Al-Mustansiriyah Journal of Science
ISSN: 1814-635X (print), ISSN:2521-3520 (online)
Volume 29, Issue 1, 2018
DOI: http://doi.org/10.23851/mjs.v29i1.288

Research Article

Effect of some *Ricinus communis* Secondary Metabolites on *Phytophthora Infestans* and *Fusarium Solani*

Adel Hamdan Alwan
Department of Biology, College of Sciences, Mustansiriyah University, IRAQ.
*Email: phdadel@yahoo.com

ArticleInfo

Received
23/Oct./2017

Accepted
25/Dec./2018

Abstract

This study was conducted to investigate the effect of two types of *Ricinus communis* plant tissue culture extract on two fungal plant pathogens *Phytophthora infestans* and *Fusarium solani.* The result showed detected several secondary metabolites component from the plant extracts like Flavonoid, Saponins, Tannins and Glycosides; and the high effect of the alcoholic extract of the plant on the fungi with 91.10% and 89.90% respectively, the percentages of inhibition are significantly increased by increasing the concentration of the extract.

Keywords: *Ricinus communis, Phytophthora infestans,Fusarium solani,* tissue culture.

الخلاصة

اجريت هذه الدراسة لفحص تاثير نوعين من المستخلصات للمزروع النسيجي لنبات الخروع والمحضرة بتقنية زراعة الانسجة هما المستخلص المائي الحار والمستخلص الكحولي على نوعين من الفطريات الممرضة للنباتات وهما*Phytophthora infestans*والفطر*Fusarium solani*. حيث اظهرت النتائج تحديد العديد من منتجات الايض الثانوي للنبات ضمن المستخلصات مثل الفلافونويدات والسابونيات والتانينات والكلايكوسيدات وكذلك اظهرت النتائج التاثير العالي للمستخلص الكحولي على الفطريات وهو 91.10% و89.90% على التوالي, كما ان النسبة المعنوية للتثبيط تزداد بزيادة تركيز المستخل المضاف.

Introduction

Fungi diseases have important effect on human life because they cause damage to plants and their products. The most important diseases are late plate disease caused by fungi *Phytophthora infestans* and soft root disease caused by fungus *Fusarium solani* on the crops of cucumber and tomatoes, especially planted in greenhouses. Fungi have been detected in every plant species examined to date [1]. They live on and within tissue of all plant species and aided in their initial invasion in terrestrial ecosystem [2]. These relationships vary between saprophytes, pathogens and beneficial mutualism that enhance ecological fitness of plants in native ecosystem [3].Chemical pesticides where use to combat these diseases, which played a key role in reducing infection, but they have the ability to pollute the ecosystem, resulting in harmful effects of human and animals, so currently interested in the safety of the environment and Biosphere to return to the use of anti-plant origin Which contain phytotoxins, such as medicinal and toxic plants. These plants contain secondary metabolites such as Alkaloids, Glycosides, resins, fats, volatile oils and others[4]. Natural plants are still an essential element and we use many of them in our daily lives. *Ricinus communis* [5, 6] belongs to the large Euphorbiaceae family of dicotyledon, which is one of the important oily plants [7][8]; the plant produces unique oil seeds that have multiple industrial applications and the native habitat of the Tropics regions. It has been spread in many areas; the future promise is great in the field of biodiesel production [9][10].Some techniques, such as plant tissue culture, have already been developed, and it is possible to produce plants with required specifications. This technique allows for increased biomass or production of

metabolic compounds using several techniques in callus or morphogenetic culture [11].

These include Bioreactor, Micropropagation, and genetic [12]. Plant tissue culture technology can produce whole plants economically important and disease-free without relying on a given season [13].

For these characteristics and their importance, the aim of this study is to:

1. Extract the tissue culture of plant using two types of solvents.

2. Study the biological activity of these extracts on fungi in the culture medium and identify the most efficient plant extract.

