

# Photochemical Study and Biological Activity of Phenolic Compounds of Three Varieties of Durum Wheat (*Triticum Durum Desf.*) Subjected to Water Stress

Ghania Chaib, and Marwa Ghorab et Sabira Djaaleb

**Abstract**—Our work has focused on the quantitative and qualitative study of polyphenols both with and without water deficit treatments (WD) and (NWD) respectively, applied on three varieties of durum wheat (*Triticum durum Desf.*): Haurani, Hedba and GuemgoumRkham. The results of the quantitative analysis of ethanol extracts showed that the polyphenols content is considerable, in the three varieties under the two treatments. The qualitative study of polyphenols begins with allocations between four solvents with different polarities. This leads to the obtaining of different phases. Their compositions were identified by UV-Visible Spectrophotometer and Thin Layer Chromatographic Analysis (TLC). The results distinguished four groups of phenolic compounds at WD treatment (1, 5, 6 and 8). While it shows, only three groups at NWD treatment(3, 5 and 6). Which indicates that the majority of polyphenols detected are single phenols, phenolic acids and flavonoids (mainly flavone and flavonol-type)? The antimicrobial activity test revealed that the methanol extracts have a strong antibacterial activity especially *Bacillus* (WD treatment). Whereas in fungi there is no resistance against *Fusarium sp.*

**Key words:** Wheat (*Triticum Durum Desf.*), Polyphenols, Water Deficit, UV-Visible Spectrophotometer, Antimicrobial, TLC.

## I. INTRODUCTION

The environment in Algeria is marked by drought, cold and hot weather which are often present. These production constraints are also combined with each other, worsening the performance of durum wheat [1].

The plants which are capable of producing a wide variety of products do not participate in their basic metabolism, but rather represent products with secondary metabolism [2]- [3]. Among these compounds, polyphenols are one of the largest groups due to their low toxicity and numerous biological benefits including therapeutic [3] pharmaceutical, cosmetic and nutritional.

In the recent years, we have witnessed a significant upsurge

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of herbalists in the products rich in polyphenols, mainly flavonoids. They showed that they had very important and very large biological properties [4].

Plants rapidly synthesize defense substances against attacks by micro-organisms [5] they have several lines of defense against pathogens. Subsequent translations include rapid production of oxygen derivatives and the synthesis of phenolic compounds [6]. Phenolic compounds play a significant part in the plants metabolism, they protect also the plants against the aggressions of different pathogenic organisms [7], and they are actively involved in the interactions of the plants with its environment; they are acting as recognition signals between plants or as a mean of resistance [8]. They contribute very effectively in the plant tolerance to various stresses. So these compounds play a vital role in the balance and the adaptation of the plant to its natural environment [3].

In humans, these molecules traces are important in acting directly on the nutritional quality of fruits and vegetables and their impact on the consumer's health (antioxidant, protective effect against the development of certain cancers ...) [9]-[10].

This study is used to compare the total polyphenol content of the extracts of the three varieties of durum wheat processed with and without water deficit (WD and NWD), to reveal the richness of these cereals in phenolic compounds and test their antimicrobial activities in vitro namely antibacterial and antifungal.

## II. MATERIALS AND METHODS

### 1. Plant Material

The study focused on three varieties of durum wheat (*Triticum durum*) Haurani (HAU) Hedba3 (HED) and Guemgoum Rkham (GGR). The planting of these three varieties was conducted January 15, 2014 in round pots containing about 3 kg of clay loam soil. Five seeds of each variety of durum wheat were seeded with an average of nine repeats for the purpose of studying two planes, phenolic compounds in normal state (NWD without water deficit) and phenolic compounds with water deficit (WD).

Seedlings were irrigated once a week during the early life stages of the plant with 1/4 field capacity. At the four-leaf stage, the test was partitioned to two treatments: the first pots were irrigated twice a week. While the remaining pots were subjected to watering stops by applying water stress. The three varieties were harvested during the bolting stage under the two treatments WD and NWD.

