

## STUDY FOR THE GROWTH RATE, VIABILITY AND MORPHOLOGICAL CHANGES OF LEISHMANIA TROPICA IN DIFFERENT CULTURE MEDIA

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### Abstract:

This study included the estimation of growth rate, viability and morphological changes in different culture media (NNN, P-Y, RPMI- 1640, and Panmed). Promastigotes cultured in RPMI-1640 showed maximal growth rate after (2, 4, 6) days of cultivation ( $27.26 \pm 0.05$ ), ( $172.20 \pm 0.1$ ) and ( $343.81 \pm 1.48$ ) million parasites / ml for each day respectively, while P-Y media gave the highest mean of growth rate after (8 and 10) days of cultivation ( $307.16 \pm 1.67$ ) and ( $303.5 \pm 4.96$ ) million parasites / ml for each day respectively.

P-Y medium showed the maximal percentage of viability after (2, 4, 6, 8, and 10) days of cultivation ( $99.76 \pm 0.5$  %), ( $98.30 \pm 0.17$  %), ( $96.1 \pm 0.1$  %), ( $92.5 \pm 0.52$  %) and ( $87.26 \pm 0.05$ ) % for each day respectively. The maximum rates of each (flagellated spindle form, flagellated ellipsoidal form, flagellated round form and round form) was (96% in P-Y medium after 2 days of cultivation), (19% in Panmed media after 8 days after cultivation), (14% in Panmed media after 10 days after cultivation) and (22% in P-Y medium after 10 days of cultivation) respectively for each forms.

### Introduction

The genus *Leishmania* contains a number of species pathogenic for humans, including *Leishmania donovani*, *Leishmania tropica*, *Leishmania major*, *Leishmania mexicana*, *Leishmania braziliensis*, *Leishmania chagasi*, and *Leishmania infantum*. [1]. They also occur in reservoir host such as dogs, rodents, and other mammals [2]. *Leishmania* species transmitted by the bite of female sand flies, which are of the genus *Phlebotomus* in the Old World and *Lutzomyia* in the New World [3]. The genus *Leishmania* has two morphological forms in its lifecycle: the amastigote within macrophages of mammalian host and promastigote in the gut of invertebrate host. [4]. More than 12 million people in 88 countries are known to be infected with leishmaniasis, but the true burden remains largely hidden. Two million new cases – 1.5 million of cutaneous leishmaniasis over 90% occur in Afghanistan, Algeria, Iran, Iraq, Saudi Arabia, Syria, Brazil and Peru, 500 000 of the visceral form of the disease – occur annually, but declaration of the disease is compulsory in only 32 countries and a substantial number of cases are never recorded. [5,6]. Many studies of the

antigenic structure, biochemical properties, and infective capabilities of *Leishmania* species that can cause leishmaniasis in the Mediterranean, the Middle East, and tropical regions are being carried out, a great number of promastigotes are needed and in vitro cultivation of both amastigote and promastigote forms is carried out by using many different media [7,8]. A variety of media have been used for the culture of *Leishmania*, these can be divided into three main categories: semi solid, biphasic and liquid. [9].

### Materials and Methods:

#### Parasites:

*Leishmania tropica* promastigotes produced by cultivating biopsy material taken from the skin lesion of a patient with cutaneous leishmaniasis in NNN culture medium.

#### Culture Media:

1-Biphasic medium (NNN):

- Solid phase [10]: Brain Heart infusion, 33.3 gm; Agar, 16 gm; D-Glucose, 8 gm; Defibrinated Rabbit Blood, 100 ml; Gentamycine, 1.25 ml.
- Liquid phase [11]: NaCl, 9 gm; KCl, 0.42 gm; Ca<sub>2</sub>Cl.H<sub>2</sub>O, 0.32 gm; NaHCO<sub>3</sub>,

0.2 gm; Gentamycin , 80 mg/ml ;Glucose, 2 gm and distilled water 100 ml.

