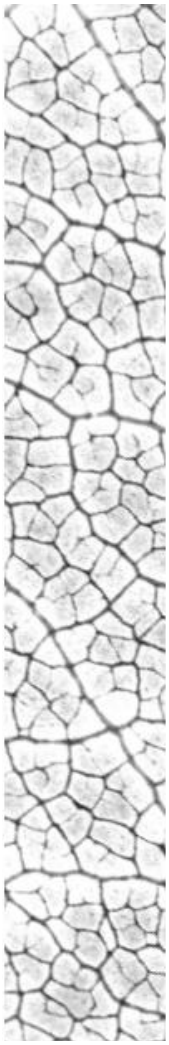

EurAsian Journal of BioSciences
Eurasia J Biosci 14, 161-166 (2020)

The synergistic effect of fungus filter *Aspergillus terreus* and aqueous extract of *Fucus vesiculosus* on some growth characteristics of the *ocimum basilicum* and its content of active substances

Abdul-Hameed M. Hamoody [1*], Jwan. N. Abood [1], Batol I. Dheeb [2]

[1] Biology Department, College of Education, Samarra University, IRAQ
[2] College of Applied Science, Samarra University, IRAQ
*Corresponding author: drhameed57@gmail.com

Abstract
This study included the synergistic effect of *A. terreus* fungi and aqueous extract of *F. vesiculosus* on some growth qualities of basil plant and its content of active substances. Three concentrations of (25%, 50% and 75%) were tested. The highest values of germination and plant height were at the concentration 50% (89.6%, and 19.5cm respectively). The number of plants branches, soft and dry weight was 75%. The highest value was 6.3 branches, 1.6 g and 0.73 g respectively, and the use of the mixture at a concentration of 75% was best in increasing the plant content of flavonoids, phenols, tannins, carbohydrates and lipids, with the highest value of 9.7%, 542.8 mg / 100 g and 541 mg / 100 g, 89.4% and 4.7%, respectively.

Keywords: aspergillus terreus, aqueous extract of fucus vesiculosus, active substances

Hamoody AM, Abood JN, Dheeb BI (2020) The synergistic effect of fungus filter *Aspergillus terreus* and aqueous extract of *Fucus vesiculosus* on some growth characteristics of the *ocimum basilicum* and its content of active substances. Eurasia J Biosci 14: 161-166.

INTRODUCTION

Basil is a widespread annual plants in tropical, subtropical and temperate regions of the world, especially West Asia, Africa and the Pacific Islands (Sabnis 1986, Kirtikar and Basu 1961). It is belong to Lamiaceae family plants, up to a height of 50-60 cm (El-Gendy et al. 2001) it has simple leaves embraced oval-shaped and side flowers symmetrical grouping inflorescences (Al-Hassani and Tahani 1990). It has many medical uses. It works as a colic, cough, digestion tonic, antiseptic gum and antispasmodic (HARRIS 2000) because it contains a large number of effective medicinal compounds, including glycosides, alkaloids and turbines. Its high content of vitamin A and C make it very useful to protect cells from damage (Small 1997).

Recent studies have shown that excessive use of chemical fertilizers has led to the emergence of various diseases, prompting the consumer to move towards the consumption of organically produced foods because it is safer health and free of pathogens if the high content of nitrates and accumulation in the human body leads to the formation of the compound Nitrosamin it represents cancer-causing raw material, fetal abnormalities and genetic mutations (Seanclark et al. 1998). One of the ways to improve soil quality and support plant growth is the use of microorganisms, including fungi. Many studies have shown that fungi, whether used in biological resistance or unused, have a role in germination and stimulate plant growth, especially species of the genus *Aspergillus,* which possesses high enzymatic capabilities From the analysis of complex carbohydrates and converted to simple form (Dhillion 1994) It also produces plant-like hormones compounds increase the susceptibility of roots in the absorption of nutrients (Sharitamadari 2011) Many studies have revealed useful applications in the effect of algae extracts on plants such as plant germination in early time and improve the performance of crops and its products, and elongated the power of products can be harmful after the harvest (Sengar et al. 2010, Szabo and Hrotko 2009).

This study aimed to investigate the effect of synergy between fungus *Aspergillus terreus* and aqueous extract of *Fucus vesiculosus* in the growth of basil plant and its content of active substances.

