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Effect of Some Ecological Factors on Stomata and Phenolic Compounds Production in *Aloe Vera* L. Gel and Effect Gel to the Adult of Flour Beetle: *Tribolium Castaneum* (Coleoptera : Tenebriodae)

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ABSTRACT

This search presents the effect of some ecological factors such as (ultraviolet –B radiation , temperature , salinity) on stomatal index and stomatal frequency and phenolic compounds after exposure to *Aloe vera* L. UV-B radiation , temperature and salinity . Ultraviolet – B radiation is one of the important abiotic factors that can stimulate the production of secondary metabolites, including phenolic compounds. *Aloe vera* (Asphodelaceae) is a medicinal plant used in Iraq for treating sunburn, wounds, and heartburn. The flour beetle: *Tribolium castaneum* is one of the inset causes of loss storage food yield in the world. The study aims the effect of some ecological factors such as (ultraviolet –B radiation , temperature , salinity) on stomatal index and stomatal frequency and phenolic compounds after exposure to *Aloe vera* L. UV-B radiation , temperature and salinity and investigate the insecticidal and repellency effect of leaf gel for *Aloe vera* to estimate the percentage of the insecticidal effect for concentrations 25, 50, 75, and 100 % of alcoholic extract within seven days on adult insects. The gel in leaf exposure to UV-B radiation contained a higher diversity of phenolic compounds and flavonoids. This search showed a high percent kill for an adult with 100% of the alcoholic extract compared with control and an excellent effect of *Aloe vera* gel at a concentration of 50 % for a repellency effect with a value of 10.842%. Phenolic compounds showed after exposure to UV as : (dioxyflavone ,kampferol, catechuic acid, flavonol glucuronic acid, gabardine isoflavone, isoquercitrin, p-coumaric acid, hispaglabridine) with a high percentage in the gel after exposure to UV-B compared by control.

KEYWORDS: phenolic compounds, HPLC, *Aloe vera*, *Tribolium castaneum*.stomata frequency

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1. INTRODUCTION

The morphology, anatomy, and chemical composition of plants and crops have changed recently as a result of the ozone hole being filled by Ultraviolet rays [1] .The effects of UV-B radiation (280-315 nm) on secondary plant metabolites such phenolic, alkaloids, and glycosidic chemicals resulted in several alterations in plants. Worldwide, UV-B damage to crops and agriculture results in severe yield losses. One abiotic stress factor, ultraviolet B, temperature and salinity has a direct impact on plant and crop output [2]. For instance, the 2012 drought , strong UV-B exposure and salinity resulted in significant losses of 40 and 80 million tons of *Zea mays* production [3]. The development, growth, and stress tolerance of plants depends on a vast class of chemicals known as phenolics [4]. Plants develop defense systems in response to this kind of environmental stress when their exposure to UV-B increases. [5] Among the secondary plant metabolites, phenols are widespread in leaves, fruits, and vegetables. The promoting properties of phenolic acid compounds include anti-mutagenic, antioxidant, and anticancerogenic properties [6]. The ability of phenols to bind free radicals and the prevention of degenerative diseases, such as cancer and cardiovascular disorders, are linked [7]. The phenolic chemicals found in *Aloe vera* L. are rich , The high amount of flavonols (derivatives of myricetin and quercetin) [8] and anthocyanins (derivatives of delphinidin and cyanidin) in leaves of *Aloe* sp. is the basis for their high antioxidant activity [9, 10]. Additionally, albeit to a lesser extent, hydroxybenzoic acid (gallic acid and hydroxybenzoic acid) and hydroxycinnamic acids (caffeic acid and coumaric acid) also contribute to antioxidant activity [11]. The effect

of cultivars, seasonal timing, and cultivation techniques have been the main areas of focus in studies on antioxidant phenolic plant components in black currant up until this point [12]. However, research on how UV-B radiation affects the phenolic content and antioxidant activity of black currants is lacking [13].

