STAINING TECHNIQUE FOR HELMINTH PARASITES BY USE RED BEET (*BETA VULGARIS* L.) EXTRACT

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(Received 3 April 2012,Accepted 26 April 2012)

Key words: Helminthes, Trematoda, red beet.

ABSTRACT

Experimentally red beet (*Beta vulgaris* L.) extract had been used to stain different types of parasites *Lecithochirium acutum* (Trematoda), *Taenia* spp.*Dipylidium caninum* and cysticercus cyst for *Taenia* spp. (Cestoda), *Toxascaris leonina* and *Toxocara canis* (Nematoda) and *Neoechinorhynchus iraqensis* (Acanthocephala). Stained helminthes were acquired a good pigmentation with distinction their internal structure, fish's Trematoda *Lecithochirium acutum* appear well –defined of its internal structure, the oral and ventral sucker, cirrus sac, uterus, testes, ovary and vitallaria were appeared pink in color. The whole of mature and gravid segments of *Taenia* spp. were acquired the yellow to brown colour in both cirrus sac and vas deference besides differentiation of eggs in gravid segment, Scolex of *Taenia* spp. get brown in color.in addition segment of *Dipylidiun caninum* acquired yellow colour. The cysticercus cyst was taken pink in color with distinguish of its hooks, while both *Toxascaris leonina***,** *Toxocara canis* and *Neoechinorhynchus iraqensis* were appear pink to red in colour .

INTRODUCTION

The red beet (*Beta vulgaris*) plant belong to Chenopodae family, it has a huge roots with have contained different substantial elements like amino acid , sucrose, minerals , vitamins and dyes in fruit and roots (4).

(10) were mentioned the *Beta vulgaris* have advantage in medicine such as it had a strong lipotrophic and antioxidant affectivity inside human body, (16) run to use extraction of red bet as active substance were given mice to protection them from exposed to gamma irradiation. Furthermore(8) been revealed the red beet appeared as inhibitor effect in lung and skin tumor produced the Epstein- Barr virus that experimented in vivo.

In addition the red bet have been used as natural colourant in food costumers which it could be healthier or at least not harmful. Both (12) and (9) were referred they many consumers in USA are replaced the artificial dyes by natural dyes of red bet. In such side the juice of it contain the all types of natural colorants which include yellow, orange, red and purple colours (1).

The aim of study include prepare dye extract from red beet to stain some types of helminthes as substitution cheap and easy getting material.

MATERIALS AND METHODS

The following steps are :

1-In this experimental study the parasites had been choosed which included**,** Trematoda is *Lecithochirium acutum*, Cestoda (Scolex , mature and gravid segment of *Taenia* spp , gravid segment of *Dipylidium caninum*, cysticercus cyst. Nematoda is *Toxocara canis* and *Toxascaris leonina* and Acanthocephala *Neoechinorhynchus iraqensis***.**

2- prepare extract solution from red bet : the plant of red bet were cut to small portion and boiled (1 kg with 1 liter normal saline) and set to filtration.

3- Stain of the specimens similar steps for aceto carmin which clarified in Palm (2004).

A- Transferred the specimens from formalin 10% to the ethanol 70 % for at least 15 minutes .

B- The specimens were transferred to red beet solution and was kept in it for 1-2 days .

C- The specimens were transferred to 70% ethanol for 15 minutes to wash out excess stain .

D- placed the specimens in acid ethanol (2 ml of concentrated HCL in 100 ml of 70% alcohol) in order to remove excessive stain without loss of pigmentation .

E-Specimens again transferred to 70 % ethanol for 15 minutes .

F- They were transferred in to 90 % ethanol for 15 minutes .

G- The specimens were transferred to 100 % ethanol for 30 minutes and to ascending dish of 100 % ethanol.

H-The specimens were cleared by clearing agent (methyl salicylate).

4- mounted the specimen on slides by canada balsam .

RESULT

Through the picture under microscope helminthes samples were evidently stained by red beet extraction and have been well-defined in external and internal structure , whence the following point well be giving more details :

1- Cestoda : Scolex of *Taenia* spp appear brown in color - fig (1). The whole of mature and gravid segment appear yellow in color, in addition the cirrus, cirrus pouch, vas deference been very definitely and brown in color -fig (2) .The eggs of gravid segment also showed aggregation like chains in dark yellow appearance fig (3) . The eggs in gravid segment of *Dipylidium caninum* appear as multiple cell masses through their acquired yellowish in color fig (4). The cysticercus cyst were appear pink in staining with prominent of its hooks, fig (5,6) .

2- The anterior part of both *Toxocara canis* , *Toxascaris leonina* were appear red in colour by this stain, fig (7,8) whether the posterior end of *Toxascaris leonina* showed pink or slightly red in color with well –defined of anus pore fig (9).

