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Effects of Ewe Oil in Media for Mass Production of *Tricephalobus* (Nematoda: Rhabditida)

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ABSTRACT

The Media WA 2%, WA 2% + Ewe oil, WA 2% + K_2HPO_4, SDA (Sabouraud 4% Dextrose Agar), SDA + K_2HPO_4, SDA + Ewe oil, NA, NA + K_2HPO_4 and NA + Ewe oil were used for mass production of *Tricephalobus*. One mL Ewe oil extracted from milk was added to the media 2% WA + Ewe oil and NA + Ewe oil and incubated at 25°C for one week. The media were monitored every 24 h. Juvenile and mature nematodes in WA 2% amended with Ewe oil and the other media were counted after one week. These results showed that the reproduction of nematodes in WA 2% + Ewe oil was much more in comparison with other media. The experiment was repeated three times. These results indicated that Ewe oil was a better medium than that of cholesterol for mass production. This is the first report of using Ewe oil in mass production of this nematode and this method is advisable for it's mass production.

Key Words: *Tricephalobus;* Ewe oil; K_2HPO_4

INTRODUCTION

The ability to culture organisms is always of great benefit to their scientific study and sometimes simply essential. Culture ensure that a steady supply of live material is available for teaching and research, eliminating the need for repeated sampling of inaccessible or temporally fluctuating habitats in search of living specimen. *Caenorhabditis elegans* is by far the most widely cultured nematode species being maintained in systems ranging from tiny petri dishes to huge bio-reactors (Gbewonyo *et al*., 1994). Many methods have been developed for axenizing nematodes (Krusberg & Sardanelli, 1984). The free living nematodes can be cultivated axenically in a chemically defined medium (Nicholas, 1962; Sayre *et al*., 1963). The nematode belong to Pnagrolaimidae is a use-full bait for studying nematophagous fungi and has therefore been used for their isolation from soil (Dackman *et al*., 1987). Freeliving nematode (Panagrellus redivivus) was axenically grown in neutralized soya peptone as in jansson and nordbring-hertz (1979). In the present study, Ewe oil was extracted from milk and its in reproduction and population growth of *Tricephalobus* was investigated.

MATERIALS AND METHODS

Sampling was carried out in the Faculty of Agriculture in Tehran Province, Iran. Nematodes were extracted using the funnel technique according to Beier and Traunspurger (2003) from leaf dust. The samples were sieved through a 38 micrometer sieve. In this study females of *Tricephalobus* sp were maintained on 2% WA in 100 mm diam plastic petri plates. Distilled water (5 - 10 mL) was added to the plates. Six media were prepared for mass production of nematodes as following: WA 2%, WA 2% + Ewe oil, WA 2% + K_2HPO_4, SDA (Sabouraud 4% Dextrose Agar), SDA + K_2HPO_4, SDA + Ewe oil, NA, NA + K_2HPO_4 and NA + Ewe oil. One mL of Ewe oil extracted from milk was added to WA 2% and NA. Three gravid females were added to each plate. Plates were viewed under a microscope one week after inoculation and the number of larvae and females were counted. To ensure that the nematodes in the cultures were *Tricephalobus* three or five adults were randomly picked and placed in a water mount during the experiment and identified. Experiments were arranged in a factorial design with four replicates. The data were analyzed with SAS software.

RESULTS AND DISCUSSION

Effect of these media on production of females and juveniles were significant ($P \leq 0.01$) (Table I & Table II). Results showed that the populations of nematodes in the media WA 2% + K_2HPO_4 and WA 2% + Ewe oil significantly increased over time, when starting with three nematodes. The population of nematode in media amended with Ewe oil in comparison with the media without Ewe oil was different at 1% level. The numbers of juvenile in media WA 2%, WA 2% + k_2HPO_4 and WA 2% + Ewe oil were more than the others. In the media NA, NA + Ewe oil and SDA the population was very low (Table III).