In addition to morphological and anatomical modifications, the protective stress response results in significant alterations in the plant's secondary metabolism, which leads to an accumulation of different phytochemicals and their antioxidative activities (such as phenolic compounds) [14]. Anthocyanins and flavonoids have UV protective properties and serve a variety of physiological purposes, including antioxidant and anticancer activities [15]. In general, UV radiation can also enhance the production of phenolic compounds in postharvest, as evidenced by reports for quercetin in onion and strawberries, flavonoids in broccoli, and black currant [15]. However, the length of the stress and adaptability determine how the phenol profile and antioxidant activity are affected by UV stress. In general, the synthesis of phenolic compounds can also be stimulated by UV exposure in post harvest as it was reported for example for quercetin in onion and strawberry, and flavonoids in broccoli and black currant [15]. However, the duration of the stress, the plants' ability to respond to the stress, and the physiological state of the plant at the time of UV exposure all play a role in the changes in phenol profile and antioxidant activity caused by UV stress [16]. The phenolic content and antioxidant activity of black currants are used as an example in this study to highlight the short-term UV-B stress-caused impact on the dynamics of secondary plant metabolites in post harvest [17]. Additionally, attention is being paid to the effects of tailored UV exposure in post harvest for boosting health-promoting phytochemical components and, consequently, the nutritional content of fruits and vegetable products [18], [25]. high temperature affect the phenolic content clearly , and high salinity also affects the phenolic compounds significantly [26].through previous studies , it has been shown that there is an effect of temperature , uv radiation and salinity on the phenolic content of some wild plants such as *Brassica nigra* and *Eruca sativa* [27].

2. MATERIALS AND METHODS

2.1 Plant material and experiment

One-year-old *Aloe vera* L. plants were grown in 20 L containers in two groups: control (before UV-B , temperature and salinity exposure) and (after UV-B , temperature and salinity exposure). The leaf harvest took place in August 2021. After being picked, one portion of the leaves samples (100 g of gel) was exposed to UV-B radiation for 10, 20 and 30 days using a UV-B fluorescence light source (FL 17SE, 300-315 nm) at a distance of 25 cm from the leaves. The leaves were stored at -25 °C in a deep freeze after a 20-hour UV-B exposure before being freeze-dried. The phenolic content and phenolic composition of dried samples were extracted and analyzed by HPLC [19], [24].

The plants were exposed to high temperature of up to 30,40 and 50 degrees celsius .measuring the amount of salinity in the soil before and after adding salt (NaCl) at concentration of 25% , 35% and 45%.

2.2 Identification of the phenolic compounds using HPLC:

By using HPLC, phenolic acid components were identified in the current study. It was carried out using an analytical Shimadzu 101 series HPLC system with an HPLC pump and detector. phenolic compounds were separated analytically on 250 mm x 4.6 mm, 4 m, 18 columns using water/acetic acid/acetonitrile as the mobile phase (94.5+ 0.5 + 5; v,v,v).

According to [20], [22], phenolic compounds were identified using the distinctive absorption wavelength of the chemical group. The extract was divided into two-milliliter portions (each containing 50 mg of DM mL-1) and concentrated by nitrogen drying before being redissolved in 500 L of HPLC-grade methanol. The flow rate was 1 mL min-1, and the injection volume was 25 L. Phenolic chemicals are present (detection wavelength: 520 nm). From the concentration of the above-mentioned compounds in a 1 mM standard solution, the total concentration of each representative subclass was computed. Dry matter (DM) was used as the unit of measurement for the results [21].

2.3 Used gel *Aloe vera* L. against insect :

Aloe vera gel was employed in the study to combat the flour beetle, *Tribolium castaneum*, in stage adults. Gel applied before and after exposure to leaf UV-B extract. The purpose of the study is to determine the percentage of the insecticidal impact of *Aloe vera* leaf gel at concentrations of 25, 50, 75, and 100% of the alcoholic extract on adult insects for seven days [20].

2.4 Statistical analysis

SPSS 13.0 (SPSS Inc., USA) was used to statistically analyze the data using the ANOVA method. Tuckey's test produced significant differences (p 0.05). The standard deviation revealed the mean variability.

