



Nanostructured flax seeds-based fatty acids effect on genotype of *Leishmania tropica*

Maksood Adil Mahmoud Al-Doori^{1,*}, Asaad T. Al-Douri^{1,2,*}, Nahedh Ayad Faris³

¹College of Health and Medical Techniques Al-Dour, Northern Technical University, Al-Dour Salaheddin, Iraq

²Department of Biotechnology, College of Applied Science, Samarra University, Iraq

³Department of Biology, College of Education for Pure Sciences, Tikrit University

*) Email: asta7062@gmail.com

Received 30/3/2025, Received in revised form 25/4/2025, Accepted 25/5/2025, Published 15/7/2025

The effect of fatty acids extracted from flax seeds (*Linum usitatissimum*) on the genotype of *Leishmania tropica* concentrations 100 micrograms/deciliter dl are prepared by means of dissolving fatty acids with Ethylen glycol to attain the above concentrations. These fatty acids had been used as capacity antileishmanial retailers in vitro, and the results showed that the awareness of 100 micrograms/deciliter gave an entire inhibition of the parasite boom in the promastigote form in 48 hours and as much as one hundred% as compared to the manage group that witnessed the parasite growth at all times. In this study, 8 non-specific primers are used (table below), and these primers search for mutations in the entire genome, both coding and non-coding, and the use of these primers is the first time on the *Leishmania* parasite. Molecular study on the parasite DNA using Random Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD-PCR) technique has proven the ability of these fatty acids to cause lethal mutations in the parasite. The 0.5 % concentration of fatty acids is used in the treatment of patients infected with cutaneous leishmaniasis, and it led to their complete recovery within 10 days without leaving a scar, compared to the group of patients treated with Pentostam who needed 6 weeks to recover. Scanning electron microscopy (SEM) has been employed to characterize this work before and after fatty acids effect, followed by analysis of absorption and Fourier transform infrared spectroscopy (FTIR).

Keywords: Nanostructured seeds; *Leishmania tropica*; SEM; FTIR.

1. INTRODUCTION

This experiment is applied for the first time in Iraq and the Arab world because there is no available literature on this investigation; the above results showed that the flax seed extract has an effect on the genome of the cutaneous leishmaniasis parasite in all treatments. The RAPD-PCR indicators showed tall competence in diagnosing these mutations with a small number of produced primers, amounting to 8 primers. The treatments presented a change in the number and excellence of the mutant band. The results showed that altogether actions have a result on the genetic material DNA of the pest, but there are dissimilar percentages depending on the treatment. The first treatment K1, which is considered the highest attentiveness of the extract used, presented the uppermost number of separate mutant band, amounting to 19 distinct bands that are divided into two types of bands, including 16 absent bands that are first current in the switch example and disappear afterward the action, and 3 unique bands that are originate after the action that are not first current in the switch example. Followed by the second treatment K2, which is characterized by 14 separate band that are divided into 9 inattentive band and 5 unique bands. Followed by the fourth treatment K4, which had 12 bands divided into 7 absent band and 5 unique bands, and finally the third treatment K3, which is characterized by 10 distinct bands divided into 5 absent bands and 5 unique bands. From the results of the four treatments, the increase in the attentiveness of the extract is straight relative to the increase in the effect on the DNA by increasing the number of distinct mutant bands. The uppermost number of mutant bands is for the highest attentiveness K1, and the number of absent mutant bands is also higher than the number of unique mutant bands, which amounted to 34 bands compared to the unique one, which amounted to 18 bands. From the absent mutant bands, it is clear that all concentrations of the extract have a strong effect on the parasite genome, and the effect increases with the increase in the attentiveness of the extract, which led to an increase in the obliteration of DNA sequences, and the indication is the disappearance of the recognition site of the primer first current in the control sample, so the original band disappeared in the treatments. On the other hand, increasing the concentration of the extract is considered destructive and fatal to the cells because the effect is high, and the best evidence for this is the large number of absent mutant bands, and this is consistent with the results obtained by [1-2].

2. EXPERIMENTAL

The original stock culture of the promastigote parasite MHOM/IQ/1992/MREC3 isolated from patients infected in Iraq are obtained from the Medical Research Center at the College of Medicine/Al-Nahrain University as the followings;

- 1- RPMI 1640 liquid culture medium, which is a ready-made culture medium industrialized by This medium covers a quantity of vitamins and amino acids.
- 2- Fetal calf serum rendering to [3].
- 3- Antibiotic: Ceftriaxone. Agencies are as follow:
9.5 ml of RPMI 1640 + 0.5 ml Fetal calf serum + 5 microliters of Ceftriaxone as an antibiotic to prevent bacterial growth. Filter the above mixture by passing it finished a 0.22 micrometer filter membrane to ensure the purity of the culture.

Growth of the pest in vitro and analyzing the impact of flaxseed fatty acids. The cutaneous leishmaniasis parasite is grown in vivo and the result of dissimilar attentions of flax seed fatty acids became studied, as follow:

- 1- Place 4.3 ml of culture medium in 25 sterile test tube and divide them into 5 groups.
- 2- An amount of 0.6 ml of different concentrations of the extract is added to 4 group, leaving the first group as a control example.
- 3- An amount of 0.1 ml of the medium containing the growing parasites containing 1080 parasites is additional to the diverse group.

4- The tubes are placed in the incubator at a temperature of 23-26 °C.

Parasitic boom in all tubes became monitored each 24 hours by means of taking 0.02 and including it to 0.4 of everyday saline containing 1% formalin to kill vermin, and the amount of vermin become counted on a Neuburger hemocytometer slide.