### 2. Phytochemical Study

### 2.1. Quantitative Study (polyphenol content)

The determination of total phenols helps the identification of phenolic compounds content in 1g of the vegetal material. This latter is ground in a water / ethanol mixture (50/50) and macerated for 24 hours [11].

The content of phenolic compounds of our extracts is estimated by the Folin Ciocalteu method [12] and determined by spectrophotometry following the protocol of Miliauskas et al. [13]. The quantification of the phenolic compounds is calculated based on a linear calibration curve prepared by a standard extract of Gallic acid [14]. The results are expressed in milligrams Gallic acid equivalent per gram of the plant dry weight.

### 2.2. Qualitative Study (Extraction, identification and separation of phenolic compounds).

#### A. Extraction

Plant material with a 20 g weight is cut into small pieces. It is macerated in a mixture of ethanol-distilled water (50/50). The ratio of plant material / aqueous-alcoholic solution is 1/10 ml / g [15]. The whole rests for 72 hours with renewal of solvent 3 times every 24 hours with filtration. The extracts obtained are faced by various organic solvents from the less to the most polar. The clashes lead to the obtaining of four phases: diethyl ether phase (DE) ethyl acetate phase (AE), butanone phase (MEK) and aqueous or residual phase (H<sub>2</sub>O). All phases are evaporated to dryness at 50 °C, except the solvent in the diethyl ether layer is evaporated in the open air [16]. The recovery of the residues is carried out with 5 ml of methanol.

#### B. Identification

##### Spectral Analysis

The reported phases obtained and the residual phases are passed in a spectral analysis to identify phenolic compounds dissolved in each solvent.

The analysis of phenolic compounds by a scrolling UV-Visible spectrophotometer is between 220 and 400 nm [14]. This device is calibrated by methanol. Then, a drop of the phase to be analyzed is added to the methanol. The spectrum is obtained with its peaks.

##### Thin Layer Chromatographic Analysis

The plates used are made of glass (20/20 cm and 20/10). The selected adsorbent is silica gel TLC. The system chosen for the three-phase Diethyl ether, ethyl acetate and butanone is 50/20/25/2: distilled H<sub>2</sub>O / n Butanol / Ethanol / acetol. Whereas, the aqueous phase is carried out by the solvent system 50/20/25: distilled H<sub>2</sub>O / n Butanol / EtOH. The eluent was poured to a height of 1 cm in an elution vessel sealed to vapor saturation. The deposition of samples is evenly using a capillary glass pipette without digging the solid support [17], where a line of 3 mm is marked with a pencil about 2 cm from the bottom side of the plate. The diameter of the spot produced is dried quickly between each application; the plate placed vertically in the tank should remain closed and not to be moved when the solvent front reaches approximately 1 cm from the upper end, the plate is removed from the vessel. The level reached by the solvent is characterized by a thin line. The plate

is dried in the open air [18] reinforced with a dryer. The distances traveled by different spots are measured by the front ratio (FR).

$$\text{FR} = \text{Distance traveled by the substance} / \text{solvent front traveled distance}$$

To the naked eye it is very difficult to identify all the spots. For this reason, the plates are passed to the dark room to visualize them using a UV lamp and with a spray of sulfuric acid, acetic acid and distilled water. The revelation made the spots more remarkable

### 3. Biological Activity (Antibacterial and Antifungal):

The microorganisms tested in this study are the bacteria's *Escherichia coli* and *Bacillus*, and the fungus *Fusarium sp* which were isolated from the pathological laboratory products of the university's hospital in Constantine, Algeria. Evaluation of the antimicrobial activity was performed by disk diffusion method [19] - [20].