3- Acanthocephala (*Neoechinorhynchus iraqensis*) acquired red in color, fig (10).

4- Trematoda (*Lecithochirium acutum*) appear well –defined of its internal structure that the oral and ventral sucker , cirrus sac yellowish in color , uterus show coils and brown color, testes, ovary pink in color) and vitallaria pink in color and like rose in apparent .fig (11,12) .

Figure (1) : Scolex of *Taenia* spp x 300

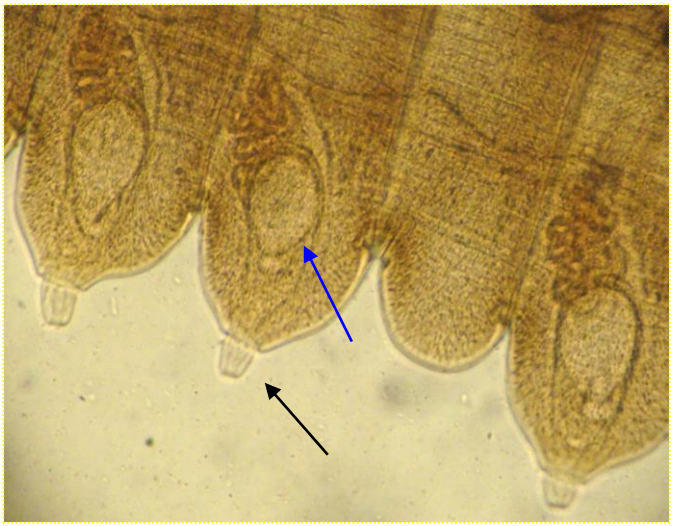

figure (2) : mature segments of *Taenia* spp, ( ) cirrus pouch , ( ) cirrus x 400.

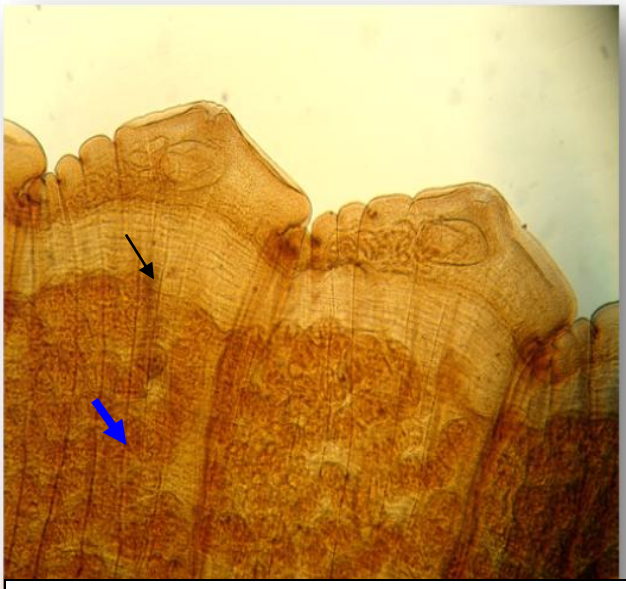

figure (3) show gravid segmenst of *Taenia spp* , eggs as masses ( ) , genital pore ( ) X 300