Received: July 2019
Accepted: February 2020
Printed: February 2020

MATERIALS AND METHODS

The experiment was carried out in the glass house of the Department of biology, University of Samarra. plastic pots was used for this purpose containing 2 kg of sterile mixture soil planted with seeds of basil plant by 15 seeds per pot, and five replicates per treatment were transactions as follows:

1- Control: Treatment of plant with distilled water.

2- Soil with filter solution which was added at a concentration of 25%, 50% and 75%, two weeks after the date of planting and the amount of 150 ml per 1 kg soil and repeated the process after two weeks of the first addition

3- Soil with algae extract which was added at a concentration of 25%, 50% and 100%, two weeks after the date of planting and the amount of 150 ml per 1 kg soil and repeated the process after two weeks after the first addition.

4- Soil with mixture of fungus filter and algae extract which was added by mixing equal proportions of each concentration, after two weeks after the date of planting and the amount of 150 ml per 1 kg soil and repeated the process after two weeks after the first addition.

Prepare Fungus Filter

The medium potato dextrose broth was use for this purpose and was prepared according to the manufacturer's instructions, then distributed in 250 ml glass jars and sterilized in autoclave at degree of (121) and pressure 1 atm and after sterilization and cooler, it was vaccine using a cork piercer colony diameter (4 mm) taken from a colony aged a week on a PDA medium, the flask was placed in a 25 °C incubator for 28 days, shaking the flask every 3 days, then filtered using Watman NO1 filter paper, then re-filtered using a 0.22 milipore filter type nylon, and kept in the refrigerator until use.

Preparation of Aqueous Extract of Algae

The algae was obtained from the local markets of Samarra city and the water extract of fucus algae was prepared to obtain of each concentration, 25 g, 50 g and 100 g were obtained respectively from the algae powder and mixed with 100 ml of distilled water to obtain concentrations of 25%, 50% and 100%. Place each mixture in the electric mixer for half an hour, then leave for 24 hours at laboratory temperature, then filtered with three layers of gauze, then Watman filter paper NO1. Sterilized using microbial membranes and placed in tight and sterile bottles and stored in the freezer until use (Mersie and Singh 1987b).

Preparation of Concentrations of Fungus Filter

Concentrations used in the experiment were prepared according to the dilution law to obtain concentrations of 25%, 50% and 100%.

Table 1. Effect of different concentrations of fungus filtrate and algae extract and their mixture on seed germination percentage (%)

Concentrations%	fungus filter	aqueous extract	mixture	Control
25.0	82.7b±5.1	70.7c±5.1	87a±5.1	82.1 b±5.1
50.0	86.7b±5.5	82.3c±5.5	89.6a±5.5	82.1 c±5.5
75.0	88a±5.2	76.3c±5.2	87.4a±5.2	82.1 b±5.2

*numbers with small similar characters in the row have no significant differences according to Duncan polynomial test at probability level 5%
* Each number represents a rate of 3 replicates.

Prepare the Mixture of Fungus Filter Solution and Algae Extract

Prepare a known volume of fungus leachate at a concentration of 25% to a similar volume at a concentration of 25%. and so as for all dilutions with shaking for tow mints to mix well.

Estimation of Seed Vitality

30 seeds of basil was took and sterilized with 5% sodium hypochlorite solution and washed with distilled water several times, and placed 10 seeds in each dish (3 plates) containing sterile filter paper and incubated dishes at 25 ° C under dark conditions for 7 days with the addition of sterile distilled water to the seeds. The vitality were estimated according to the following equation:

$$\text{Germination percentage} = \frac{number\ of\ germenation\ seeds}{total\ number\ of\ seeds} \times 100$$

Measurement of Plant Height

Plant height was measured from the soil surface to the highest point in the main branch using the ruler.

The number of branches in each stem was calculated.

Dry and Soft Weight of the Plant

The soft weight of the plant was taken two months after the date of planting and then the plants were dried at 80 °C and weighed using a sensitive balance to know the dry weight of the plant.