Previous studies have proven that flax seeds contain acids: (Lauric acid, Myristic acid, Palmitic acid, Oleic acid, Stearic acid, Arachidic acid, and Behenic acid).

Concentrations 100 µg/dl are prepared with the aid of melting fatty acids with ethylene glycol to obtain the overhead attentions. These fatty acids had been usage as capacity antileishmanial sellers in vitro. The effects presented that the awareness of 100 µg/dl gave a whole reserve of the increase of the pest inside the promastigote shape inside a period of 48 hours and with the aid of 100 % in comparison to the switch organization that the boom of the parasite has been saw at altogether time.

In this study, 8 non-specific primers are used (table below). These primers search for mutations throughout the coding and non-coding genome. The use of these primers is the first time on the cutaneous Leishmania parasite. A molecular study on the parasite's DNA using Random Amplified Polymorphic DNA Polymerase Chain Reaction RAPD-PCR technology has proven the ability of these fatty acids to cause fatal mutations to the parasite.

The solutions are prepared according to what is [4];

1-Extraction buffer CTAB2X:

Prepare by dissolving the components 0.08g Tris-HCL, 0.001g NaCl, 0.04g Na₂EDTA, 200 µg CTAB, 10µl 2-mercaptoethanol in 6 mL of distilled water and adjusting the pH of the solution to 8 by using NaOH, completing the volume to 10 mL and sterilize with autoclave.

2-Sodium dodecyl sulfate (SDS) 20 %:

prepare by dissolving 2 g of (SDS) in 10 ml of purified water and heat to 68°C.

3-Phenol - Chloroform

Add 100 g of phenol to 100 mL of Tris-Cl (50mM) and regulate the pH to 8, mix the solution gently and leave until the removed top layer has separated, add 100 mL of Tris-Cl 50mM again, adjust the pH to 8 and mix the solution gently The upper layer is removed, the process is repeated 3 times until the pH of the upper layer is fixed at 7.5, 100 ml of saturated phenol pH 8 is mixed with 100 ml of chloroform and placed in falcon tubes of 10 ml for each tube and stored in the refrigerator after being covered with aluminum foil.

4-Absolute Ethanol 100 %: Use ready-made.

5-Ethanol 70 %: It is prepared by addition 30 ml of distilled water to 70 ml of absolute ethanol.

The method is conducted according to [4], and the experiment is repeated with three replicates.

The treated groups are coded according to the concentration as K1 treated with a concentration of 100 µg/ml, K2 for the 75 µg/ml treatment group, and K3 for the 25 µg/ml treated group.

1-The parasite is grown on liquid medium in test tubes covering 10 ml of liquid nutrient medium at 37 °C for 24 hours.

2-Transferring 1 ml of the parasite-containing media (Second paragraph above) to a 1.5 ml Eppendorf tube with a pipette, and discarding the suspension in a centrifuge at 14000 rpm for 3 minutes, then discarding the clear liquid and washing the precipitate with 200 µl of TE buffer.

3-200 µL of 2XCTAB Extraction Buffer solution is added to the precipitate after washing and mix with a vortex mixer, then 100 µL of Sodium dodecyl Sulfate 20 % (SDS) is additional and the mixture is placed in a water bath at 65 °C for 20 minutes.

4-After the incubation period, 200 microliters of phenol - chloroform - isoamyl alcohol are additional to the mixture in an Eppendorf tube, and with a slight stirring of the tubes (stirring), then the tubes are located in a centrifuge and the mixture is centrifuged at 10,000 revolutions / minute for ten minutes.

5-After the end of the discarding time, the higher (aqueous) layer became transferred with the aid of a micropipette to a new Eppendorf tube, and the equal preceding volume of chloroform solution changed into brought and discarded on the equal pace once more.

6-The upper (aqueous) layer became transferred through a micropipette to a new Eppendorf tube and a double extent of a hundred % absolute ethanol become added to precipitate the DNA.

7-The mixture placed in Eppendorf tube is stirred about 5-6 times, then the tubes are located in a centrifuge and centrifuged at 10,000 revolutions/min for ten minutes, then the clear liquid is discarded and 1 ml of 70 % ethanol is added to the sediment. The tubes are placed in a centrifuge and centrifuged at 10,000 cycles/min for five years minutes.

8-The supernatant is discarded, then the tubes are left to dry from alcohol for 10-15 minutes using clean absorbent papers, then the precipitated DNA is dissolved by adding 100 μ L of sterile TE to dissolve the DNA.

9-Stock sample is kept at -20 °C until use.

3. RESULTS AND DISCUSSION

The DNA concentration dimension manner turned into achieved as an estimate of its purity using the nanodrop tool, wherein one drop of the formerly extracted genomic DNA changed into taken, then positioned inside the sample vicinity, then the device changed into ordered to degree after calibrating the device on the identical dissolved solution, then the device gave the attention in nanograms/microliter and purity as it should be, then the sample is diluted until the concentration became 50 ng/ μ L and kept frozen until use. Solutions, necessary Substances and gels are organized and samples are load into the electrophoresis procedure in step with the technique reported by [5].

The RAPD reactions are performed based [6] on a control sample and the four treatments of the Leishmania parasite using 8 primers, as well as the reactions are performed.

1-The DNA awareness in all studied samples became adjusted by means of dilution with TE solution to obtain the concentration required to behavior RAPD reactions, which is approximately 50 ng/microliter for each sample.