2-RPMI-1640 medium [11]: RPMI-1640 powder, 10.4 gm; sodium bicarbonate, 2 gm; double distilled water 1000 ml; Hepes solution, 10 ml; Fetal Calf Serum, 10%; penicillin, 1 U / 100 ml and streptomycine, 1 gm / 100 ml.

3- P-Y medium [9] : Peptone , 1gm ; yeast extract , 0.25 gm ; Na<sub>2</sub>HPO<sub>4</sub> , 0.75 gm, NaCl , 0.80 gm ; distilled water 100 ml; penicillin 100 U/ ml ; streptomycine , 0.1 mg/ml and 10% Fetal Calf Serum.

4- Panmed Liquid Medium[12]: Panmed, 2 gm ; KCl , 0.23 gm ; NaCl , 8.5 gm; NaHCO<sub>3</sub>, 2 gm ; Glucose 3 gm; Gentamycine , 1.5 ml ; Fetal Calf Serum, 10%; diluted Defibrinated Rabbit Blood, 50 ml (dilution 1 Blood : 2 Distilled water) and Distilled water , 950 ml.

The pH of all the above media adjusted to 7.2.

Twenty universal tubes of each of the above media were inoculated with 10<sup>6</sup> parasites/ml, 15 tubes were used for examination and five as backup in case of contamination.

All of the universal tubes were incubated at 26°C, then the following follow up tests were done:

1. Growth Rate: Promastigotes were counted with a Thoma microscope slide (hemocytometre).
2. Viability: Viability of parasites in different culture media was estimated by using 0.04% Erythrocin-B. [13].
3. Morphological Changes: Morphological changes of parasites were checked in different culture media, the morphological changes include the transformed forms (flagellated spindle form, flagellated ellipsoidal form, flagellated round form and round form).

All of the above criteria were checked at (2, 4, 6, 8, and 10) days of cultivation

## Results

1) Growth Rate: Figure (1) describe the growth rate of *Leishmania tropica* in different culture media (NNN , P-Y , RPMI- 1640 , Panmed), the maximum

mean of growth rates in these culture media after 6 days of cultivation were (114.43 ± 1.56 ), (338.06 ± 1.15), (343.81 ± 1.48) and (331.48 ± 0.65) million parasites / ml for each culture media respectively .

RPMI-1640 media gave the highest mean of growth rate after (2, 4, 6) days of cultivation (27.26 ± 0.05), (172.20 ± 0.1) and (343.81 ± 1.48) million parasites / ml for each day respectively, while P-Y media gave the highest mean of growth rat after (8 and 10) days of cultivation (307.16 ± 1.67) and (303.5 ± 4.96) million parasites / ml for each day respectively.

2) Viability: Figure (2) describe the viability of *Leishmania tropica* in different culture media (NNN, P-Y , RPMI- 1640, Panmed ), the maximum mean of viability in each culture media were after 2 days of cultivation (96.85 ± 0.69) % , (99.76 ± 0.05) % , (99.7) % and (97.26 ± 0.5) % for each culture media respectively .

The viability gradually declined and reached the minimum value after 10 days of cultivation (83 ± 1) % , (87.26 ± 0.57) % , (86.23 ± 0.30) % , (84.66 ± 0.49) % for each culture media respectively.

The results also showed that P-Y media gave the maximum mean of viability after (2, 4, 6, 8, and 10) days of cultivation (99.76 ± 0.5) % , (98.30 ± 0.17) % , (96.1 ± 0.1) % , (92.5 ± 0.52) % and (87.26 ± 0.05) % for each day respectively.

3) Morphological Changes: Figure (3) showed the transformed forms (flagellated spindle form, flagellated ellipsoidal form, flagellated round form and round form) in different culture media (NNN, P-Y , RPMI- 1640 , Panmed ) , the maximum rate of flagellated spindle form (95 , 96 , 94 , 93 ) % after 2 days of cultivation in each culture media respectively.