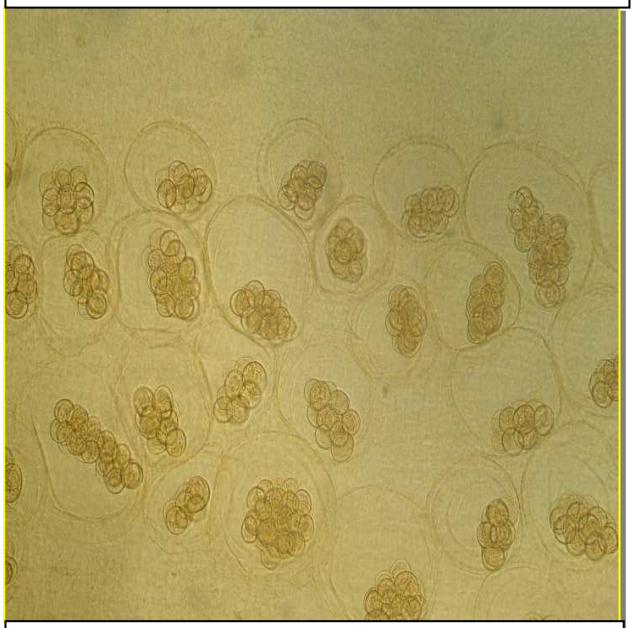

figure (4) : gravid segment of *Dipylidium caninum* engorgement with eggs. X 400

figure (5) : cysticercus cyst , arrow in hooks X 300

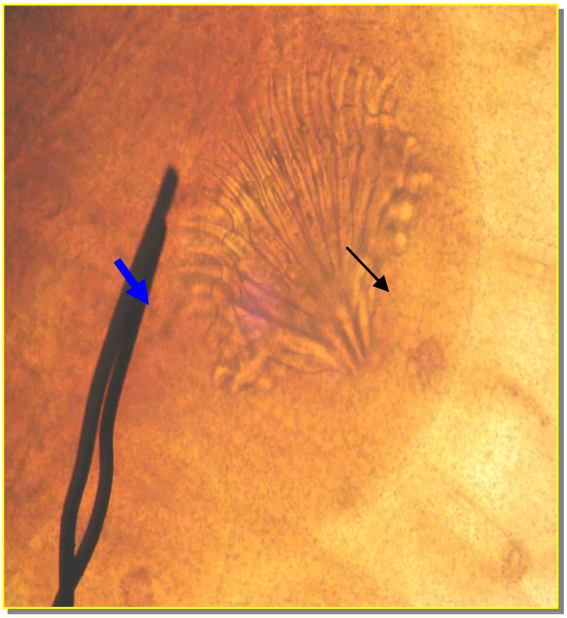

figure (6) : hooks dendrites of Cysticercus cyst X 400

figure (7) : Anterior end of *Toxacara canis* X 300

figure (8): Anterior end **of** *Toxascaris leonina* X 400

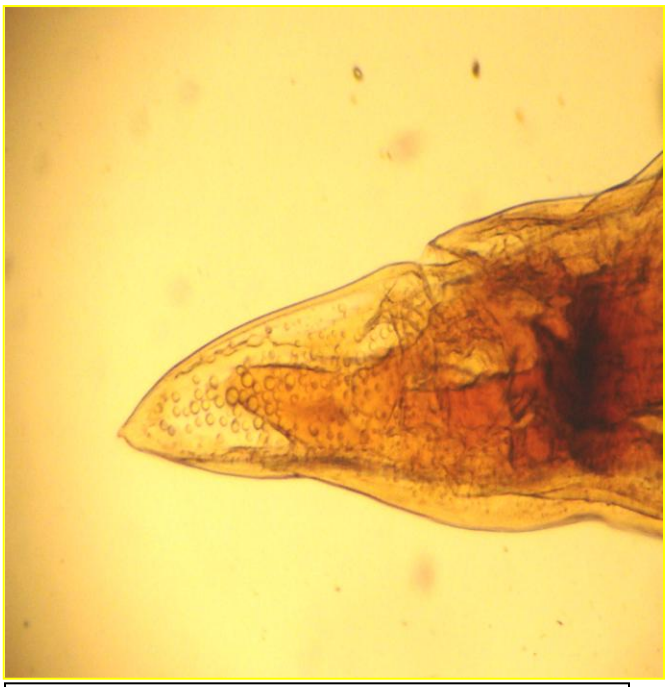

Figure (9): posterior end of *Toxascaris leonina* ( ) anus region . x 400

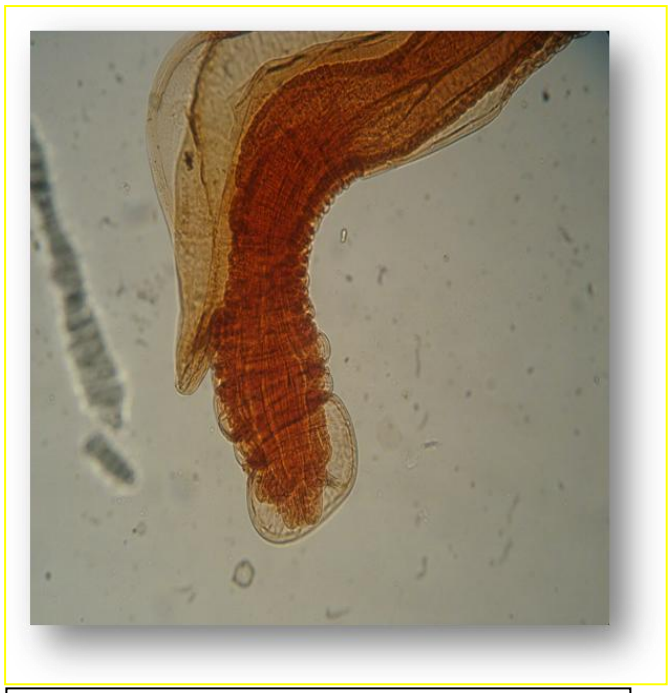

figure (10) : *Neoechinorhynchus iraqensis* x 400

figure (11) : anterior end of *Lecithochirium acutum* ***( )*** arrow refer to testes x 400