Determination of Chemical Compounds in Plants

1- Determination of flavonoid and tannin content: Determined according to (Boham) (Boham and Kocipai 1994)

2- Determination of plant phenol content was determined Spectrophotometrically according to (Mattila) (Mattila and Hellström 2007)

3- Determination of plant carbohydrate content: Spectrophotometrically according to Thayumanavan (Thayumanavan and Sadasivam 1984)

4- The lipid content of the plant was determined according to (Chopra). (Chopra and Kanwar 1991)

RESULTS AND DISCUSSION

The results showed that filtrate of *A. terreus* and aqueous extract of *F. vesiculosus* have effects on germination percentage (**Table 1**) for the different

Table 2. Effect of different concentrations of fungus filtrate and algae extract and their mixture on plant height (cm)

Concentrations%	fungus filter	aqueous extract	mixture	Control
25.0	14 b±1.7	13.5c±1.7	15.5a±1.7	9.3d±1.7
50.0	19.3a±1.4	15.6 b±1.4	19.5a±1.4	9.3c±1.4
75.0	19.1a±1.6	15.8b±1.6	18.8a±1.6	9.3c±1.4

*numbers with small similar characters in the row have no significant differences according to Duncan polynomial test at probability level 5%
* Each number represents a rate of 3 replicates.

Table 3. Effect of different concentrations of fungus filtrate and algae extract and their mixture on number of plant branches

Concentrations%	fungus filter	aqueous extract	mixture	Control
25.0	3.5b±0.4	3.7b±0.4	4.7a±0.4	3.3b±0.4
50.0	5.5a±0.3	4.6b±0.3	5.7a±0.3	3.3c±0.3
75.0	6.1a±0.4	4.4b±0.4	6.3a±0.4	3.3c±0.4

*numbers with small similar characters in the row have no significant differences according to Duncan polynomial test at probability level 5%
* Each number represents a rate of 3 replicates.

Table 4. Effect of different concentrations of fungus filtrate and algae extract and their mixture on plant soft weight (g)

Concentrations%	fungus filter	aqueous extract	mixture	Control
25.0	1.3a±0.1	1.1b±0.1	1.4a±0.1	0.6c±0.1
50.0	1.4a±0.2	1.2b±0.2	1.5a±0.2	0.6c±0.2
75.0	1.3a±0.2	1.4b±0.2	1.6a±0.2	0.6c±0.2

*numbers with small similar characters in the row have no significant differences according to Duncan polynomial test at probability level 5%
* Each number represents a rate of 3 replicates.

concentrations. The highest germination percentage was obtained when the mixture was used at a concentration of 50% (89.6 germination), while the lowest germination rate was 25.7% when the extract was used at 70.7% compared to the control treatment (82.1%).

Table 2 shows the effect of the treatments on plant height. The results showed that the concentration of 50% for the filter and extract ant their mixture is the best in increasing the high of plant comparative with control treatment. The effect of using the filter alone didn't show any significant differences comparative with the using the mixture, while there were significant differences with the using of extract alone and control treatment at the same concentration. The highest length of plant was 19.5 cm when treated with 50% concentration. The lowest length was 13.5 cm when using the extracted with (25% concentration) compared to control treatment.

The results of the effect of treatments on the number of branches of the plant (**Table 3**) showed that the concentration of 25% gives a significant difference only when treated with the mixture. While there was no significant differences when using the mixture at the concentration (50 and 75%). But there was significant differences when use the extract alone. The highest number of plant branches was 6.3 branches when the mixture was using at a concentration of 75%, while the lowest number of plant branches was 3.7 when the using of extract treatment at concentration 25% comparative to control treatment which was 3.3 branches.

Table 4 shows the effect of treatments on the soft weight of the plant. The results showed that the addition

Table 5. Effect of different concentrations of fungus filtrate and algae extract and their mixture on dry weight of plant (g)

Concentrations%	fungus filter	aqueous extract	mixture	Control
25.0	0.33a±0.2	0.20 b±0.2	0.31a±0.2	0.11b±0.2
50.0	0.4b±0.1	0.22c±0.1	0.5 a±0.1	0.1c±0.1
75.0	0.48b±0.1	0.3c±0.1	0.73 a±0.1	0.1d±0.1

*numbers with small similar characters in the row have no significant differences according to Duncan polynomial test at probability level 5%
* Each number represents a rate of 3 replicates.