In this experiment, 100 ml of the ethanolic extract was used in each variety. After evaporation to dryness, the residue is recovered with 5 ml of ethanol. A Whatman paper sheet is cut into discs of 6 mm diameter sterilized in an autoclave at 120 °C for 20 min. Then they are soaked in three sterilized tubes each containing an ethanol extracts of each variety. The nutrient media employed are respectively Potato Detrox Agar (PDA) for fungi and nutrient agar for bacteria GN.

The same procedure is used for sterilization of the GN. Agar (GN and PDAs) are melted and cast in Petri dishes in half. Once the agar is solidified completely, A suspension of either bacterial or fungal of 10 µl is spread over the agar by a sterile rake (The discs impregnated in the ethanolic extracts are carefully deposited half dried by means of a clamp on the suspension). The well closed dishes are incubated respectively in ovens at 30 °C for 72 hours for fungus and at 37 °C for 24 hours for the bacteria. The diameters of the inhibition zones are measured using a caliper. An extract is considered active when measuring a zone of inhibition around the disc is in diameter greater than 6 mm [21].

### 4. Statistical analysis

The results obtained represent the average of three replicates for both phytochemical and biological studies. The statistical test performed is the variance analysis of two factors, followed by a comparison test of Newman-Keuls mean (SNK) at a 95% confidence threshold performed by the software stat Excel 2014 release.

## VI. RESULTANTS AND DISCUSSION

### 1. Phytochemical study

#### 1.1. Determination of polyphenols (Quantitative aspect)

The levels of total phenols varies among the three varieties of durum wheat in the elongation stage at WD from  $41.86 \pm 15.91$  mg / g Gallic acid equivalent (eq AC) to  $23, 24 \pm 2.42$  mg / g eq AC. The maximum value is recorded in the Hau variety, while the minimum value is recorded in the Hed variety. The variety GGR marks a  $37.20$  mg / g AC eq intermediary holding  $\pm 8.87$  mg / g AC eq. On the other hand; the NWD phase

Polyphenols contents vary among the three varieties from  $46.28 \pm 12.93$  mg / g AC eq at GGR as maximum value to  $13.07 \pm 4.10$  mg / g AC eq at Hed as minimum value. The variety Hau marks an intermediate grade of  $29.24 \pm 4.46$  mg / g AC eq.

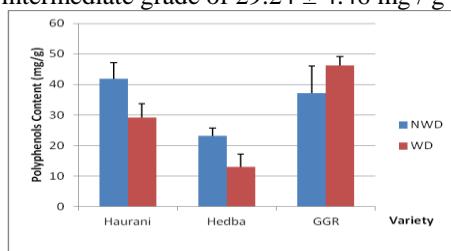


Fig. 1 Polyphenols Content In The Three Wheat Varieties In Two Treatments NWD And WD.

In relation to the contents recorded in the two varieties treatments studied, total polyphenols reduced in the NWD by 1/4 phase and by half in both HAU and HED varieties respectively from the WD phase. But this content slightly increased in the GGR variety by range of 1/6.

The two factors variance analysis revealed a significant difference between the three studied varieties. But it does not reflect any statistical significant difference between the two WD and NWD treatment.

According to the SNK test, tow groups include the three studied varieties with a simple difference in the average between them.

$$\text{HED} \leq \text{HAU} \approx \text{GGR} \Leftrightarrow 18,153 \leq 35,512 \approx 41,740$$

Also, the SNK test combines the two treatments in one group:

$$\text{WD} \approx \text{NWD} \Leftrightarrow 29,529 \approx 34,075.$$

### 1.2. Identification and extraction of polyphenols (qualitative aspects)

#### A. Extraction

The difference in color phases of diethyl ether, ethyl acetate and MEK may be related to different concentrations of polyphenols and their types included in each phase (Table I).