3. RESULTS AND DISCUSSION

3.1. Effects of UV-B radiation , temperature and salinity on Stomata frequency:

The current study showed a clear variation of environmental factors on the stomata frequency, as the frequency of stomata decreased in the upper and lower surface of the leaves of the *Aloe vera* in plants treated with ultraviolet rays (Uv-B), high temperature and salinity compared to control plants, as it was noted that the number of stomata in the upper surface is less than the lower surface in the majority of treatments.This study showed that there is a change in the number of stomata in mm^2 (stomata frequency

) , dimensions of stomata in upper and lower leaves of *Aloe vera* .as shown in Table 1 and Figure 1.

Ultraviolet rays led to reducing the frequency of stomata more than the effect of High temperature and salinity , after exposing the plants to radiation 30 days, as the stomata frequency reached 50 stomata per mm^2 in the upper surface and 65 stomata per mm^2 in the lower surface, while it was 86 and 92 stomata per mm^2 in plants not exposed to radiation, it was also noted that the rise in temperature to 50 degrees Celsius led to a reduction in the number of stomata in both the upper and lower surface, as it reached 62 and 71 stomata per mm^2. As for salinity, it led to a reduction in the stomatal frequency in the upper and lower surface more than UV rays and high temperature, as the number of stomata reached 46 and 49 stomata per mm^2 in the upper and lower surfaces, respectively, while 86 and 92 stomata per mm^2 were in the control group, which indicates its impact on vital activities such as photosynthesis, water absorption, gas exchange and other physiological processes.

A clear variation in the dimensions of the stomata in the upper and lower surfaces of the leaf before and after exposure to ultraviolet radiation, heat and salinity was also observed as shown in Table 1 and Figure 2.The stomata were larger in size in the lower surface than the upper surface due to the exposure of the upper surface to environmental factors such as heat, light, etc., and it was also noted that all stomata are of the divergent type, as the guard cells are surrounded by three auxiliary cells graded in size from small to large, as in Figure 1.

Table 1: effect of some ecological factors on stomata frequency and dimension of stomata in upper and lower leaves of *Aloe vera* .

Characteristic of stomata	Before ecological factors	After ecological factors								
		Uv-B radiation By day			Temperature C0			Salinity %		
	control	10	20	30	30	40	50	25	35	45
Stomata frequency (upper leaves)	86	76c	65b	50a	83 c	75b	62a	60c	52b	46a
Stomata frequency (lower leaves)	92	82c	79b	65a	89 b	78a	71a	64c	58b	49a
Length stomata (upper leaves)	20	18c	17b	15a	15 b	15b	18a	18c	15b	13a
Width stomata (upper leaves)	15	12c	12b	10a	11 c	13b	12a	11b	10a	10a
Length stomata (lower leaves)	22	20c	18b	14a	19 b	20c	18a	19c	17b	16a
Width stomata (lower leaves)	15	15c	13b	11a	12 b	13c	15a	12b	11a	12b

