronellol
3	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	D-Fructose, 1-O-methyl-	Cyclotetradecane
4	Methyl 9,12,15-octadecatrienoate	Methyl palmitate	Pentadecanoic acid, 14-methyl-, methyl ester
5	Methyl trans-8-octadecenoate	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Methyl linoleate
6	Dihydro-.beta.-agarofuran	Methyl linolenate	Methyl 9,12,15-octadecatrienoate
7	Phytol	Methyl oleate	trans-.delta.(sup 9)-Octadecenoic acid
8	Octadecanoic acid, methyl ester	trans-.delta.(sup 9)-Octadecenoic acid	Phytol
9	.alpha.-Monopalmitin	2,5-Diaminobenzophenone	Hexadecanamide
10	1-Heneicosyl formate	Phytol	9-Octadecenamide, (Z)-
11	Diisooctyl phthalate	Octadecanoic acid, methyl ester	Cyclohexane, 1-(1,5-dimethylhexyl)-4-(4-methylpentyl)-
12	.gamma.-Sitosterol	9-Octadecenamide, (Z)-	N-ethyl-1,3-dithioisindoline
13	Control	1,3-dimethyl-4-azaphenanthrene	Cyclotrisiloxane, hexamethyl-
14	2-Propenoic acid, 2-methyl-, 1,2-ethanediyl ester	Bis(2-ethylhexyl) sebacate	.gamma.-Sitosterol
15		Cyclotrisiloxane, hexamethyl-	Amox.250
16		.gamma.-Sitosterol	2-Propenoic acid, 2-methyl-, 1,2-ethanediyl ester
17			.beta.-Citronellol

CONCLUSIONS

It was discovered that only two materials were consistently present across all three models under study: Phytol and gamma-Sitosterol. This finding highlights the commonality of these two compounds despite the variations in the other components analyzed in each model.

REFERENCES

- Kumar, S., Kumari, P., Kumar, U., Grover, M., Singh, A. K., Singh, R., & Sengar, R. S. (2013). Molecular approaches for designing heat tolerant wheat. *Journal of plant biochemistry and biotechnology*, 22, 359-371. doi: 10.1007/s13562-013-0229-3
- Nawaz, R., Ahmad, H., Din, S. U., & Iqbal, M. S. (2013). Agromorphological studies of local wheat varieties for variability and their association with yield related traits. *Pak J Bot*, 45, 1701-1706.
- Kizilgeci, F., Yildirim, M., Islam, M. S., Ratnasekera, D., Iqbal, M. A., & Sabagh, A. E. (2021). Normalized difference vegetation index and chlorophyll content for precision nitrogen management in durum wheat cultivars under semi-arid conditions. *Sustainability*, 13(7), 3725. doi: 10.3390/su13073725
- Khalid, A., Hameed, A., Shamim, S., & Ahmad, J. (2022). Divergence in single kernel characteristics and grain nutritional profiles of wheat genetic resource and association among traits. *Frontiers in Nutrition*, 8, 805446. doi: 10.3389/fnut.2021.805446
- Mohammad Nisar, M. N., Naeem Khan, N. K., Nausheen, N., Zakia Ahmad, Z. A., & Abdul Ghafoor, A. G. (2011). Genetic diversity and disease response of rust in bread wheat collected from Waziristan Agency, Pakistan. *Int J Biodivers Conserv*, 3, 10-18. doi: 10.5897/IJBC.9000067
- Riaz, M. W., Yang, L., Yousaf, M. I., Sami, A., Mei, X. D., Shah, L., ... & Ma, C. (2021). Effects of heat stress on growth, physiology of plants, yield and grain quality of different spring wheat (*Triticum aestivum* L.) genotypes. *Sustainability*, 13(5), 2972.
- Ward, H. A., Musa, T. M., & Nasif, Z. N. (2022). Synthesis and Characterization of some transition metals complexes with new ligand azo imidazole derivative. *Al-Mustansiriyah Journal of Science*, 33(2), 31-38.