TABLE I

PHASES OBTAINED AFTER CONFRONTATIONS IN TWO TREATMENTS (NWD AND WD)

	solvant	variety	Color	Phase	solvant	variety	Color
No Water Deficit (NWD)	EP	HAU	greenish yellow	Water deficit (WD)	EP	HAU	greenish yellow
		HED	greenish yellow			HED	greenish yellow
		GGR	greenish yellow			GGR	greenish yellow
	DE	HAU	Light green		DE	HAU	Light green
		HED	Light green++			HED	Light green++
		GGR	Light green			GGR	Dark green
	EA	HAU	Light greenish yellow		EA	HAU	Light greenish yellow
		HED	Light greenish yellow			HED	Light greenish yellow
		GGR	greenish yellow			GGR	greenish yellow
	MEK	HAU	Light yellow		ME	HAU	Light yellow
		HED	brown			HED	greenish yellow
		GGR	yellow			GGR	greenish yellow

The phase's colors are clearly different which helps to conclude that each phase contains some phenolic compounds. The residual aqueous phases from the clashes are shown in Table II.

TABLE II  
RESIDUAL PHASES (NWD AND WD)

	Variety	NWD	WD
H <sub>2</sub> O	HAU	Light brown	Dark brown
	HED	Light brown	Dark brown
	GGR	Dark brown	Light brown

#### B. Identification

##### Spectral Analysis

In the UV-visible range, the methanolic solutions of diethyl ether phase in both WD and NWD treatments for the majority give four peaks located between 222 nm and 276 nm. This allows us to deduce that this phase does not contain flavonoids and assume that these peaks may represent simple phenols and Phenolic acids.



Fig. 2. Specters Of Phases In NWD Treatment.

The methanolic solutions of the acetate phase were found for most flavonoic solutions giving four peaks situated in the 222-397 nm intervals. The revealed spots of ether diethylic phase give more distinct peaks (therefore more pure) which are strongly nearing those of the butanone phase. The first two peaks found in between 222 and 225 nm characterizes a simple phenol or phenolic acid. The other two peaks are between 225 and 397 nm. This interval is characteristic of flavonoids. Bousmid [14] found that after the spectral analysis of pure compounds of the phases of ethyl acetate and butanone, pure compounds of these phases are flavonoids. Our results agree with those of Wagner and Bladt [22]-[15] and Chaib et al. [23], who report that flavonoids are found by two peaks. The first is between 230 and 280 nm, the second appears at about 300 and 385 nm.

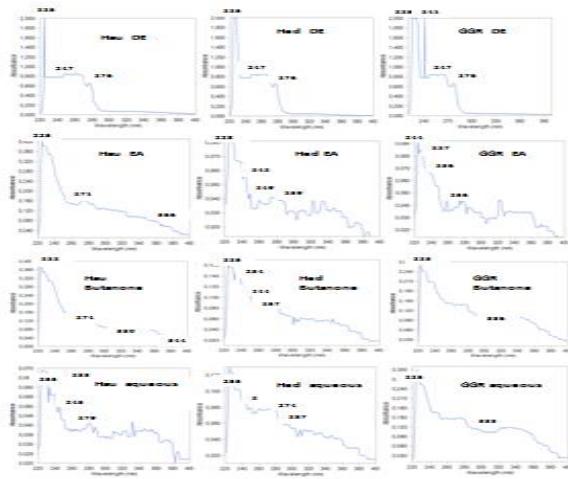
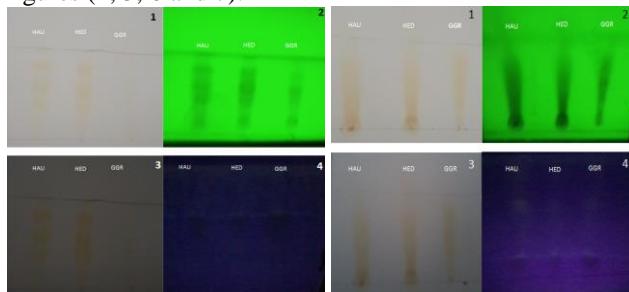