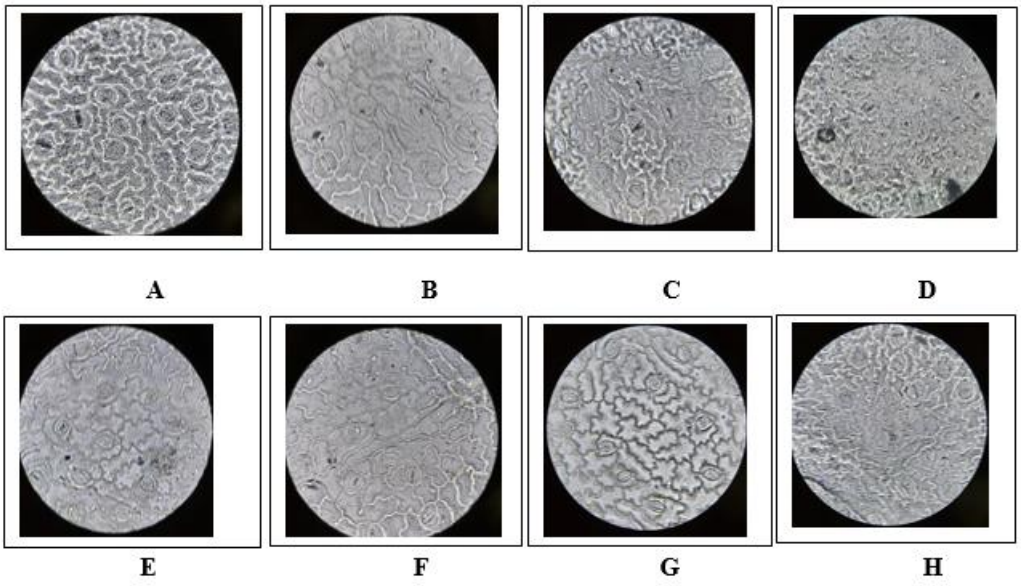

Figure 1: effect of some ecological factors on stomata frequency in upper and lower leaves of *Aloe vera* .A,B - control , C,D-under UV-B radiation , E,F- under temperature , G,H-under salinity.

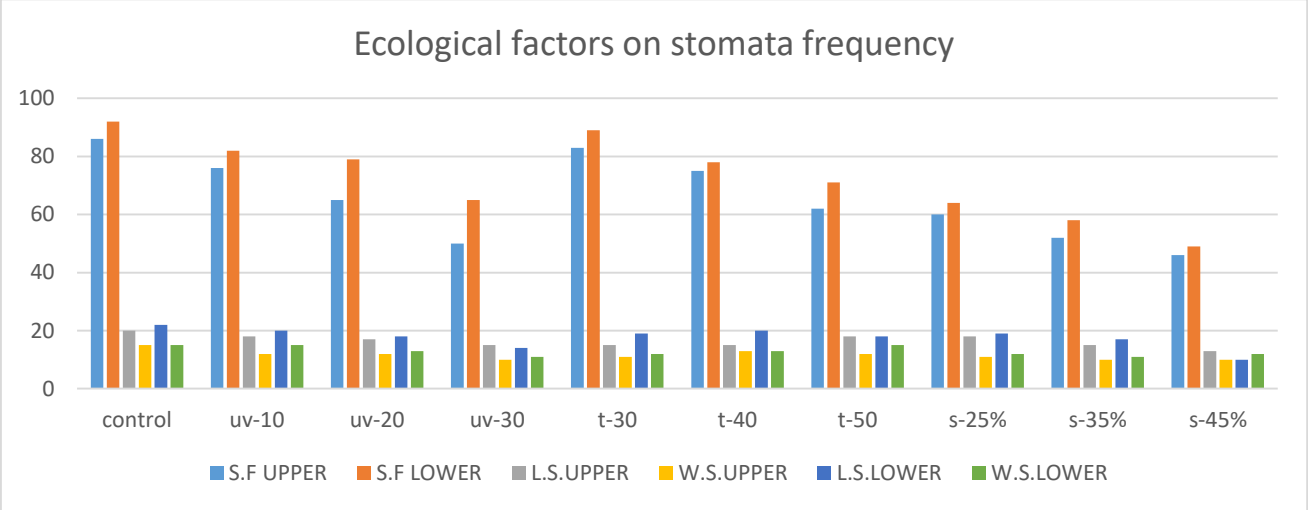

Figure 2: effect of some ecological factors on stomata frequency and dimension of stomata in upper and lower leaves of *Aloe vera*

3.2 Effects of UV-B on gel features of *Aloe vera* (Phenolic compounds)

The exposure of *Aloe vera* plants to UV-B radiation led to significant increases in phenolic compounds. This species is rich in active substances as phenolic compounds that have biological activities, including anti-fungal, antibacterial, and anti-inflammation properties. we investigated the effect of UV-B radiation on the polyphenol and flavonoids in leaf gel. exposure UV-B radiation enhanced the total phenolic, analysis by HPLC of control and plants exposure UV-B radiation revealed quantitative and qualitative differences in their phenolic profiles. the gel in the leaf exposed to UV-B radiation contained a higher diversity of phenolic compounds and a larger amount of phenolic and flavonoids.

