Natural date vinegar as a natural dye in staining and preservation of some tapeworm specimens and other parasite's eggs

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Abstract

Preparation of Specimens is one of the important techniques in the investigation study of parasites infection and this process is complicated so this study deals with staining and preservation of tapeworm's specimens (Moniezia expansa adult worm) and preserve other parasite's eggs (Diphyllobothrium latum and Toxocara canis) by using a simple method and inexpensive, available, non toxic, unharmed and locally produced material (Natural Date Vinegar). The results showed that the Natural Date Vinegar had the ability to preserve eggs specimens that were used in the study (100 %) ; it also showed that the Natural Date Vinegar had the ability to stain and preserve about (75%) of tapeworm's specimens which kept their shape and their internal structure after 6 years from preparation and they were compared with other specimens stained by Aceto Carmin which their internal structure appeared unclearly after the same period of time .

Key words : Diphyllobothrium latum, Toxocara canis, Moniezia expansa, Natural Date Vinegar, staining, preservation .

Introduction

One of the biggest problems that faced the study of parasites are the difficulties of obtaining, preparing and preserving of parasites specimens for along period of time , in addition to the complexity of these methods and the expensive materials used in them and In order to decrease these difficulties so many studies deal with using natural material to stain and preserve parasitic specimens such as El – Edressy (2005) [1]. This study deals with naturally produced Vinegar which is a liquid condiment, made and used by people for thousands of years. Traces of it had been found in Egyptian urns from around 3000 years B.C. The word "vinegar" derives from the Old French vin aigre, meaning "sour wine." Louis Pasteur showed in 1864 that vinegar results from a natural fermentation process[2]. It produced from an anaerobic fermentation of sugar–materials in dates by yeasts (glucose and fructose 55:45) to produce ethanol then the oxidation of ethanol to acetic acid by Acetobacter [3], acetic acid concentration in vinegar is not less than 4 % (w/v) [4], vinegar also contains small amounts of tartaric acid, citric acid, and other acids. The pH of it is typically falls in the range of 2 to 3.5, depending on the concentration of acetic acid but Commercially available vinegar usually has a pH of about 2.4 [2]. Vinegar is a useful material used in many purposes, it used as a detergent, a herbicide [5], in food preparation (as a condiment for fishes and flavoring for potato chips, sauces and lemon juice) [2]. The medical uses of it include the inhibition of serum total cholesterol and triacylglycerols [6] also, It had a role in blood glucose control, diabetic management [7],[8] and diet control [9]. Acetic acid the major component of vinegar was used in scientific researches, in the process of fixation and preservation because it is one of the components of many fixation agents such as Zenker's fluid and Bouin's fluid and in the process of tissue's other biological samples including parasites specimens staining because it is one of the components of staining dyes such as Carmin [10]. The parasitic worms specimens are so important in the study of parasites, such as tapeworms specimens (Moniezia expansa) which is the common tapeworm of sheep, it lives in the intestine of sheeps and reach a length of 600cm (20 feet), the mature proglottides are wider.
than long and some other parasite's eggs such as fish tapeworm Diphyllolothrium latum which they are ovoid, yellowish to golden, operculated 55-76 × 41-56 μm and unembryonated [11] and Toxocara canis eggs, which is the cosmopolitan parasite of domestic dogs, the eggs are light brown, have a thick rough proteinaceous coat [11], [12]. These specimens were fixed mostly with formalin and stained with carmin and because of the destruction of it's internal structure of these specimens after a period of time, there will be a continuous requirement to renewed these specimens. So the aim of this study is to develop a reliable, inexpensive and a simple method for staining and preservation of tapeworms specimens and preservation of other parasite's eggs using locally produced Natural date vinegar for this purpose. All the samples were collected and stained in 2001 and vinegar ability to preserve them examined after 6 years from preparation in 2007.

Materials and Methods

1. Eggs samples:
Eggs of D. latum & T. canis (which reported in Iraq by El-Emarah 2007) [13] were collected from fecal samples of infected dogs they were isolated by the floating technique [14], then they were identified [15]. The collected eggs were placed on slides and left until the normal saline dried. Then two drops of natural date vinegar placed on it, covered with a thin layer of Canada Balsam and a cover slide. After making sure there is no spaces for air to enter under the cover slide or vinegar leak, the slides were labeled and examined. (this part of the research performed in 2001). In order to examine the ability of vinegar to preserve the specimens, the slides which made in 2001 were reexamined, identified and photographed in 2007.

2. Tapeworm's specimens:
Tapeworm adult specimens were collected from the infected sheeps in Basrah slaughter house, kept in normal saline and took to the laboratory. Tapeworms washed with normal saline for five times to remove the stocking remains then they were cut into 1 cm long pieces from several parts of the worm body, (20 pieces of the tapeworm specimens). Each piece put between two slides and pressed on it hardly and gently to avoid tearing it up; the two slides were bound by a cotton thread and placed in a glass jar containing 10% formalin for 3–7 days. After that slides were opened and the worms specimens were placed in a glass jars contained natural date vinegar the jars were closed and left for 7–15 days, then the specimens were took from the jars and each one placed on a new slide and covered with a thin layer of Canada balsam and a cover slide after making sure that there is no air bubbles, slides examined and identified according to [14] another specimens were stained with aceto carmin and represent the control specimens. (this part of the research performed in 2001). In order to examine the ability of vinegar to preserve the specimens, the slides which made in 2001 were reexamined, identified and photographed in 2007.