The results showed that the maximum rate of flagellated spindle form was 96% after 2 days of cultivation in P-Y media, while the maximum rate of flagellated ellipsoidal form was 18% after 8 days of cultivation in NNN media, 15 % after 6 and 8 days of cultivation in P-Y media, 17% after 6 days of cultivation in

RPMI-1640 and 19% after 8 days of cultivation in Panmed media. While the maximum rate of flagellated round form was (12, 8, 10) % after 8 days of cultivation in (NNN, P-Y and RPMI- 1640) respectively, and 14 % after 10 days of cultivation in Panmed media.

Finally, the maximum rate of round form was (22, 18, 21, and 20) % after 10 days of cultivation in (NNN, P-Y, RPMI-1640, and Panmed) respectively.

### Discussion:

*Leishmania* have been readily cultured as promastigotes (the insect stage) in a variety of media at temperatures below 28 °C [14].

In our study we evaluated (growth rate, viability and morphological changes) of *Leishmania tropica* in different culture media (NNN, P-Y, RPMI- 1640, and Panmed). Although NNN medium and other diphasic media are routinely used for maintenance and production of *Leishmania* [15], RPMI-1640 media gave the highest mean of growth rate after (2, 4, 6) days of cultivation, while P-Y media gave the highest mean of growth rate after (8 and 10) days of cultivation, the excessive production of promastigotes depend largely on the serum and serum components present in (RPMI-1640 , P-Y and Panmed) and absent in NNN media [16].

The viability gradually declined in all culture media used in our study and reached the minimum value after 10 days of cultivation. The minimizing of viability may be due to the food insufficiency to all parasites in culture media [17].

The maximum rate of flagellated round form was (12, 8, 10) % after 8 days of cultivation in (NNN, P-Y and RPMI- 1640) respectively, and 14 % after 10 days of cultivation in Panmed media and the maximum rate of round form was (22, 18, 21, and 20) % after 10 days of cultivation in (NNN, P-Y, RPMI- 1640, and Panmed) respectively. these tow form of *Leishmania* (flagellated round form and round form) found in old media which characterized by the presence of metabolic products (toxic materials) [18].

Thus, there has been a preference for liquid media for promastigote cultivation, although NNN medium and other biphasic-type media are routinely used for maintenance and producing parasites [19]. Indeed, the origin formulation of NNN medium remains useful for establishment of strains in culture [15].