The current research, a clear variation was observed in the Total phenolics and flavonoids content in the gel of the leaves of *Aloe vera* , as it was noted that phenols were more than flavonoids in general and their concentration increased with the greater the duration of exposure to ultraviolet rays, as exposure for 30 days to radiation recorded high values shown in Table 2 and Figure 3.

Table 2: Total phenolic and flavonoid compounds in the gel of *Aloe vera* leaves before and after exposure to Uv-B .

Total Phenolic compounds	Total flavenoids compounds	Period of exposure to UV-B (days)	Concentration of total Phenolic compounds	Concentration of total flavenoids compounds
Before exposure UV-B (mg/ 100g)	Before exposure UV-B (mg/ 100g)		After Exposure UV-B (mg/ 100g)	After Exposure UV-B (mg/ 100g)
109b	98b	10	278a	128a
145b	112b	20	291a	165a
218b	135b	30	308a	187a

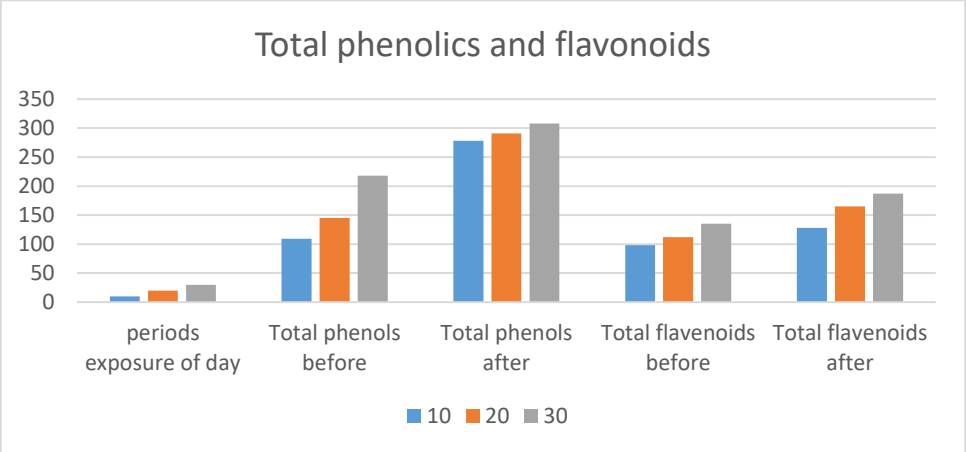

Figure 3: Total phenolic and flavonoid compounds in the gel of *Aloe vera* leaves before and after exposure to Uv-B .

3.3 Identification of phenolic compounds and flavonoid in gel of *Aloe vera* plant.

Phenolic compounds showed after exposure to UV as : (dioxyflavone ,kampferol, catechuic acid, flavonol glucuronic acid, gabardine ,isoflavone, isoquercitrin, p-coumaric acid, hispaglabridine) with a high percentage in the gel after exposure to UV-B compared by control as shown in figure 4-6 .

Phenolic compounds were identified in the gel of *Aloe vera* before and after exposure to ultraviolet rays (UV-B) and showed nine compounds with high concentrations compared to the control group, which indicates the effect of radiation in increasing compounds and their concentration.