Results showed that WA 2% + k_2HPO_4 and WA 2% + Ewe oil with 289.25 and 609.50 larvae and 70.25 and 89.25 female were the best media for production of Tricephalobus and showed significantly differences at 1% level comparison with other media (Table III). The number of larvae and females in the media SDA, SDA + K_2HPO_4, SDA + Ewe oil, NA, NA + K_2HPO_4 and NA + Ewe oil were very low and could not show any significantly differences at 1% level with the others (Table III).

Table I. Analysis of variance of some media and amended additives on production of larvae of *Tricephalobus* after one week under dark condition at 25°C

Treatment	DF	Mean Square
Medium	2	813.200**
Amended	2	106.018**
Medium × Amended	4	125.090**
Error	27	1.765

** Significantly different (1%)

Table II. Analysis of variance of some media and amended additives on production of females of *Tricephalobus* after one week under dark condition at 25°C

Treatment	DF	Mean Square
Medium	2	157.071**
Amended	2	7.984**
Medium × Amended	4	12.164**
Error	27	0.454

** Significantly different (1%)

Table III. Effect of some media and amended additives on production rate of Larvae and Female of Tricephalobus after 1 week under dark condition at 25°C

Treatment[a]	Larvae	Female
WA	35.00 c[b]	16.00 b
WA+ K2HPO4	289.25 b	70.25 a
WA+ Ewe oil	609.50 a	89.25 a
NA	1.50 d	1.00 c
NA+ K2HPO4	1.00 d	0.00 c
NA+ Ewe oil	0.00 d	0.00 c
SDA	5.00 d	1.25 c
SDA+ K2HPO4	2.25 d	0.75 c
SDA+ Ewe oil	3.75 d	0.75 c

[a] WA, Water Agar 2%; Ewe oil, extracted from milk ; NA, Nutrient Agar 2%; SDA, Sabouraud 4% Dextrose Agar 6.5%.
[b]Mean values followed by the same letter are not significantly difference according to Duncan test at 1% significance level.

In this study adults and juveniles of *Tricephalobus* proved relatively easy to be maintained *in vitro* with media WA 2% + K_2HPO_4 and WA 2% + Ewe oil. Our results showed that *Tricephalobus* needs moisture as observed in preliminary culturing plates. The availability of a new source of nematode growth factor from Ewe oil extracted from milk provides a useful supplement for culture of free living nematodes and may be of value in culture of other organisms. This material (Ewe oil) can be used either as a supplement or as a complete medium. De ley (1998) used cholesterol in medium for *in vitro* culturing of free living nematodes. Cholestrol starvation on free living development at both embryonic and post-embryonic stages by examining brood size, embryonic lethality, growth rate and worm size (Yhong-Hee Shim *et al.*, 2002). Nematodes, including free-living *Caenorhabditis elegans*, require sterol for their growth as a nutritional source (Hieb & Rothstein, 1968). It is well known that some free living nematodes such as *C. elegans* is un-able to bio-synthesize sterol de novo (Rothstein, 1968). However, apparently *C. elegans* is capable of obtaining cholestrol or cholestrol-like sterol (e.g. 7-DHC) for their growth by metabolizing natural sterols such as 24-alkyl sterol present in many plants (Chitwood *et al*., 1995) or sterols from the animal body in soil. Our results showed that Ewe oil is easily available and can be a useful substitution for cholesterol as a lipid source for nematode. It is well established that exogenous factors such as temperature play an important role in altering the rate of development in nematodes (Tahseen *et al*., 1994). The results showed temperature (25°C) had effective role in reproduction and population of *Tricephalobus.* Some sterol that already have been used for studying of Panagrolaimids nematodes played important role in growth and reproduction of this group, because of they are not able to synthesis the sterol. This demonstration also confirms a vital function in these nematodes (Cole & Dutky, 1969). As Ewe oil is the great source of cholesterol, it can be used for preparation of media for mass production of these nematodes. The ability to culture *Tricephalobus* allows further examination of the ecological, biological and molecular investigation free living and plant parasitic nematodes.

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