Table 6. Effect of different concentrations of fungus filtrate and algae extract and their mixture on flavonoid plant content (%)

Concentrations%	fungus filter	aqueous extract	mixture	Control
25.0	4.5b±0.03	2.3c±0.03	5.1a±0.03	2.1c±0.03
50.0	6.4b±0.05	4.1c±0.05	8.5a±0.05	2.1d±0.05
75.0	8.2b±0.06	4.9 c±0.06	9.7a±0.06	2.1 d±0.06

*numbers with small similar characters in the row have no significant differences according to Duncan polynomial test at probability level 5%
* Each number represents a rate of 3 replicates.

of filter and mixture give significant differences comparative with extract treatment and control treatment for all concentration, the highest weigh was 1.6gm when treated with mixture at 75% concentration, while the lowest weight was 1.1gm when using the extract treatment alone at 25% concentration comparative to control treatment which was 0.6gm.

When examining the effect of treatments on dry weight (**Table 5**), the results showed that the significant differences between the filtrate, extract and mixture were shown at 75% concentration. while, when adding 25% and 50% concentrations, the significant difference did not appear between the treatment with fungi and the mixture, but between them and the treatment with the extract. The highest dry weight was 75% when the mixture was 0.73. The lowest weight among the three treatments was 0.2g when the extract use at 25% concentration compared with the control treatment which was 0.1.

Table 6 shows the effect of the treatments on the flavonoid content of the plant. The results showed that the treatment at a concentration of 25% did not give a significant difference between the fungus and the mixture, but between them and the extract. Treatment with concentrations of 50% and 75% gave a significant difference for all treatments. The highest content was 9.7% when mixture treatment was at 75%, the lowest content between the filtrate and the extract and their mixture was 2.3 when treated with extract at a concentration of 25% compared to control treatment 2.1.

The results of the effect of the treatments on the plant content of phenol (**Table 7**) showed that there were significant differences between the treatment with filtrate and the extract and their mixture for all concentrations. The highest content of phenol was found in the mixture treatment of 75% (542.8 mg / 100 g), while the lowest content between the treatment of filtrate and extract and

Table 7. Effect of different concentrations of fungus filtrate and algae extract and their mixture on plant phenol content (mg / 100 g)

Concentrations %	fungus filter	aqueous extract	mixture	Control
25.0	310c±0.06	401.2b±0.06	430.4a±0.06	210.9d±0.06
50.0	316.7c±0.04	465.7b±0.04	527a±0.04	210.9d±0.04
75.0	327.6c±0.06	487.5b±0.06	542.8a±0.06	210.9d±0.06

*numbers with small similar characters in the row have no significant differences according to Duncan polynomial test at probability level 5%
* Each number represents a rate of 3 replicates.

Table 8. Effect of different concentrations of fungus filtrate and algae extract and their mixture on plant content of tannins (mg / 100 g)

Concentrations %	fungus filter	aqueous extract	mixture	Control
25.0	293b±0.05	180.5c±0.05	301.1a±0.05	146.5d±0.05
50.0	366.2b±0.03	194.7c±0.03	439a±0.03	146.5d±0.03
75.0	512b±0.04	219.8c±0.04	541.7a±0.04	146.5d±0.04

*numbers with small similar characters in the row have no significant differences according to Duncan polynomial test at probability level 5%
* Each number represents a rate of 3 replicates.

Table 9. Effect of different concentrations of fungus filtrate and algae extract and their mixture on plant carbohydrate content (%)

Concentrations%	fungus filter	aqueous extract	mixture	Control
25.0	68.8b±0.07	67.5b±0.07	80.5a±0.07	51.3c±0.07
50.0	74.9b±0.03	68.2c±0.03	84.3a±0.03	51.3d±0.03
75.0	80.6b±0.05	71.6c±0.05	89.4a±0.05	51.3d±0.05

*numbers with small similar characters in the row have no significant differences according to Duncan polynomial test at probability level 5%
* Each number represents a rate of 3 replicates.

their mixture was 25% (310 mg / 100 g) compared to the control treatment which is 210.9 mg / 100 g.

The content of tannins was estimating, the results showed in **Table 8** that there were significant differences between all treatments and for all concentrations, and that the highest value was when treated with mixture at a concentration of 75% at which was 541.7 mg / 100 g. while the lowest value of the three treatments was108.5m g100 gm when using the extract at 25% concentration compared to control treatment which is 146.5 mg / 100 g.