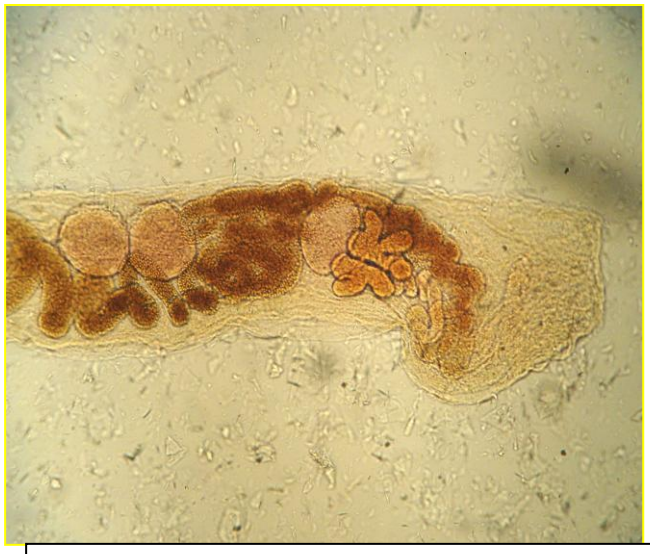

figure (12) : posterior end of *Lecithochirium acutum* ***( )*** arrow refer to vitallaria x 400

DISCUSSION

The applied of this study by used red bet plant extract for staining different type of helminthes (Trematoda, Cestoda, Nematoda and Acanthocephala). (15) referred that necessary to explicit the internal and external parts of parasites in order to laid in classification and identical key. Recently it ran many research to evaluation the effect of natural dyes to stain different parasites, therefore (3) were used the aqueous extraction from red bet and Kujurat flower for staining Scolex of hydatid cyst in order to detection the viability.

The specimen were appeared well pigmentation with distinction of their internal structure. So that the successful staining has been ability the stain to distribution in the tissues of helminthes in different degree for permanent mount. The pigmented specimens were kept without decay for more than one year, also the Cestoda was acquired yellow in color while the Nematoda and Acanthocephala acquired red in color that due to the juice of red bet have red and yellow dyes with a strong stability. whence the(14) , (7) and (5) were referred the red beet have many pigment component like Betacyanin (red color) and Betanin , isobetanin and betanidin (yellow color).

Nevertheless the variances helminthes structure might be given another reason to acquisition the dye at different color degree.(13) were mentioned the body or tegument of cestoda composed from muscle bundles, layer of glycocalyx and calcareous corpuscles and other substance, but the internal body of Nematoda have been pseudocoelom, therefore it would be high probably to acquire the red color .

The present result agreeable with (2) when found the kujurat red flower could stain of *Diphyllobothrium latum*, with purple color but the *Ancylostoma* spp. and *Enterobius* sp. were taken a red in color.

The structure of Trematoda like Cestoda but different from its digestive system and bodies more elastic than Cestoda, This Trait might be reason to acquired pink colour, therefore present result agree with (6), when found red colour of whole structure of *Fasciola hepatica* by alizarin and henna.

Conclusion From all above mention the regard red beet extract or its juice has prosperous stain for pigmentation different type of helminthes and as natural dye material

that it would be cheap dye than expensive artificial one and capable to developed it by using some modification to other groups of helminthes such as Leeches.

Aknownlogment : I would like to thank Dr Atheer ali in agriculture college \ basrah unviersity who help me for got some specimen and other effort .

تقنية تصبيغ الديدان الطفيلية باستخدام مستخلص الشوندر *Beta vulgaris* L.

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الخلاصة

تضمنت الدراسة استخدام خلاصة الشوندر (*Beta vulgaris* L.)لتصبيغ بعض انواع من الطفيليات وهي المثقوبة *Lecithochirium acutum* والشريطيات .*Taenia* spp و *Dipylidium caninum* و الطور المتكيس لـ .*Taenia* spp و الديدان الاسطوانية *Toxascaris leonina* و *Toxocara canis* و شوكية الرأس *Neoechinorhynchus iraqensis*.الديدان المصبغة اكتسبت الصبغة بشكل جيد مع وضوح تراكيبها الداخلية، مثقوبة الاسماك *Lecithochirium acutum* حيث وضحت تراكيبها الداخلية والمتضمنة المحجم الفمي والبطني و كيس الذؤابة والامعاء والرحم والخصى والمبيض مع بروز الغدد الحية باللون الوردي. اكتسبت القطع الناضجة والحبلى لـ . *Taenia* spp اللون الاصفر- مع ظهور كل من كيس الذؤابة والناقل المنوي باللون الرمادي اضافة وضوح البيوض في القطع الحبلى ،اما رؤيس الدودة الشريطية .*Taenia* spp اكتسب اللون الرمادي.بالضافة ان قطعة طفيلي *Dipylidium caninum* اكتسبت اللون الاصفر الطور المتكيس اكتسب اللون الوردي مع وضوح الاشواك ، بينما ظهر كل من الطفيلي *Toxascaris leonina* و *Toxocara canis* و *Neoechinorhynchus iraqensis* اللون الوردي الى الاحمر .

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