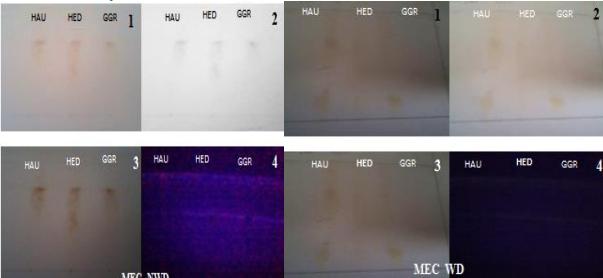
Fig. 3. Specters Of Phases In The WD Treatment.

#### Chromatographic Analysis

The results of the chromatographic analysis are introduced in Figures (4, 5, 6 and 7).

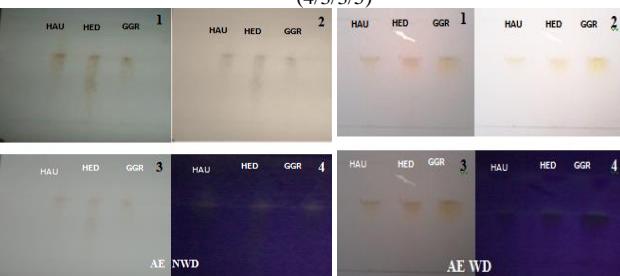


NWD: No Water Deficit; WD: Water Deficit; DE: Diethyl ether; EA: Ethyl Acetate; MEK: Methyl Ethyl Ketone. 1: Visible, 2: Under UV, 3: Visible after pulverization with sulfuric acid 50%, 4: UV after pulverization.

Fig. 4. The three varieties aqueous phase TLC in two treatments NWD and WD, in the system H<sub>2</sub>O distilled/n Butanol/EtOH (50, 20, 25)..

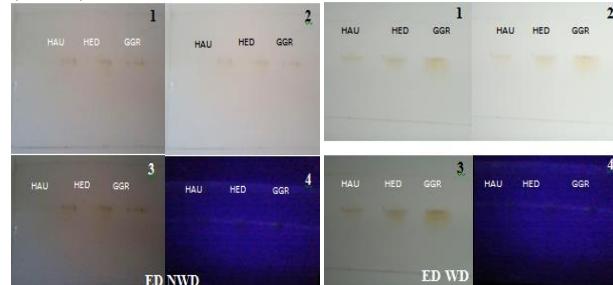
NWD: No Water Deficit; WD: Water Deficit; DE: Diethyl ether; EA: Ethyl Acetate; MEK: Methyl Ethyl Ketone. 1: Visible, 2: Under UV, 3: Visible after pulverization with sulfuric acid 50%, 4: UV after pulverization

Fig. 5. Durum wheat three varieties Methyl Ethyl Ketone Phase TLC, in two treatments (WD and NWD) in the system Tol / MEK / ETOH / Ether Petrol (4/3/3/5)



NWD: No Water Deficit; WD: Water Deficit; DE: Diethyl ether; EA: Ethyl Acetate; MEK: Methyl Ethyl Ketone. 1: Visible, 2: Under UV, 3: Visible after pulverization with sulfuric acid 50%, 4: UV after pulverization.

Fig. 6. Durum wheat three varieties Ethyl Acetate phase TLC in the two treatments WD and NWD in the system Tol / MEK / ETOH / Ether Petrol (4/3/3/5).



NWD: No Water Deficit; WD: Water Deficit; DE: Diethyl ether; EA: Ethyl Acetate; MEK: Methyl Ethyl Ketone. 1: Visible, 2: Under UV, 3: Visible after pulverization with sulfuric acid 50%, 4: UV after pulverization.

Fig. 7. Durum wheat three varieties diethyl ether phase TLC in two treatments (WD and NWD) in the system Tol / MEK / ETOH / Ether Petrol (4/3/3/5).