2-The Master Reaction mixture is prepared by mixing the response components in a sterile 2 ml Eppendroffe tube. The mixture is discarded in the Microfuge device for 3-5 seconds to complete the mixing of the reaction components, taking into account that the work must be inside a sterile hood, wearing gloves and placing the tubes on. Inside snow.

The RAPD-PCR reaction program is applied, where the initial denaturation temperature is 94 for 4 minutes, one cycle followed by 40 cycles consisting of the denaturation temperature 92 for 30 seconds and the initiator association temperature 36 for 30 seconds. The elongation temperature 72 for 1 minute and the final elongation temperature 72 for 7 minutes, one cycle. After the end of the reaction time, the tubes are removed from the thermospolymer device and kept in the freezer. 5 microliters are withdrawn from the tubes and the mixture is loaded onto the previously prepared agarose gel at a attentiveness of 1.5 % with a volumetric indicator. The examples are then transferred onto the agarose gel. The gel is then dyed by submerging it in ethidium bromide dye for one hour while rousing, after which it is exposed to an ultraviolet light basis on a UV-Transilluminator and the gel is photographed.

The variations within the genetic fabric (DNA) that may be acquired from the utility of Random Amplified Polymorphic DNA Polymerase RAPD signs can be used to become aware of mutations attributable to treatments and compare them with the control pattern with a view to be acquired via changing the effects that we attain and that seem inside the gel into description tables by way of setting 1 while the bundle is gift and 0 whilst the package is absent, that means that the package that looks within the remedy and isn't always current in the switch example expresses a mutation and vice versa.

Likewise, the package deal this is absent inside the remedy and is current within the switch example too couriers a mutation and comparisons may be made amid the result. For the molecular results of Random Amplified Polymorphic DNA Polymerase Chain Reaction RAPD-PCR indicators. 8 primers are used as shown in Table 1 RAPD indicators. All primer originates compulsory site on the genome and produced diverse band that are detected on the crossed gel in the attendance of the size guide DNA ladder 100bp DNA Marker. This primer varied in producing main bands. /Monomorphic bands and polymorphic bands.

The results of the primer presented in Table 1 and exposed in picture 1, 2, 3, 4 show diverse patterns of band. The overall total of sites recognized by the primers on the genome of the sample is 43 sites, of which 7 are overall sites for altogether sample and 36 differentiated sites, and the P-7 primer is characterized by the uppermost number of creating site, which reach 8 sites. The overall variety of bands comprised of these sites turned into 129 general bands, including 35 general bands and 94 polymorphic bands. The primer P-three produced the very best number of produced bands, which amounted to 23 bands. The percentage of popular variant of the produced primers become 75 %. The phenomenon of variation of the 4 remedies in comparison to the switch sample is an indicator of the result at the parasite genome. The extra the variant after the treatment, the more the impact of the remedy [7-8].

Table 1 Number of productive sites and their type, total number of packages and their type, number of distinct packages and their type, and percentage of variation resulting from Random Amplified Polymorphic DNA RAPD interactions.

1	P-1	3	2	1	11	10	1	1	-	33
2	P-2	5	-	5	10	-	10	6	1	100
3	P-3	5	3	2	23	15	8	-	2	40
4	P-4	3	-	3	9	-	9	1	-	100
5	P-5	7	2	5	22	10	12	2	5	28
6	P-6	7	-	7	18	-	18	1	13	100
7	P-7	8	-	8	18	-	18	10	4	100
8	P-8	5	-	5	17	-	17	3	6	100
Total		43	7	36	129	35	94	24	31	75%

Greatest of the treatments, as exposed in Tables 2 and 3 designated by arrows in Figures 1-4 are characterized by distinctive bands (Unique band, Absent band). The total number of distinctive distorted groups subsequent from the primer shaped in this study is 55 distinctive bands likened to the switch example, of which 18 are sole bands and 37 are inattentive band. The primer P-2 shaped the uppermost number of sole bands, which amounted to 5 bands, while the primer P-6 shaped the highest number of absent bands, which amounted to 13 bands. As for the treatments, the K1 treatment obtained a percentage of distinctive bands, which amounted to 19 bands, of which 3 are sole bands and 13 are absent bands, followed by treatment K2, which obtained 14 distinctive bands, which are distributed into 5 unique bands and 9 absent bands, then treatment K4, which obtained 12 distinctive bands, which are divided into 5 Unique bands and 7 Absent bands. Finally, the K3 treatment had 10 separate bands dispersed into 5 unique bands and 5 absent bands. These bands are a distinguishing feature and diagnosis for these treatments, as they designate that treating the parasite with an extract touches the genetic material DNA, as the arrival of differences in these bands between one treatment and another results from the difference in the effect of the concentration used, as the increase in concentration is proportional to the increase in mutant bands, which makes the increase in concentration spread the grade of complete murder of the parasite. It is concluded from this that the occurrence of a mutation in an exact site led to the recognition of the primer and the appearance of the unique band, as for the absent bands, the occurrence of a mutation in the site that recognizes the primer only in that treatment led to the cancellation of that recognition and the concealment of the appearance of the band, and this is consistent with the results of many previous researchers [9-10]. The primers varied in the sizes of the resulting bands, ranging between 75-1750bp. The smallest molecular size is 75bp in primer P-5, and the highest molecular size is (1750 bp) in primer P-7.