Materials and methods

Preparation of the culture medium

The preparation of the MS solid media [14] by dissolving all composition materials in amount of distilled water with agar 8g / L and sucrose 30g / L, then placed on the hot plate magnetic stirrer for the purpose of moving media, boiled a little cold and measured the PH 5.6-5.8. Fill the recorded medium in vials with dimensions of 8 * 2.5cm by 10ml / vials and media sterilization with Autoclave at 121C^{o} and 1.04 kg / cm^{2} for 20 min.

Sterilization of plant parts

Leaves and stems separated from mature *Ricinus communis* washed with tap water for 15 min, submerged with 70% ethanol for 30 sec and washed with sterilized distilled water several times then submerged with sodium hypochlorite solution with 1.5% concentration added to Twee-20 as a diffuser for 10 min., the plant parts were washed several times with sterile distilled water to remove the effect of the sterile material and cut off the plant parts of both plants distributed in pre-prepared seedling[15].

Callus development

Leaves and stems separated from the sterile plants were planted in the prepared solid MS media, which were supported by plant hormones 2, 4.D (0, 1, 2, 3 mg / L) and BA (0, 0.5, 1, 1.5mg / L). 10 replicates of each type where done; the induced callus were maintained by 3-4 weeks. All seedlings were incubated at 25 ± 1 ° C in light and dark conditions.

Preparation of plant extracts

Preparation of hot water extract

For the purpose of preparing the hot water extract of the *Ricinus communis* plant, the method [16] was followed with some modification and as follows: Weight 100 g of tissue in and add 500 ml of boiling distilled water, placed in a shaking incubator for 30 minutes at a temperature (30 ° C). After 72 hours, the solution was filtered using filter paper. Dissolve the filtrate on the central centrifuge tubes at 3000 cycles / min for 5 minutes and leave the leak to the rotary evaporator for concentrate the raw extract.

Preparation of Ethanolic extract

The same steps were followed to prepare the hot water extract to obtain a dry plant extract. Ethanol was used with 80% concentration instead of distilled water, as mentioned [17].

Materials for preliminary chemical detection of active compounds in plant extracts

Preliminary and sedimentary reagents are used to determine the quality of secondary compounds in hot water and alcohol extracts:

Detection of glycosides

The method of [18] was followed by mixing two parts of the Fehlink Detector and the plant extract, then left in boiling water bath for 10 minutes and indicated the positive detection through the appearance of red color.

Test of Tannins

A solution of ferric chloride 1% Prepared by dissolving 1 g of Fecl2 in 100 ml of distilled water and indicate the positive detection by appearance of the bluish green color when mixed with an equal amount of plant extract and used to detect tannins and phenols[19].

Test of Saponins

Mercuric chloride reagent: This reagent indicates the presence of Saponinsif 1-2 ml of 1% mercuric chloride is added in 5 ml of plant extract and the appearance of a white deposit is evidence of the presence of Saponins[19].

Test of Flavonoides

Prepare by adding 4 ml of ethanol 95% to 1 ml of extract, leave in a water bath for 25-30 minutes at boiling point then add drops of 50% Potassium hydroxide to 5 ml of the form, the appearance of yellow color evidence of flavonoids [20].

Test of Terpenes and Steroids

1 g of ethanolic extract is diluted in a small amount of chloroform with a drop of concentrated sulfuric acid. The brown color indicates containing the extract Terpenes, and if after a period of dark blue color appearance refers that the extract contains steroids [20].

Isolation of fungi

The fungi were isolated according to the method of Brown 1999, which is called soil drop-method, where 1 g of dry soil was taken from an area infected with late blight diseases and the soft roots of the plants were added to a 250ml of distilled water and applied to the electric vibrator for 3 minutes. Water agar, prepared 3 days ago inoculated with 1 ml of soil solutionincubated at 25C° for two days, then took a piece of medium containing the fungus and planted on the center of PDA incubated for two days then isolated the isolates through transfer to the center of a new PDA and diagnosed according to the diagnostic methods used.

Culture media PDA

The Potato Dextrose Ager was used in all the experiments and 200 g of potatoes + 20 g of Ager and 20 g of dextrose in a liter of distilled water sterilized at autoclave under 121 ° C/ 15 lb / For 20 minutes.