TABLE III  
THE FRONTAL REPORTS AND COLORS OF THE SPECKS IN THE TLC (NWD)

Variety	Phase	Nº Spots	R <sub>f</sub>	Color
HAU	ED	1	0,61	yellow
	AC	2	0,64 0,96	bright yellow mauve
	MEK	3	0,16 0,67 0,94	Yellow green bright yellow Yellow green
	H <sub>2</sub> O	4	0,16 0,37 0,67 0,88	White Yellow White orange
HED	ED	1	0,63	yellow
	AC	2	0,64 0,96	Yellow mauve
	MEC	3	0,28 0,51 0,69	Yellow green Yellow brown
	H <sub>2</sub> O	3	0,28 0,51 0,69	Yellow Yellow green White
GGR	ED	1	0,63	Yellow green
	AC	2	0,65 0,96	bright yellow mauve
	MEK	3	0,14 0,68 0,91	Yellow green Yellow green
	H <sub>2</sub> O	4	0,9 0,65 0,77 0,86	White Yellow green Yellow green orange

According to Tables III and IV, the spots number in the two phases of NWD and WD treatments are the same.

The diethyl ether phases showed a single spot color, indicating that they are rich in phenolic acids and simple phenol in both WD and NWD treatments. The Ethyl Acetate phases showed two spots for the majority. On the other part, the Butanone Phase records three spots in the WD process and a single spot in the NWD process. The aqueous phases are rich in flavonoids and showed for all four varieties three spots at both treatments.

TABLE IV  
THE FRONTAL REPORTS AND COLORS OF THE SPECKS IN THE TLC (WD)

variety	Phase	N° Spots	R <sub>f</sub>	Color
HAU	ED	1	0,73	orange
		1	0,61	yellow
		1	0,66	bright yellow
	H <sub>2</sub> O	4	0,08	White
			0,27	Yellow
			0,65	Yellow green
			0,76	orange
HED	ED	2	0,73	Orange
			0,92	Dark yellow
	AC	2	0,37	Orange
			0,67	Dark yellow
	MEK	2	0,66	Orange
			0,88	bright yellow
	H <sub>2</sub> O	3	0,06	Yellow
			0,56	White
			0,62	orange
GGR	ED	1	0,77	Yellow green
			0,37	orange
	AC	2	0,69	Dark yellow
			0,66	bright yellow
	H <sub>2</sub> O	3	0,09	Yellow
			0,59	White
			0,65	orange

We note that the frontal Reports range from 0.61 and 0.96 for WD .They are very close to those of the stadium NWD varying between 0.06 and 0.88 (Table V).

Table V: Frontal reports intervals for the four phases.

Treatments \ Phases	NWD	WD
diethyl ether	0.61-0.63	0.73-0.77
Ethyl acetate	0.64-0.96	0.09-0.37
Butanone	0,14-0.94	0.66-0.88
H <sub>2</sub> O	0.9-0.88	0.06-0.77

According to the FR the identification of flavonoids in each phase is possible [24]. Bandykova and Shinkarenko [25] claimed that the flavonols and flavanones are characterized by

an FR between 0.3 and 0.5. Therefore and according to the two tables II and IV, it can be assumed that the phases contain the following types of flavonoids (the numbers in Table VI are used in Table VII to replace the types of flavonoids mentioned in the same line)

TABLE VI  
RELATION BETWEEN FLUORESCENCE UNDER UV AND FLAVONOIDS STRUCTURE [26]