Table 9 shows the effect of the treatments on the plant content of carbohydrates. The results showed that there are no significant differences when using filtrate and extract separated at a concentration of 25%. But using theme as a mixture appear significant differences at the same concentration 25% comparative with using theme separately and comparative with control treatment. The concentration of 50% and 75% gave significant differences between all treatments and the highest content was when using the mixture at 75% concentration at 89.4% while the lowest content was at 25% concentration at 67.6% compared to the control treatment 51.3%.

In the study of plant fat content (**Table 10**), The results showed that there were significant differences between treatments for all concentrations and that treatment with fungus, extract and mixture at

Table 10. Effect of different concentrations of fungus filtrate and algae extract and their mixture on plant fat content %

Concentrations%	fungus filter	aqueous extract	mixture	Control
25.0	2.5b±0.2	1.8c±0.2	3.9a±0.2	2c±0.2
50.0	3.8b ±0.4	2.2c±0.4	4.8a±0.4	2c±0.4
75.0	4.6a±0.17	3.1b±0.17	4.7a±0.17	2c±0.17

*numbers with small similar characters in the row have no significant differences according to Duncan polynomial test at probability level 5%
* Each number represents a rate of 3 replicates.

concentration 25% and 50% showed significant difference between each of them and no significant difference between treatment with extract and control treatment. The results showed that there was no significant difference between the fungus and the mixture while the difference was found between the extract and the control treatment. The highest fat content was 50% when the mixture was treated. The lowest content was 1.8% when the extract was treated at 25% concentration comparative with Control 2%.

DISCUSSION

The use of naturally occurring fertilizers has increased in recent times, helping to reduce the use of fertilizers of chemical origin, which negatively affect the quality of soil and beneficial neighborhoods. It also leads to decreased fertility, loss of nutrients, accumulation of salts and other toxic elements.

Numerous studies have indicated that fungi can act as growth promoters (PGPF) and vital factors in stimulating seed germination and ending its dormancy period (Park et al. 2015, Zhang et al. 2014). We have noted in the results of the present study that the fungus filtrate stimulated seed germination and acceptable proportions compared with the control treatment and these results came close to (Yoo et al. 2018) in the ability of fungus filtrate *A. terreus* to stimulate germination of tomato seeds as the study showed that the treatment of fungus filtrate alone or in concert with the extract contributes to increase the length of the plant and the number of branches and weight of dry and wet content. And Some chemical compounds if many of the Studies have shown that the use of fungi as growth-enhancing agents is beneficial for the plant because it produces hormones similar to plant hormones such as gibberellin and oxygen, and works to change the levels of plant hormones associated with growth and production of phosphates (Priyadharsini and Muthukumar 2017).

Although there have been several studies that indicated the role of seaweed as probiotic of plant growth, including study (Jothinayagi and Anbazhagan 2009, Ramya et al. 2010, Sangeetha and Thevanathan 2010), the treatment with algae extract *F. vesiculosus* gave discouraging results when added individually as it showed a decrease in germination rates and for all concentrations compared to other treatments. Its effect on plant height, number of branches, soft and dry weight and its content of some chemical compounds was slight

compared with filtrate fungus, mixture and distilled water, but it gave better results than filtrate fungus when estimating plant content of phenol ,the reason for this may be that the content of this algae of secondary metabolic products such as tannins, turbines, flavonoids, proteins and coumarin slightly, although it contains many mineral elements such as potassium, magnesium, sodium, iron and zinc and this confirmed (Samurai 2013) in its study of the chemical content of algae as noted by (Michalak et al. 2017) that Brown algae contain fluorotanin, a complex polymeric phenolic compound produced only by brown algae as well as minerals and vitamins. However, its concentrations may be insufficient to support plant growth when used alone, while synergy between it and the fungus leachate yielded better results and all factors considered that the synergies contributed to increase the diversity of the chemical content of the mixture and increase its concentration.

REFERENCES

Al-Hassani M, Tahani M (1990) Medicinal plants Cultivation and components and therapeutic uses. Ibn Sina Library for Publishing & Distribution, Cairo. P119.

Boham AB, Kocipai AC (1994) Flavonoid and condensed tannins from Leaves of Hawaiian vaccininum vaticulum and V.calycinium''. Pacific Sci 48: 458-463.

Chopra SL, Kanwar JS (1991) In Analytical Agricultural Chemistry. Kalyani Publications- New Delhi- IV: 297.