Most of the treatments, as exposed in Tables 2 and 3 designated by arrows in Figures 1-4 are characterized by distinctive bands (Unique band, Absent band). The total number of distinctive mutant bands subsequent from the primers shaped in this study is 55 distinctive bands likened to the control example, of which 18 are unique bands and 37 are absent bands. The primer P-2 shaped the uppermost number of unique bands, which amounted to 5 bands, while the primer P-6 shaped the highest number of absent bands, which amounted to 13 bands. As for the treatments, the K1 treatment obtained a percentage of distinctive bands, which amounted to 19 bands, of which 3 are unique bands and 13 are absent bands, followed by treatment K2, which obtained 14 distinctive bands, which are distributed into 5 unique bands and 9 absent bands, then treatment K4, which obtained 12 distinctive bands, which are divided into 5 Unique bands and 7 Absent bands. Finally, the K3 treatment had 10 distinct bands distributed into 5 unique bands and 5 absent bands. These bands are a distinguishing feature and diagnosis for these treatments, as they indicate that treating the parasite with an extract affects the genetic material DNA, as the appearance of differences in these bands between one treatment and another results from the difference in the effect of the concentration used, as the increase in concentration is proportional to the increase in mutant bands, which makes the increase in concentration reach the degree of complete killing of the parasite. It is concluded from this that the occurrence of a mutation in a specific site led to the recognition of the primer and the appearance of the unique band, as for the absent bands, the occurrence of a mutation in the site that recognizes the primer only in that treatment led to the cancellation of that recognition and the concealment of the appearance of the band, and this is consistent with the results of many previous researchers [9-10]. The primers varied in the sizes of the resulting bands, ranging between 75-1750bp. The smallest molecular size is 75 bp in primer P-5, and the highest molecular size is 1750bp in primer P-7.

Table 2 Mutant distinctive packages and their quality for the treatments compared to the control sample.

Initiator name	Molecular weight (bp)	K1		K2		K3		K4	
P-1	600-1000	-	-	-	-	-	-	-	1
P-2	300-1100	1	-	-	3	-	2	-	1
P-3	400-1050	1	-	1	-	-	-	-	-
P-4	250-500	-	-	-	-	-	1	-	-
P-5	75-1700	3	2	-	-	2	-	-	-
P-6	300-1650	6	-	3	1	2	-	2	-
P-7	450-1750	5	-	1	1	-	1	4	2
P-8	400-1500	-	1	4	-	1	1	1	1
		16	3	9	5	5	5	7	5
Total	55	19		14		10		12	

Table 3 Translation of RAPD products on agarose gel in the presence of the size indicator, where the number 1 represents the presence of the band and the number 0 represents the absence of the band.

molecular size (bp)	Control sample C	K1 transaction	K2 transaction	K3 transaction	K4 transaction	Initiator name
1000	1	1	1	1	1	P-1
900	0	0	0	0	1	
600	1	1	1	1	1	P-2
1100	1	0	1	1	1	
900	0	0	0	0	1	
700	0	0	1	1	0	
400	0	0	1	1	0	
300	0	0	1	0	0	
1050	1	1	0	1	1	P-3
950	1	0	1	1	1	
800	1	1	1	1	1	
600	1	1	1	1	1	
400	1	1	1	1	1	
500	1	0	1	1	1	P-4
400	1	0	1	1	1	
250	0	0	0	1	0	
1700	1	0	1	0	1	P-5
1600	1	1	1	1	1	
1500	1	1	1	1	1	
1400	1	0	1	1	1	
1250	1	0	1	0	1	
500	0	1	0	0	0	
75	0	1	0	0	0	
1650	1	0	0	0	0	P-6
1500	1	0	0	0	0	P-7
1250	1	0	0	1	1	
1000	1	0	1	1	1	
800	1	0	1	1	1	
500	0	0	1	0	0	
300	1	0	1	1	1	
1750	0	0	0	0	1	
1650	1	0	1	1	0	
1550	0	0	0	0	1	P-8
1500	0	0	1	1	0	
1250	1	0	1	1	0	
1000	1	0	0	1	0	
800	1	0	1	1	0	
450	1	0	1	1	0	
1500	1	1	0	0	0	
1100	0	1	0	1	1	P-8
900	1	1	0	1	1	
600	1	1	0	1	1	
400	1	1	0	1	1	

Red numbers indicate unique packages and blue numbers indicate missing packages.