Spot Colored	Flavonoids Type
<b>1-Black-Brown</b>	Flavonols 5, 6, 7 Tris-OH Free Flavonols 5, 7, 8 Tris-OH
<b>2-Brown Black</b>	3-OH Absent Or 3-OH Substituted
<b>3-Purple</b>	Flavones 5-OH Et 4'-OH Flavones 3-Or Et 5-OH, 4'-OH Flavones Ou Flavonols 5-OH Avec 4'-OH Absent Or Substituted In 3. Flavones 6- Or 8-OH Chalcones Isoflavones, Dihydroflavanols, Flavanones.
<b>4-Clear Bleu (Fluorescent)</b>	Flavones Without 5-OH Free Flavones Without 5-OH Free With 3-OH Substituted
<b>5-Dull Yellow, Yellow, Fluorescent Orange</b>	Flavonols 3-OH Free With Or Without 5-OH Substituted
<b>6- Bright Yellow Green</b>	5-OH Free Or 5-OH Substituted
<b>7- Fluorescent Yellow</b>	Flavonols With 3-OH Free
<b>8-Pale Yellow</b>	Dihydroflavanols

TABLE VII  
THE FLAVONOIDS CONTAINED IN EACH TREATMENT (WD AND NWD) PHASE

Treatment \ Phases	NWD	WD
diethyl ether	(6), (5)	(5), (8), (6)
Ethyl acetate	(6), (3), (5)	(8), (5)
Butanone	(5), (6)	(6), (5), (1)
H <sub>2</sub> O	(6), (5)	(5), (6)

It is observed that there are four groups of the phenolic compounds in the treatment WD (1, 5, 6 and 8). But, there is only three treatment groups NWD (3, 5 and 6).

It can be concluded that both WD and NWD treatments mainly contain flavones and flavonols with different substitutions. On the other hand the phases of WD are characterized by the presence of Chalcones. Flavanones and isoflavones are present exclusively in the ethyl acetate layer of the WD treatment.

### 3. Biological Activity

#### 3.1 Antibacterial Activity

The extract of GGR has the strongest activity against the development and growth of *E.coli* in WD treatment with an inhibition zone average diameter of  $1.4 \pm 0.47$  cm followed by Hau extracts with an average of  $8.7 \pm 0.06$ , and finally that of Hed with a weaker effect than the extracts from the other varieties  $0.27 \pm 0.46$  cm. In the contrary, for NWD treatment, the methanolic extract of both varieties Hed and GGR have the most vigorous activity with an inhibition zones average diameter of  $0.53 \pm 0.46$  cm and  $0.53 \pm 0.6$  cm in succession. Hau extract marks the lower zone of inhibition by an average of  $1.25 \pm 0.65$  cm diameter (Fig.8).

No statistical difference between the effect of the methanolic phase ethyl acetate on the E-coli and also between both NWD and WD treatments.

$$\text{NWD} \approx \text{WD} \Leftrightarrow 0.794 \approx 0.844$$

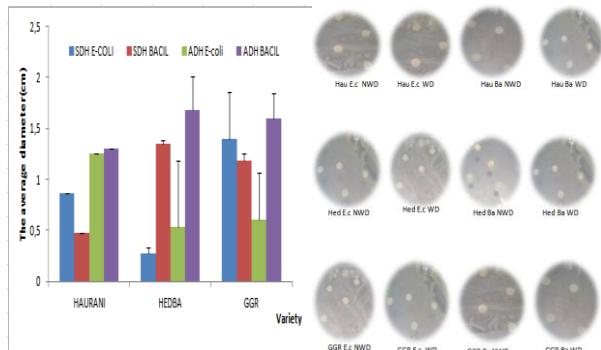


Fig 8: Disk growth inhibition zones of both *E. coli* and *Bacillus* bacteria WD and NWD treatment by extracts from three varieties of durum OF Ethyl acetate phase;

HED extract has the strongest activity against the development and growth of *Bacillus* in WD treatment with an inhibition zone average diameter of  $0.88 \pm 0.08$  cm. While in NWD treatment, the two extracts HAU and HED have a remarkable effect with an inhibition zone average diameter of  $1.22 \pm 0.25$  cm and  $1.55 \pm 0.03$  cm in succession.