Dhillion SS (1994) Effect o Trichoderma harzianum ,Beijernckia mobilis and Aspergillus niger on arbuscular mycorrhizal infection and sporulation in mazie, wheat, millet, sorghum, barley and oats . eitschrift fuer Pflanzenkheiten 101(3): 272-277.

El-Gendy SA, Hosni AM, Ahmed SS, Omer EA, Reham MS (2001) Variation in herbage yield and oil composition of sweet basil (Ocimum basilicum L.) var. 'Grande Verde' grown organically in a newly reclaimed land in Egypt. Arab Univ. J. Agric. Sci 9(2): 915-933.

HARRIS J (2000) Medicinal and herbs plants. Arab Publishing House. Second edition: 90-100.

Jothinayagi N, Anbazhagan C (2009) Effect of seaweed liquid fertilizer of Sargassum wightii on the growth and biochemical characteristics of Abelmoschus esculentus (L.) Medikus. Recent Research in Science and Technology 1: 155–158.

Kirtikar KP, Basu BD (1961) Indian Medicinal Plants. 2ndEd vol.1, International Book Distributor, India 1985: p

Mattila P, Hellström J (2007) Phenolic acids in potatoes, vegetables, and some of their products''. J. Food Compost. Anal 20: 152-60.

Mersie W, Singh M (1987b) Allelopathic effect of Lantana on some agronomic crops and weeds. Plant and Soil 98: 25-30.

Michalak I, Chojnacka K, Saeid A (2017) Plant Growth Biostimulants, Dietary Feed Supplements and Cosmetics Formulated with Supercritical CO2 Algal Extracts. Molecules 22(1): 66.

Park Y-H, Chung JY, Ahn DJ, et al. (2015).Screening and characterization of endophytic fungi of Panax ginseng Meyer for biocontrol activity against ginseng pathogens. Biol Control 91: 71–81.

Priyadharsini P, Muthukumar T (2017) The root endophytic fungus Curvularia geniculate from Parthenium hysterophorus roots improves plant growth through phosphate solubilization and phytohormone production. Fungal Ecol 27: 69–77.

Ramya SS, Nagaraj S, Vijayanand N (2010) Biofertilizing Efficiency of Brown and Green Algae on Growth, Biochemical and Yield Parameters of Cyamopsis tetragonolaba (L.) Taub. Recent Research in Science and Technology 2(5): 45-52.

Sabnis TS (1986) The Flora of Punjab Plains and the Associated Hill Region. Scientific Publishers, Jodhpur- 342001, India: p. 352.

Samurai MA (2013) Histopathological study of fucus vesiculosus and its effect on the level of thyroid hormones and antioxidants in sera of adult white rabbits. Master Thesis. College of Education, Samarra University.

Sangeetha V, Thevanathan R (2010) Biofertilizer Potential of Traditional and Panchagavya Amended with Seaweed Extract. The Journal of American Science 6: 61-67.

Seanclark M, William R, Horwarth S, Scow KM (1998) Changes in soil chemical propertiesresulting fromorganic and low-input farming practices. Agronomy 90: 190-195.

Sengar RMS, Bhadauria S, Sharma P (2010) The effect of cyanobacterial toxin on seed germination. Indian Journal of Scientific Research 1(2): 41-45.

Sharitamadari Z (2011) Study of soil blue-green algae and their effect on seed germination and plant growth of vegetable crops. Rostaniha 12(2): 101-110.

Small E (1997) Culinary herbs. Ottawa: National Research Council of Canada. Szabo.

Szabo V, Hrotko K (2009) Preliminary Results of biostimulator treatments on Crataegus and Prunus stockplants. Bulletin UASVM Horticulture 66(1): 223-228.

Thayumanavan B, Sadasivam S (1984) Qual Plant Foods. Hum Nutr 34: 253.

Yoo SJ, Shin DJ, Won HY, Song J, Sang MK (2018) Aspergillus terreus JF27 Promotes the Growth of Tomato Plants and Induces Resistance against Pseudomonas syringae pv. tomato. Mycobiology 46(2): 147-153.

Zhang Q, Zhang J, Yang L (2014) Diversity and biocontrol potential of endophytic fungi in Brassica napus. Biol Control 72: 98–108.