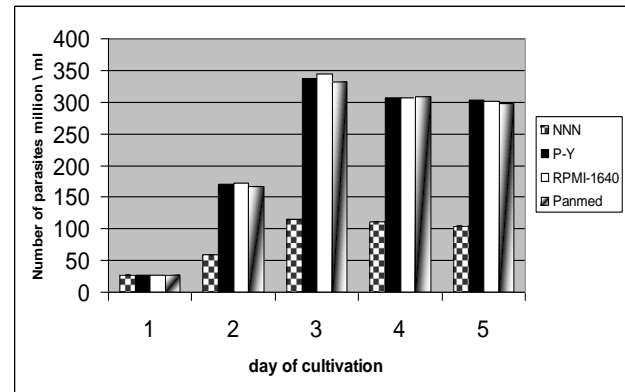


Figure (1): Growth rate of *Leishmania tropica* in different culture media

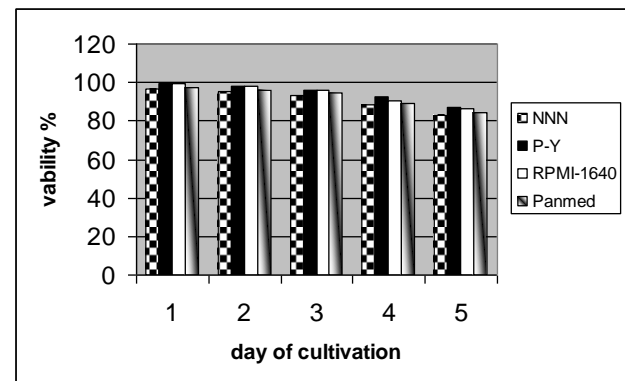
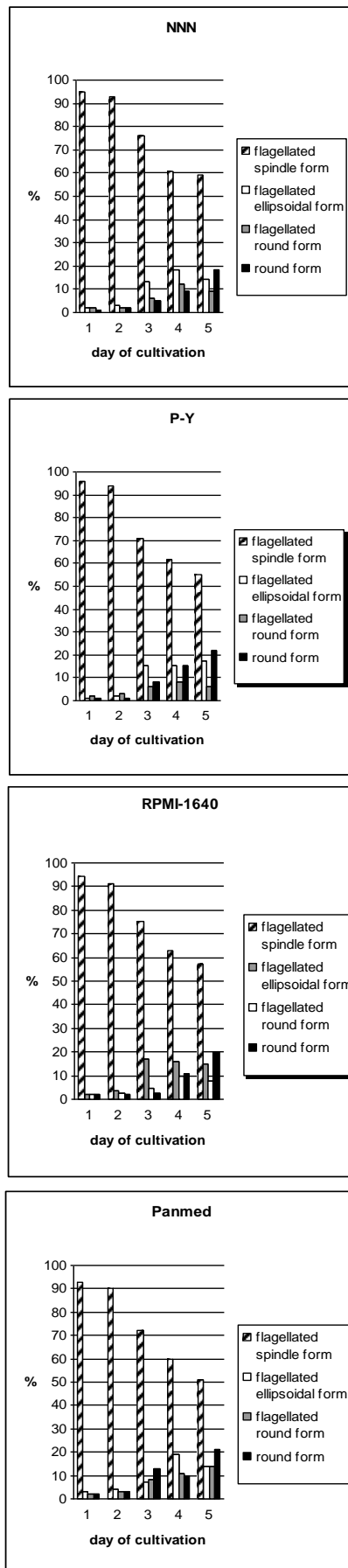


Figure (2): Viability of *Leishmania tropica* in different culture media



**Figure (3): the transformed forms (flagellated spindle form, flagellated ellipsoidal form, flagellated round form and round form) in different culture media**

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## الخلاصة:

في هذه الدراسة تم تقدير كل من معدل نمو وحيويته وشكليته طفيلي *Leishmania tropica* في اوساط زرعيه مختلفه ( RPMI-1640 , P-Y , NNN ) و ( Panmed ) .

اظهرت الطفيليات اعلى معدل نمو في الوسط الزراعي RPMI-1640 بعد (2,4,6) ايام من الزرع (  $0.05 \pm 27.26$  ) و (  $0.1 \pm 172.20$  ) و (  $1.48 \pm 343.81$  ) مليون طفيلي / مل على التوالي بينما اظهرت الطفيليات اعلى معدل نمو في الوسط الزراعي P-Y بعد (8 و 10) ايام من الزرع (  $1.67 \pm 307.16$  ) و (  $4.96 \pm 303.5$  ) مليون طفيلي / مل على التوالي كما اظهر الوسط الأخير اعلى حيويته للطفيليات بعد الأيام (10,8,6,4,2) من التتميه حيث بلغت (  $0.5 \pm 99.76$  )% , (  $0.17 \pm 98.30$  )% , و (  $0.52 \pm 92.5$  )% و (  $0.5 \pm 87.26$  )% على التوالي.

كما بلغت اعلى معدلات كل من الأشكال (المغزلي المسوط , اهليلجي مسوط , دائري مسوط ودائري) (96% في وسط P-Y بعد يومين من التتميه ) , ( 19% في وسط Panmed بعد 8 ايام من التتميه ) , ( 14% في وسط Panmed بعد 10 ايام من التتميه ) و ( 22% في وسط P-Y بعد 10 ايام من التتميه ) على التوالي .