Effect of plant extracts on fungi

Examination the effect of plant extracts on plant pathogenic fungi requires the use of method [21], by measuring colony diameter for fungi on the culture medium. In order to obtain concentrations (2.5, 5, 10, 20, and 40) mg / ml, where the original solution of the plant extracts was sterilized by passing through the Millipor filter with a diameter of 0.22 µn.

The sterile dishes were placed 90 mm horizontally on a flat surface, Repairs for single treatment.As for the control dishes, it contained the culture medium without any addition and was planted with a fungal inoculums from a 5 mm tablet from a week-old fungus. The dishes were then incubated at 25 ° C and the diameter of the developing colony were measured Daily according to the rate of inhibition by the following equation [21], Inhibition ratio=

$$\frac{\text{The colony diameter rate in the control dish - the colony diameter rate in the treatment dish}}{\text{The colony diameter rate in the control dish}} \times 100$$

Statistical analysis:

The results were analyzed by using the least significant difference (L.S.D) at a level of 0.05 to test the significance of the differences between the coefficients.

Results and Discussion

The cultivation of plant parts leaves and stems separated from adult plants affected the percentage of the development of callus, since the callus did not inducedat the stems of plant and all the interference in the light and dark conditions, so it was excluded from the results and work, but the response of leaves to the development of Callus was superior in giving the highest percentage of induced callus at 1mg /L 2.4 D and 0.5mg /L BA. This synthesis was adopted in the calcification of the callus induced to the leaves of the *Ricinus communis* plant and was maintained every 3 weeks with 10 replicates Table 1.

The quality of the callus required continuous maintenance at close intervals. The callus was a brown color due to the secretion of the phenolic material. Therefore, it was not indicated in the tables due to lack of good results for the development of callus and excluded from subsequent experiments.

Table 1: Development of Callus of leaves for *Ricinus communis* plant.

Treatment no.	Concentration of 2,4-D mg/l	Callus dry weight mg				
		Concentration Of BA mg/l				L.S.D
		0	0.5	1	1.5	
1	0	0	1	21	26	1.25
2	1	9	36	62*	82**	26.76
3	2	11	22	53	55*	13.5
4	3	7	19	24	40	11.50
L.S.D		3.25	7	13.75	24.75	

Chemical detection of active compounds

The results of chemical detection of plant extracts using reagents and solutions mentioned above showed the presence of the compounds listed in Table 2 as follows:

Table 2: Chemical compounds test in plant extract.

No.	Chemical com.	Hot water extract	Ethanolic extract
1	Tannins	+	+
2	Saponins	+	+
3	Glycosides	+	+
4	Flavonoid	+	+
5	Terpenes	-	+
6	Steroids	-	-

Effect of plant culture extracts of Ricinus communis plant on pathogenic fungi:

The results showed in Table 3 the high effect of the alcoholic and water extracts of the plant on the fungi *Phytophthora infestans*, which causes late blight and *Fusarium solani*, which causes soft root disease of plants. Figure 1, the percentages of inhibition is significantly increased by increasing the concentration of the extract as follows:

Ricinus communis L. was chosen because it is one of the important medicinal plants available and has different therapeutic properties. Two types of solvents have been taken for extraction (water and ethanol) to show the difference in their effect since the effectiveness of the plant extracts varies according to the solvent.

Table 3: Effect of plant extract (hot water, ethanolic) on fungi growth.

extract	Percentage of inhibition mm				
Concentration Mg/ml	*Phytophthora infestans*		*Fusarium solani*		LSD P>0.05
	Hot water. extract	Ethanolic extract	Hot wat. extract	Ethanolic extract	
2.5	27.00	63.37	33.10	70.10	9.15
5	29.00	75.11	40.22	74.14	11.50
10	42.91	76.77*	46.12	80.44**	13.74
20	51.66	84.30**	56.54	87.32**	9.52
40	60.30	89.90***	70.10*	91.10***	10.11
Control	0.00	0.00	0.00	0.00	0.00
LSD P>0.05	5.44	8.98	10.83	14.19	