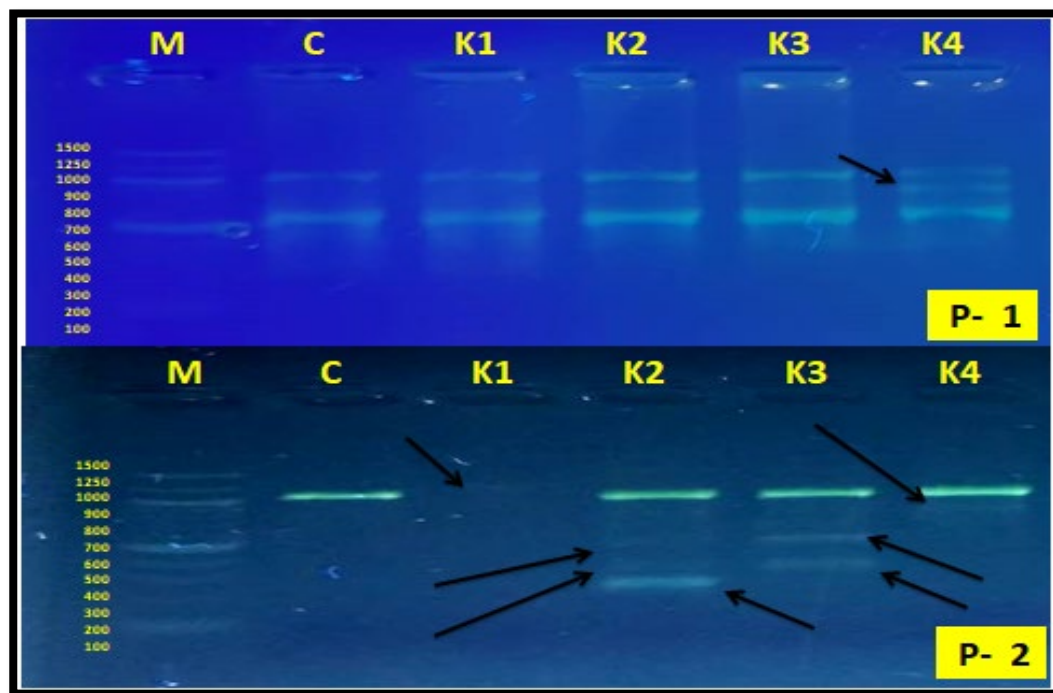


Figure 1 Represents the products of the starter P-1 and P-2 for the four fatty acid treatments on the Leishmania parasite compared to the control sample and the stage on a 1.5% agarose gel in the presence of the size indicator Marker.

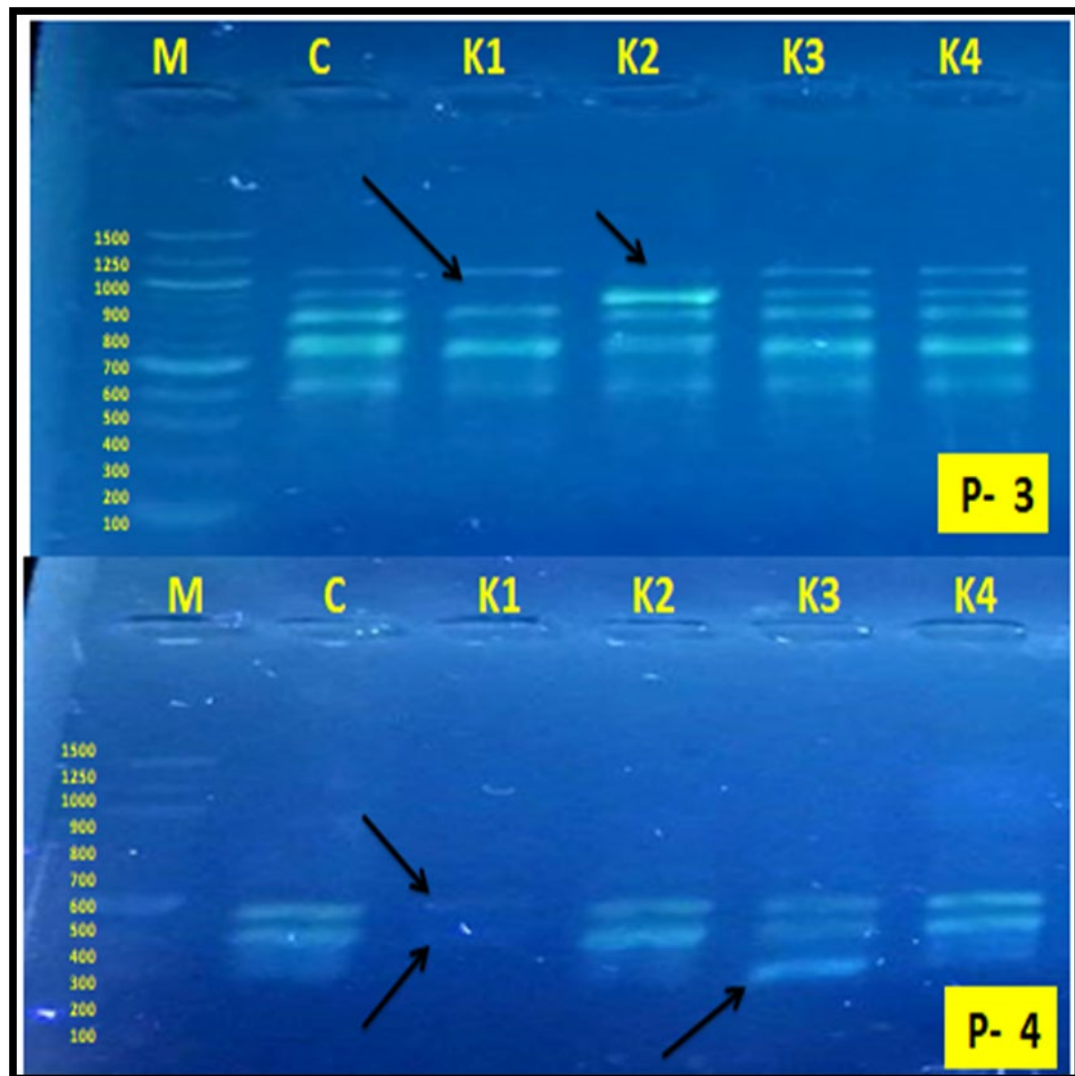


Figure 2 Represents the products of the starter P-3 and P-4 for the four fatty acid treatments on the Leishmania parasite compared to the control sample and the stage on a 1.5 % agarose gel in the presence of the size indicator Marker.