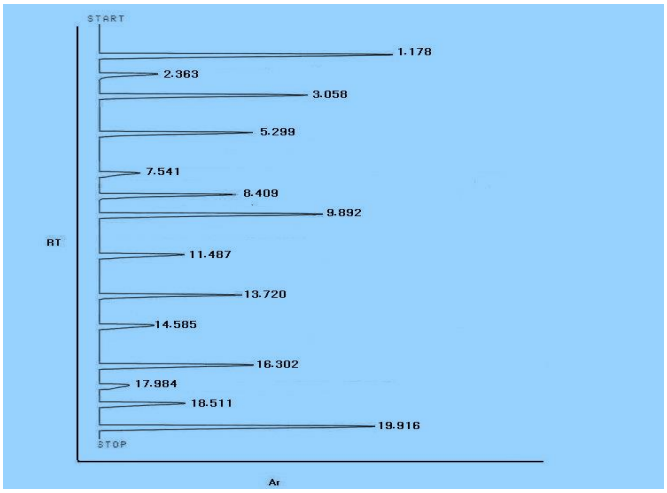

Figure 4: standard assay of phenolic compounds in *Aloe vera* by HPLC. Rt: Retention time, Ar: Area.

CHROMATOPAC C-R10A
SAMPLE NO 0 FILE 1
REPORT NO 1 METHOD 10

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	1.178	7925			12.1206	QUERCETIN
2	2.363	1583			4.9735	SPINACETIN
3	3.058	4899			9.3598	DIOXYFLAVONE
4	5.299	3510			7.9254	KAEMPFEROL
5	7.541	1179			3.5512	CATECHUIC ACID
6	8.409	3158			7.7941	FERULIC ACID
7	9.892	5034			10.0017	COUMARIC ACID
8	11.487	2772			5.8663	FLAVONAL GLUCURONIC ACID
9	13.720	3109			7.6156	TRIHYDROXY FLAVANONE
10	14.585	1254			3.7146	GLABRIDIN
11	16.302	3439			7.8573	ISOFLAVONES
12	17.984	1021			3.2736	ISOQUERCETIN
13	18.511	2316			5.0611	P-COMARIC ACID
14	19.916	6198			11.0122	HISPAGLABRIDINS
		--------			--------	
TOTAL		47397			100	

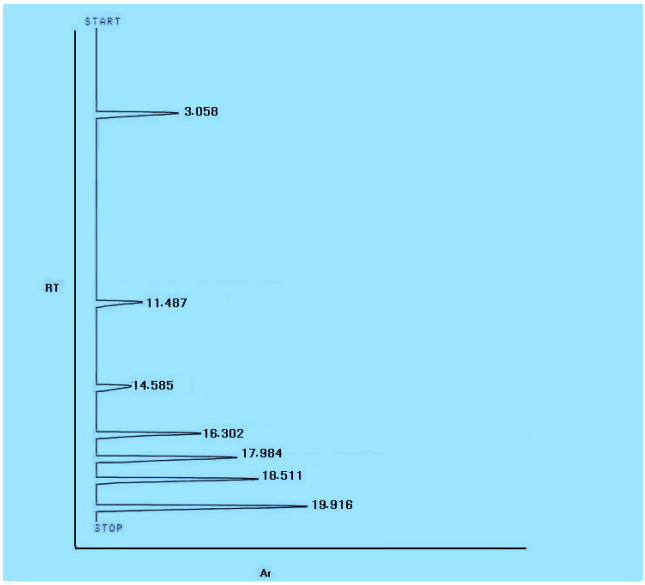

Figure 5: Identified phenolic compounds in the gel for *Aloe vera* by HPLC before exposure to Uv-B radiation (control). Rt: Retention time, Ar: Area.

CHROMATOPAC C-R10A

SAMPLE NO 2 FILE 1

REPORT NO 3 METHOD 10

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	3.058	2481			13.8201	DIOXYFLAVONE
2	11.487	1625			9.9714	FLAVONAL GLUCURONIC ACID
3	14.585	1034			7.7352	GLABRIDIN
4	16.302	4648			20.5248	ISOFLAVONES
5	17.984	2919			14.3327	ISOQUERCETIN
6	18.511	3307			16.7530	P-COMARIC ACID
7	19.916	3586			17.0435	HISPAGLABRIDINS
		– – – – – – – –			– – – – – – – –	
TOTAL		19600			100	