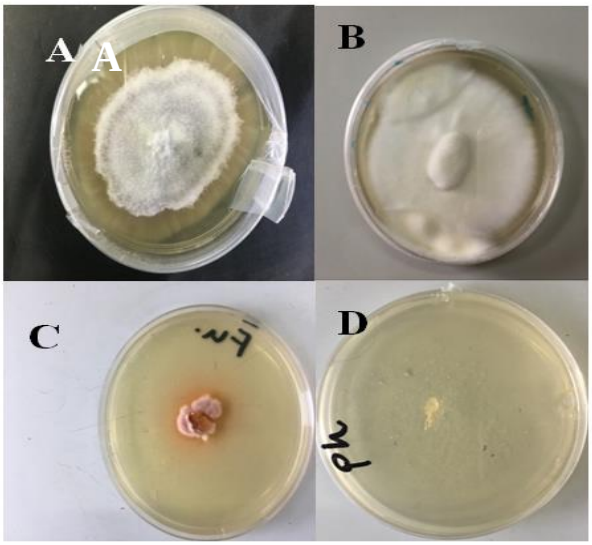

Figure 1: effect of plant extracts on fungi:a) *Fusarium solani* control ,b) *Phytophthora infestans* control) *Fusarium solani* treated with ethanolic plant extract d) *Phytophthora infestans* treated with ethanolic plant extract.

By extracting the plant using water and ethyl alcohol, all extracts of the plant's fabric were extracted in the form of crystals, which resulted in the absence of gels, dyes and waxes [23]. In this study, it was revealed that extracts of the raw plant cultivars contain many active compounds such as Flavonoid, Terpenes, Saponins,

Glycosides and Tannins. Chemical reagents used to investigate compounds and their proportion necessary to study the plant before evaluating its therapeutic efficacy. The results were identical with [24] which found that castor plant contains several active compounds, with inhibitory effect of microscopic microorganisms, especially fungus, that affect oxidative phosphorylation and reduce energy production. Depending on the percentages of inhibition, the highest sensitivity of the fungi was for the alcohol extract where 91.10 and 89.90 were for *Fusarium solani, Phytophthora infestans* respectively, and hot water extract (70.10, 60.30) for both fungi and respectively, with concentration of 40 mg / ml. This is due to the components of fungal cell walls and their relationship to the mechanism action of active compounds in plant extracts because the effect of these extracts by damage the cell membranes and their internal structure, which impedes the transfer of the basic materials into the fungus cell [25].

Conclusions

According to the results of this study, we can conclude the following: the response of leaves to the development of Callus was superior in giving the highest percentage of induced callus at 1mg / l 2.4 d and 0.5mg / l BA. extracts of the plant cultivars contain many active compounds such as Flavonoid, Terpenes, Saponins, Glycosides, Tannins and Steroids, which have inhibitory effect of microscopic microorganisms, especially fungus. The highest sensitivity of the fungi (*Fusarium solani, Phytophthora infestans*) was for the alcohol extract then hot water extract for both fungi with concentration of 40 mg / ml. These extracts are caused damage to the cell membranes and their internal structure.

References

[1] Berbee, M. L., "The phylogeny of plant and animal pathogens in the Ascomycota, "Physiol. Mol. Plant Pathol. Vol. 59, pp. 165–187, 2001.

[2] Smith, S.E. and Read, D.J., eds. Mycorrhizal symbiosis, 2nd edn. San Diego, London: Academic Press, pp. 59–60, 1997.

[3] Ishrak, K.Khafagi., "Variation of callus induction and active metabolite accumulation in callus culture of two varieties of *Ricinus_communis*_L, " Biotechnology vol. 6, no. 2,pp. 193-201, 2007.

[4] Moshkin, V.A.editor. Castor Amerind, New Delhi, 1986.

[5] Weiss, E.A. Oil seed crops. Longman group Ltd .pp31-99, 1983.

[6] Kumara, K.G., Ganesan, M. and Jayabalan, N., "Somatic organogenesis and plant regeneration in *Ricinus communis* L, "Biol plant arum. Vol. 52, pp.17-25, 2008.

[7] Ogunniyi, D.S., Castor oil: Avital industrial raw material-Biosource technology. 97:1086-1091, 2006.