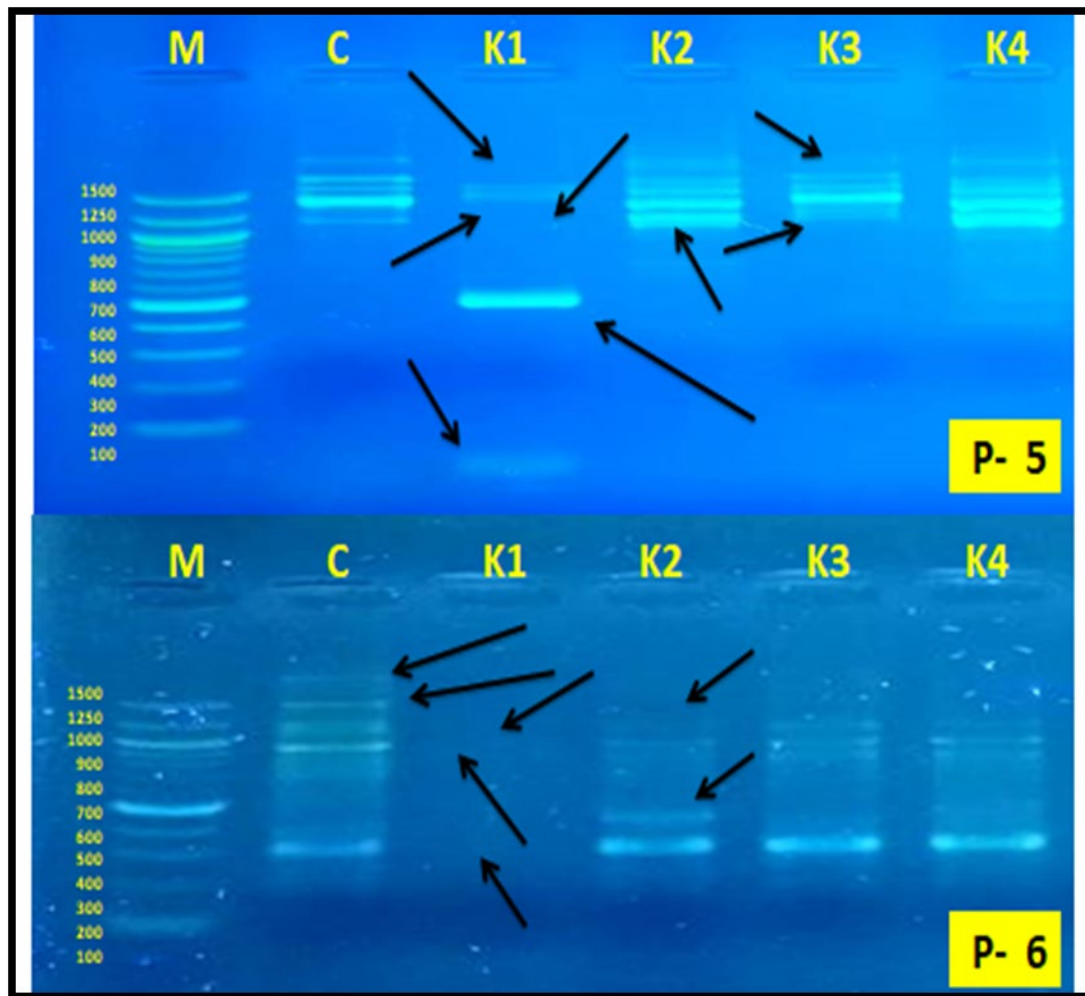


Figure 3 Represents the products of the starter P-5 and P-6 for the four treatments of fatty acids on the Leishmania parasite compared to the control sample and the stage on a 1.5 % agarose gel in the presence of the size indicator Marker.