Results and Discussion

In order to decrease the difficulties of the process of staining and preserving of parasite's specimens the aim of this study was to use natural date vinegar instead of the expensive materials and complex methods (Aceto carmine staining). The results showed that natural date vinegar had the ability to preserve 100% of the egg samples of both D. latum and T. canis for 6 years, and no changing in the eggs structures were noticed as in picture. No. (1) and (2). This ability may be explained by the presence of acetic acid, the major component of vinegar, which allow vinegar to preserve the specimens from degradation by preventing bacterial growth because many bacteria won't grow in acidic media with a pH level below 4.6 and therefore vinegar inhibits bacteria and other microorganisms growth [16]. The results showed that the tapeworm specimens belonged to Moniezia expansa
according to its typical features [15], staining with vinegar were good in 15 specimens from 20 pieces (75%). The ability of vinegar to stain can be explain in one hand by the red to brownish color of vinegar that results from colored materials and tanines which are responsible of red to brownish color of date (the source of vinegar) [3], and in the other hand by the absorptive nature of tapeworm tegument because they lack any trace of a digestive tract and therefore they must absorb all required substances through their external covering [12] so vinegar will inter the tapeworm body carrying with it the staining pellets causing specimens staining. The prepared specimens kept their structure after 6 years, the most clearly feature of it is the interproglottids glands picture No. (3) and the female reproductive system picture No. (4) while the internal structure were not clear in the specimens stained with Aceto Carmin picture No. (5) and (6). vinegar also used to stain different microorganisms such as fungi (Arbuscular-Mycorrhizal Fungi) and it also gave an excellent results [17]. The preserving results of tapeworm's specimens were good because in addition to inhibit the bacterial growth [16] mounting the specimens with Canada Balsam also helped to preserve them. Using vinegar not only gave an excellent results but also provide a safe alternatives to the hazardous, toxic, and potentially carcinogenic chemicals used in usual staining techniques, because it usually used in human nutrition and it is obviously not harmful [18], and the specimens stained and preserved with vinegar don't need to be kept in cold or special places but it can be left in the laboratory at room temperature.

Picture (1): Egg of *D. latum* preserved by natural date vinegar after 6 years

Picture (2): Egg of *T. canis* preserved by natural date vinegar after 6 year
Picture (3): Interproglottids glands of *Moniezia expansa* stained by natural date vinegar after 6 year of preservation

Picture (4): Female reproductive system of *Moniezia expansa* stained by natural date vinegar after 6 year of preservation

Picture (5): Interproglottids glands of *Moniezia expansa* stained by Aceto Carmin after 6 year of preservation
Picture(\*): Female reproductive system of *Moniezia expansa* stained by Aceto Carmin after 6 year of preservation

**References**


2. Internet: en.wikipedia.org/wiki/Vinegar


5. Internet: www.gardenstew.com/ about 319.html


خل التمر الطبيعي كصبغة طبيعية لتصبغ وحفظ بعض نماذج الديدان
الشريطية ووبوض بعض الطفيليات الأخرى

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الخلاصة
إن تحضير عينات الطفيليات من الأمور المهمة في دراسة علم الطفيليات وهي عملية معقدة، لهذا فإن هذه الدراسة تضمنت تصبـيـغ وحفظ بعض عينات الدـيدان الشريطية البالغة باستخدام طريقة سـبـيـطة وـمـادـة رخـيـصة وـمنـسـورة وـ (Moniezia expansa, Diphyllobothrium latum, Toxocara canis) الطفيليات ولـاـيـة وأبـيـرة وـهي خـل التـمـر طـيـرـيـي المصـنـع مـلـحـاـ، أظهرت النتائج قدرة خـل التـمـر طـيـرـيـي على حفظ عينات بـبـلاـيـة الطـفـيـلـيات المـسـتـخـدـمـة في الدراسة وـنـسبـة (100 %) إذ احتفظت العينات بشكلها ونتراكيماها الداخلية بعد 6 سنوات من تحضيرها كما أظهرت النتائج قدرة خـل التـمـر طـيـرـيـي على تصبـيـغ وحفظ عـيـنـات الدـيدان الشريطية البالغة ونسبة (75 %) إذ احتفظت العينات بشكلها ونتراكيماها الداخلية بعد 6 سنوات من تحضيرها وقد تم مقارنتها مع عينات أخرى تم تصبـيـغها بـصـغاـة الـكـارـمـان الحامضية وـالـتي ظـهرت تراكيماها الداخلية غير واضحة بعد مرور نفس الفترة الزمنية.

Diphyllobothrium latum - Moniezia expansa - Toxocara canis -

الكلمات المفتاحية: خـل التـمـر الطبيعي - تصبـيـغ - حفظ