[8] Kumrun, Nahar and Rita sarah Borna., "In vitro propagation from shoot tip explant of castor oil plant *Ricinus communis* L. A Bio energy plant, "Canadian Journal on scientific and industrial Research, vol. 3, no. 5, pp. 254-255, 2012.

[9] Salihu B., Gana, A. K, Apuyor B.; and Son G., "Castor oil plant (*Ricinus communis* L.) Botany, Ecology and uses, "International J. of science and research (IJSR).vol. 3, no. 5, pp. 1333-1341, 2014.

[10] Shah, A.; Naveed A., Akram, A.; Pervaiz A.; Tariq S. and Asif1, H., "Pharmacological activity of *Althaea officinalis* L, "J. Med .Plants Research., vol. 5,no. 24, pp. 5662-5666, 2011.

[11] Blumenthal M, Goldberg A, and Brinckmann J. Herbal Medicine: Expanded Commission E Monographs. Austin, Am. Bot. Council. pp. 244-248, 2000.

[12] Afshari, R.T., Angoshtari, R. and kalantari, S., "Effect of light and different plant growth regulators on induction of callus growth in rapessed Brassic anapus L, "genotypes, plant omics, J. vol. 4, no. 2,pp. 60-67, 2011.

[13] Sutovska M, Nosalova G, Sutovsky J, Franova S, Prisenznakova L. and Capek P., "Possible mechanisms of dose-dependent cough suppressive effect of *Althaea officinalis* rhamno galacturon an inguinea pig's test System, "International Journal of Biologica Macromole-cules,vol. 45, pp. 27-32 , 2009.

[14] Murashige, T. and Skoog, F., " A revised medium for rapid growth and bioassays with tobacco tissue culture, "Physio. Plan. Vol. 15, pp. 473-497, 1962.

[15] Kordestani, K. G. and Karami, O., "Piclo-ram-induced somatic embryogenesis in leaves of strawberry (*Fragaria ananassa* Duch.) , " Acta Biologica Cracoviensia Series Botanic vol. 50,pp. 69-72, 2008.

[16] Hage-Sleiman R., Mroueh, M., and Daher C.R., "Pharmacological evaluation of aqueous extract of *Althaea officinalis* flower grown in Lebanon, "Pharm Biol, vol.49, pp. 327-333, 2011.

[17] Ramachandra, R.S., and Ravishankar, G.A., "Plant cell culture chemical fact-ories of secondary metabolites, "Biotech-nol Adv ,vol. 20, pp.101-153, 2002.

[18] Smetanska, I, "Production of secondary metabolites using plant cell culture, "Adv Bio chem. Eng Biotechnol, vol. 111, pp. 187-228, 2008.

[19] Shihata, I.M. A pharmacological study of *Anagalli sarvensis* M.D. Vet. Thesis. Cairo University, 1951.

[20] Harbone, J.B. Photochemical Methods. Champo and Hall. (2nd). London, 1984.

[21] Rai, M.K., Qureshi, S. and Pandey, A.K., "In vitro susceptibility of opportun-istic *Fusarium* spp. to essential oils, ". J.Mycoses India., vol. 42, pp. 97-101, 1999.

[22] Sheriff, N., Sudarshana, M.S., Umesha, S. and Hairipra S., "Antimicrobial activi-ty of Rauvolfia tetra phyla and physalis minima leaf and callus extracts, "African Journal Biotechnology,vol. 5, pp. 946-950, 2006.

[23] Hussein, A.O., Hameed, I.H., Jasim, H., and Kareem, M.A., "Determination of al-kaloid compound of *Ricinus communis* by using gas chromatography-mass spec-troscopy (6C-MS) , " J. of medicinal plants Research, vol. 9, no. 10, pp. 349-359, 2015.

[24] Smetanska, I., "Production of secondary metabolites using plant cell culture, ". Adv Biochem Eng Biotechnol, vol. 111, pp.187-228, 2008.

[25] Alaa, J.Taha, "Effect of abiotic elicitation in some secondary compound of callus *Nerium oleander*, ". World journal of pharma sci. vol. 4, no. 5,pp. 288-293, .2016..