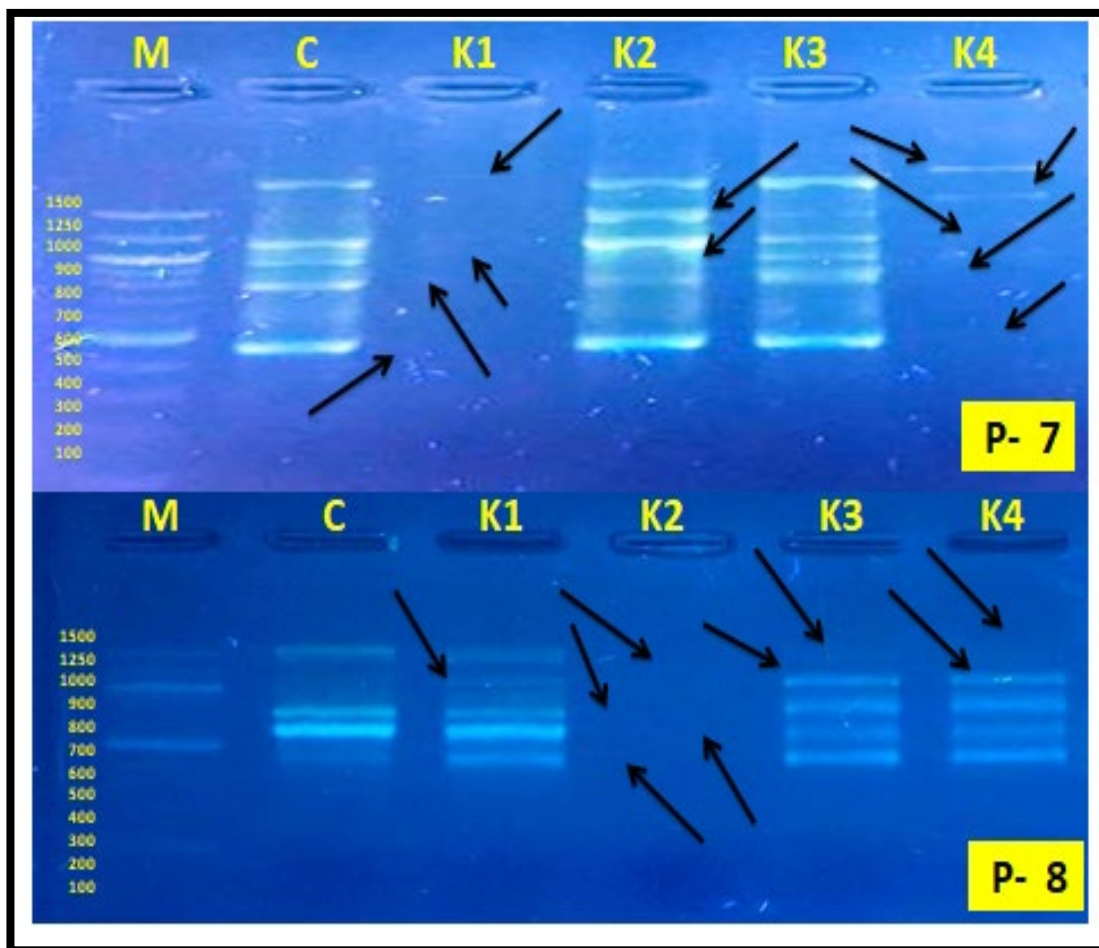
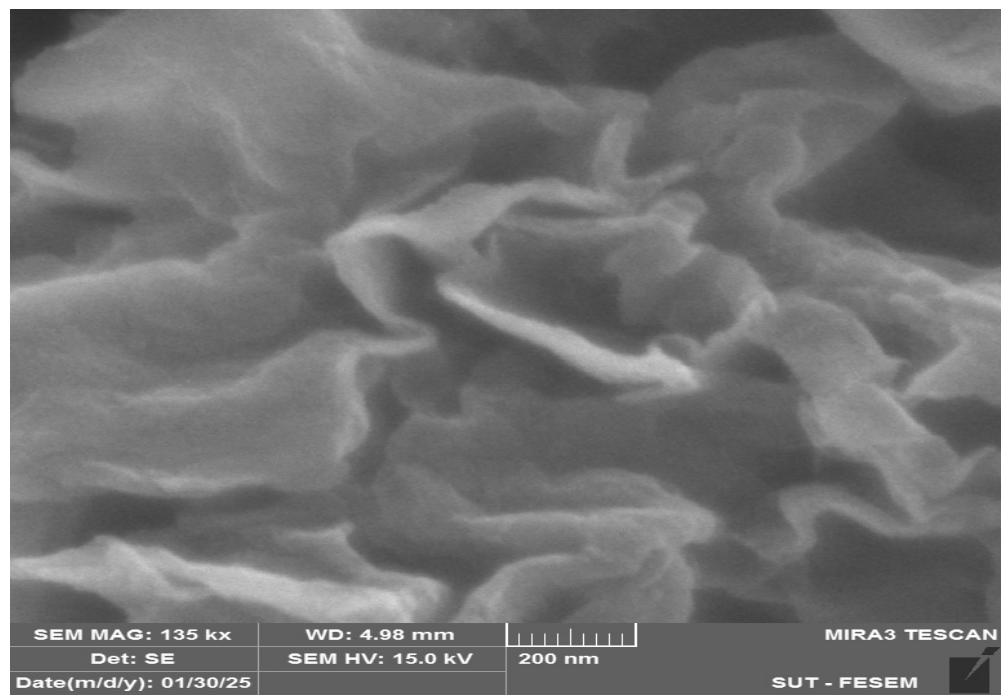
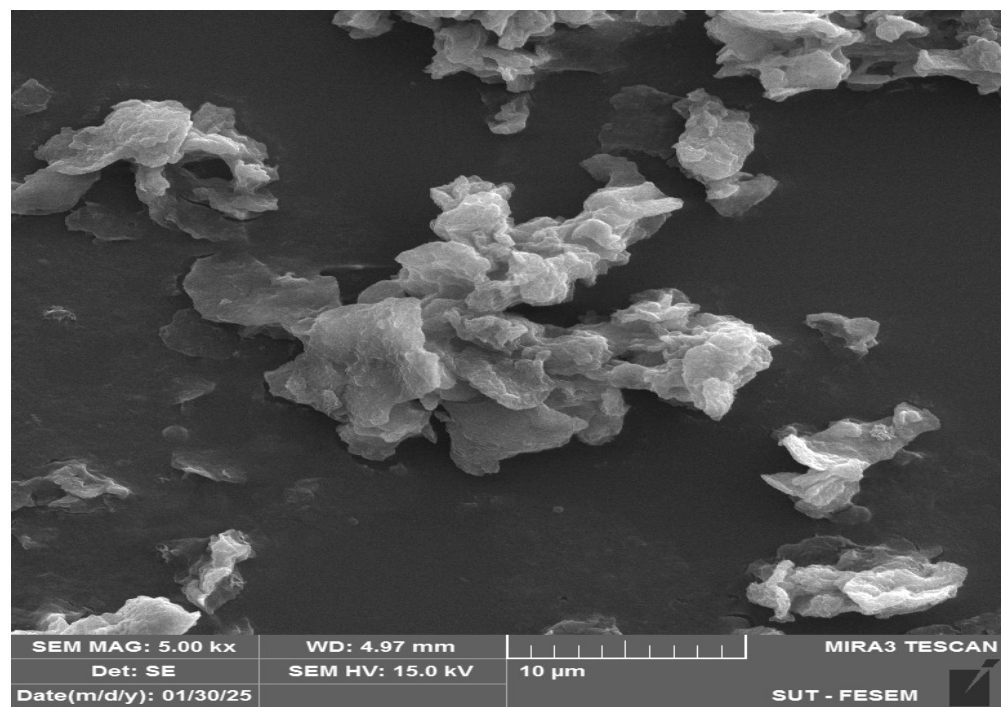