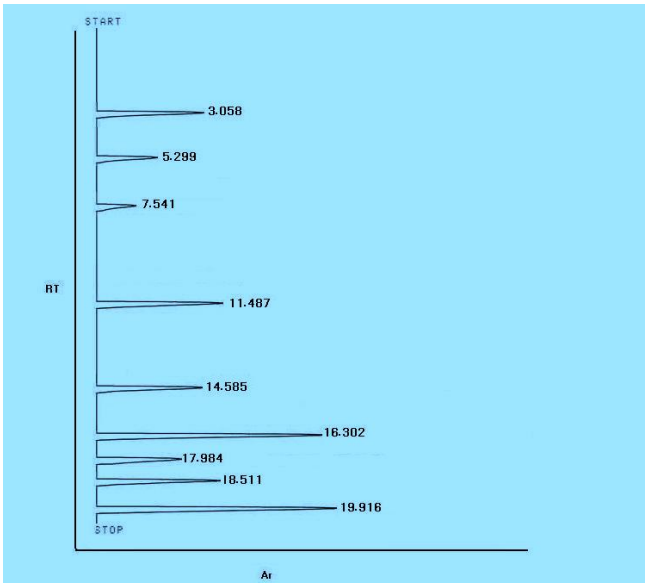

Figure 6: Identified phenolic compounds in the gel for *Aloe vera* by HPLC after exposure to Uv-B radiation after 7 days. Rt: Retention time, Ar: Area.

CHROMATOPAC C-R10A
SAMPLE NO 1 FILE 1
REPORT NO 2 METHOD 10

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	3.058	2731			10.8196	DIOXYFLAVONE
2	5.299	1277			7.5935	KAEMPFEROL
3	7.541	1085			7.1372	CATECHUIC ACID
4	11.487	3649			11.8924	FLAVONAL GLUCURONIC ACID
5	14.585	2653			10.6473	GLABRIDIN
6	16.302	4767			15.0507	ISOFLAVONES
7	17.984	2063			10.2981	ISOQUERCETIN
8	18.511	3512			11.4128	P-COMARIC ACID
9	19.916	4898			15.2145	HISPAGLABRIDINS
		–––––––			–––––––	
TOTAL		26635			100	

3.4 Effect the gel after exposure Uv-B on adults of flour beetle: *Tribolium castaneum:*

This search showed a high percent kill for adults with 100% of the alcoholic extract compared with the control and excellence effect of *aloe vera* gel at a concentration of 50 % for repellency effect with a value of 10.842%, these results according to [20] .this research showed importance secondary metabolites (phenolic compounds) in *Aloe vera* gel and effect these compound against insects.

(a) (b) (c)

Figure 4: effect gel for *Aloe vera* used to control the adult of flour beetle: *Tribolium castaneum* (Coleoptera: tenebriodae), (a)- adult,(b)- used gel for *Aloe vera* 50% percent, (c)- used gel for *Aloe vera* 100% percent showed kill adult beetle.

4. CONCLUSION

Environmental factors such as ultraviolet rays, high temperature and salinity clearly affected the frequency of stomata in the upper and lower leaf surfaces, and it was also noted that ultraviolet rays affected more than the rest of the other factors in the frequency and dimensions of stomata.

Phenolic compounds showed after exposure to UV as : (dioxyflavone ,kampferol, catechuic acid, flavonol glucuronic acid, gabardine isoflavone, isoquercitrin, p-coumaric acid, hispaglabridine) with a high percentage in the gel after exposure to UV-B compared by control. This search showed a high percent kill for adult flour beetle with 100% of alcoholic extract of gel aloe vera compared to the control.

5. ACKNOWLEDGMENTS

To the best of our knowledge, this is the first study on the effect of phenols on insects in Iraq.

Author's contributions

Mwafaq Inhab Salih Al-Hamdany: conceived the idea.
Mohammad A. Hashem AL-Blesh: Wrote abstract and planned methodology and concluded the research.
Huda Damin Abduljabbar Al-Tikrity: wrote the introduction, results, and discussion.

Conflict of interest

The authors have declared no conflict of interest.

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