Statistical analysis indicates a significant difference between the effect of the three varieties extracts of *Bacillus* bacterium and also a highly significant difference between both WD and NWD treatments. The SNK test classes the studied varieties in two groups:

$$\text{GGR} < \text{HED} \approx \text{HAU} \Leftrightarrow 0.408 < 1.050 \approx 1.198$$

While extracts of NWD treatment have a more powerful effect than extracts of WD treatment

$$\text{WD} < \text{NWD} \Leftrightarrow 0.750 \approx 0.944$$

Statistical analysis revealed no difference between the effect of methanolic extracts of ethyl acetate Phase on the both bacteria's on both WD and NW treatments.

$$\text{E.coli} \approx \text{Bacillus} \Leftrightarrow 0.819 \approx 0.875$$

Concerning the comparison between the two bacteria, the three-factor ANOVA showed no difference between the effects of the three varieties extracts but saves a significant difference between the effect of the two treatments and both bacteria. According to the SNK test treatments and bacteria have two different groups with the NWD extracts exert a stronger activity than the WD extracts. *Bacillus* also has a more rigorous effect than *E. coli*. Our results are consistent with those of Katarrzyna and collaborators [27] and Harikrishna et al. [28] which have demonstrated the antibacterial activity of a flavonoic glycoside (prunin 6'-Op coumarate) against two strains of Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus albus*) and two gram-negative bacteria (*Escherichia coli* and *Proteus vulgaris*).

$$\text{WD} < \text{NWD} \Leftrightarrow 0.704 < 1.204 ; \text{E.COLI} < \text{BACILLUS} \Leftrightarrow 0.646 < 1.263$$

### 3.2. Antifungal activity

The effect of two treatments phenolic extracts of durum wheat three varieties is negative on the growth of *Fusarium* fungus sp.

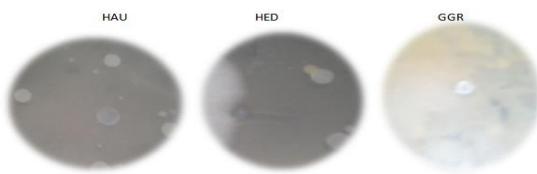


Fig 10. Results Of Antifungal Activity

### VI. CONCLUSION

The quantitative study or measurement of total polyphenols indicates that each variety has significant polyphenol content. According to the statistical analysis this content means a significant difference between the three studied varieties. But it does not reflect any significant statistical difference between both WD and NWD treatments, and it varies between 29 529 mg / g eq AG and 34 075 mg / g eq AG.

The phase analysis by UV -visible spectrophotometer shows that the varieties extracts are rich in simple phenols, flavonoids and phenolic acids.

The colors clearly differenced the phases which help to conclude that each phase contains some phenolic compounds.

The methanolic solutions of acetate layer revealed four peaks for all solutions flavone located at an interval 222-397 nm. Spots give more distinct peaks which are highly close to those of the butanone phase. The thin layer chromatography shows that flavones and flavonols are the most dominant flavonoids in this species.

The clashes four phase's spots number in WD treatment is similar to those in NWD treatment. The number of compounds is almost the same with an identical difference between WD and NWD. Four groups of phenolic compounds distinguished the NWD treatment. However, only three groups in WD treatment. It is concluded that both WD and NWD treatments mainly contain flavones and flavonols with different substitutions. On the other hand the WD phases are characterized by the presence of Chalcones, flavanones, and isoflavones that are present exclusively in the ethyl acetate layer of the WD. The results of the antimicrobial activity obtained show that the NWD extracts have a more inhibitory response than the ones of the WD. The bacterium extracts; *Bacillus* exerts a more rigorous effect against *E. coli* extracts. The *Fusarium* fungus is resistant to the extracts of the three varieties to both WD and NWD treatments. It presents a negative result.

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#### On interest areas:

Molecular biology, plant biotechnology, genetic engineering, biology and plant physiology, Secondary metabolism, water stress, cereals (*Triticum* and *Hordeum*).Biodiversity.

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