Figure 4 Represents the products of the starter P-7 and P-8 for the four treatments of fatty acids on the Leishmania parasite compared to the control sample and the stage on a 1.5 % agarose gel in the presence of the size indicator Marker.

Scanning electron microscopy (SEM) images of nanostructured flax seeds-based fatty acids effect are shown in Figure 5. This presents agglomerated grains and small cubes. The formation of crystallites is dense. Figure 5 has showed agglomerated particles, good and large nanostructure adhere to the surface attributing to the material content, the analogy is clear. The homogeneity of cubes-like nanostructure is illustrated that reflects the crystallite size.



(a)



(b)

Figure 5 SEM images of nanostructured flax seeds (a) before fatty acid effect (b) after fatty acid effect.

The optical studies are very important for biological application to illustrate the necessity of absorption and FTIR from analytical viewpoint. The absorbance spectra of nanostructured flax seeds-based fatty acids effect are measured at ambient temperature via UV-vis in wavelength range of 350 - 1150 nm as shown at Figure 6. The absorbance spectra are illustrated clearly that are attributed to material behaviour. The correlation between absorption and wavelength is inversely [11-14].

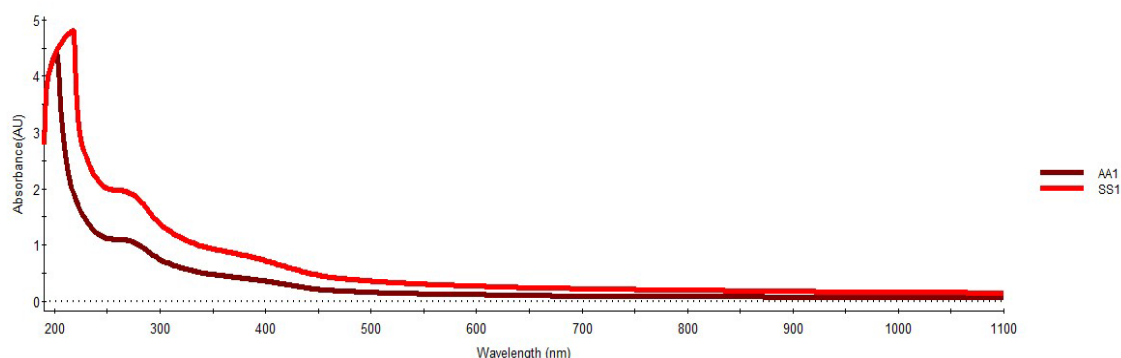


Figure 6 Absorbance of nanostructured flax seeds (red line) before fatty acid effect (brown color) after fatty acid effect.

4. CONCLUSIONS

The dissolving fatty acids have been employed to prepare successfully effect of fatty acids extracted from flax seeds of *Leishmania tropica*. 100 micrograms have given entire inhibition of the parasite boom in the promastigote form in 48 hours. 0.5% concentration of fatty acids was used in the treatment of patients infected. Scanning electron microscopy (SEM) has proved a nanostructure of our synthesized material, followed by prepared absorption successfully.

References

- [1] F.A.Atiensar, , A.J.Evenden, , A.N.Jha, , M.H.Depledge, *Biomarkers* 7 (2002) 94
- [2] Z.Zhou, *Nature* 452 (2005) 997
- [3] S.Sundar, G Agrawal, M. Rai, MK Makharia, HW Murray. *British Medical Journal* (2001) 419
- [4] A.Onasanya, H.D Mignouna, G.Thottappilly, *African Journal of Biotechnology* 8 (2003) 246
- [5] J.Sambrook, , E. R Fritsch, T. Maniatis, Cold Spring Harbor Laboratory Press (1989)
- [6] J.G.Williams, *Nucleic Acids Research*, 18 (1990) 6531
- [7] A.Hassan, A.Al-Ghamdi, *Journal of Applied Sciences Research* 5 (2009) 2153
- [8] M. R.Blair, , M. R.Watson, , R. C.Walshe, , F.Maj, *Journal of Experimental Psychology* 35 (2009). 1196
- [9] Al-Asi, Academic Excellence Prize/ Jordan University of Science & Technology (2002)
- [10] Al-Zuhairi Ph.D. Thesis, Faculty of Agriculture and Forestry, University of Mosul, Iraq (2014)
- [11] Maria S. da Dunla, *Exp. Theo. NANOTECHNOLOGY* 8 (2024) 23
- [12] Ziyad Khalf Salih, Angham Ayad Kamall-Eldeen, *Exp. Theo. NANOTECHNOLOGY* 8 (2024) 27
- [13] Ghazal Tuhmaz, *Exp. Theo. NANOTECHNOLOGY* 8 (2024) 33
- [14] A. T. Al-Douri, R. Gdoura, Y. Al-Douri, A. Bouhemadou, A. F. Abd El-Rehim, *Journal of Materials Research and Technology* 15 (2021)1487