8. Ali, A., Khaliq, T., Ahmad, A., Ahmad, S., Malik, A. U., & Rasul, F. (2012). How wheat responses to nitrogen in the field. *A review. Crop and Environment*, 3(1-2), 71-76.
9. Aswad, O. A. (2022). Evaluation some Polycyclic Aromatic Hydrocarbons (PAHs) in Eucalyptus Camaldulensis and Phragmites Australis Plants Around the Diyala-Baghdad Bridge. *Al-Mustansiriyah Journal of Science*, 33(2), 14-18.
10. Sharma, S., Shrivastav, V. K., Shrivastav, A., & Shrivastav, B. R. (2013). Therapeutic potential of wheatgrass (*Triticum aestivum* L.) for the treat-ment of chronic diseases. *South Asian Journal of Experimental Biology*, 3(6), 308-313.
11. Garg, M., Sharma, A., Vats, S., Tiwari, V., Kumari, A., Mishra, V., & Krishania, M. (2021). Vitamins in cereals: a critical review of content, health effects, processing losses, bioaccessibility, fortification, and biofortification strategies for their improvement. *Frontiers in nutrition*, 8, 586815.
12. Ikhtiar, K., & Alam, Z. (2007). Nutritional composition of Pakistani wheat varieties. *Journal of Zhejiang University Science B*, 8(8), 555-559. doi: 10.1631/jzus.2007.B0555
13. Urade, R., Sato, N., & Sugiyama, M. (2018). Gliadins from wheat grain: An overview, from primary structure to nanostructures of aggregates. *Biophysical reviews*, 10, 435-443. doi: 10.1007/s12551-017-0367-2
14. Mohamad, I. (2019). Determination and validation of tetracycline residues in poultry by high performance liquid chromatography-diode array detector technique. *Al-Mustansiriyah Journal of Science*, 30(3), 38-46.
15. Siddiqi, R. A., Singh, T. P., Rani, M., Sogi, D. S., & Bhat, M. A. (2020). Diversity in grain, flour, amino acid composition, protein profiling, and proportion of total flour proteins of different wheat cultivars of North India. *Frontiers in Nutrition*, 7, 141. doi: 10.3389/fnut.2020.00141
16. Hassan, G. H. U. L. A. M., & Gul, R. A. H. M. A. N. I. (2006). Diallel analysis of the inheritance pattern of agronomic traits of bread wheat. *Pak. J. Bot*, 38(4), 1169-1175.
17. Jassim, A. H., & Al-Newani, H. R. H. (2023, December). Chemotaxonomy Significant in Delimitation of Six Taxa of *Euphorbia* L. in Iraq. In *IOP Conference Series: Earth and Environmental Science* (Vol. 1262, No. 5, p. 052019). IOP Publishing.
18. Al-Newani, H. R. H. (2019). Systematics significance of morphological and anatomical diversity of *Portulaca oleracea*. *Iraqi Journal of Agricultural Sciences*, 50(5), 1383–1389.
19. Bibi, K., Inamullah, H. A., Din, S., Muhammad, F., & Iqbal, M. S. (2012). Characterization of Wheat genotypes using randomly amplified polymorphic DNA markers. *Pak. J. Bot*, 44(5), 1509-1512.
20. Khalid, A., Hameed, A., & Tahir, M. F. (2022). Estimation of genetic divergence in Wheat genotypes based on agromorphological traits through agglomerative hierarchical clustering and principal component analysis. *Cereal Res Commun*, 50, 1–8. doi: 10.1007/s42976-022-00287-w
21. Hameed, R. S., Mohammad, M. K., Ahmed, S. H., & Al-Kharkhi, I. H. T. (2021). Synthesis of New Iron Nanoparticles derived from Beta Vulgaris Extract and its Bioactivity against *Enterobacter cloacae*, Anti-inflammatory and House Fly. *Research Journal of Pharmacy and Technology*, 14(9), 4887-4890.
22. Ahmed, S. H., Nuaman, R. S., Al Newani, H. R. H., & Yousif, A. M. (2023). The Effect of Nanoparticles and Plant Growth Regulators on Germination of Miazee Seeds: A Comparative Study. *Journal of Medicinal and Chemical Sciences*, 6(6), 1246-1253.
23. Kemper, N. (2008). Veterinary antibiotics in the aquatic and terrestrial environment. *Ecological indicators*, 8(1), 1-13.
24. Guo, Y. (2021). Allelopathy of *Eupatorium adenophorum* extracts on seed germination and seedling growth of different strawberry varieties. *Seed*, 40(6), 96–101.
25. Deb, P., & Sundriyal, R. C. (2017). Effect of seed size on germination and seedling fitness in four tropical rainforest tree species. *Indian Journal of Forestry*, 40(4), 313-322.
26. Domic, A. I., Capriles, J. M., & Camilo, G. R. (2020). Evaluating the fitness effects of seed size and maternal tree size on *Polylepis tomentella* (Rosaceae) seed germination and seedling performance. *Journal of Tropical Ecology*, 36(3), 115-122.