FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 67760
Scriptwriter Name: Debopriya Sadhukhan
Project Page Link: https://review.jove.com/account/file-uploader?src=20666848

Title: Evaluation of Antioxidant and Anthelmintic Properties of *Tithonia diversifolia* Extracts Against Gastrointestinal Nematode Eggs Using In Vitro Assays

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

Correct X

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **NO**

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **NO**

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **August**

When you are ready to submit your video files, please contact our Content Manager, Utkarsh Khare.

Current Protocol Length

Number of Steps: 14
Number of Shots: 20

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

NOTE: Authors changed all interview answers on spot

1.1. **Griselda Meza Ocampos:** My research focuses on parasitology. Currently, studying natural compounds from plants to control internal parasites in small ruminants.
a. We want to integrate the traditional knowledge and the alternative methods to controlling small ruminant worm populations.
b. For this purpose we design effective, long-term, and environmentally sustainable parasite control strategies

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.3.1., 3.3.2. (slated 1.1 A + 1.1B)(last one)*

What technologies are currently used to advance research in your field?

1.2. **Csilla Zambori:** Nowadays, increasingly using technologies like predictive modelling, automatic counters, and molecular tools for detecting specific alleles. Moreover, several institutes around the world are developing vaccines prototypes against Haemonchus contortus, which is a major step forward. Others are using organoid models to reduce reliance on laboratory animals. This is ethically and scientifically valuable. Overall, there is a strong and coordinated global effort to advance diagnostic tools and research, aiming to develop innovative and sustainable strategies for parasite control worldwide and prevent drug resistance.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. (last one)

What are the current experimental challenges?

1.3. **Helene Desanti:** The main challenge of this technique lies in the variability of the egg hatching response. Even when following all the protocol steps, the egg's hatching may occur between 48 - 72 hours, or, in some cases, not at all.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.1.1. (last one)*

What significant findings have you established in your field?

1.4. **Griselda Meza Ocampos:** Our findings showed in vitro ovicidal activity, confirming the antiparasitic potential. Thus, representing a meaningful discovery and their potential clinical application to enhance animal health. Additionally, the low cost and ethical approach of this assay validates the used of traditional knowledge regarding future studies.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.1.1.(last one)*

How will your findings advance research in your field?

1.5. **Griselda Meza Ocampos:** Our study results delivered evidence-based insights into alternative anthelmintic strategies. Validated eco-friendly compounds to support control strategies to reduce drug dependency.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. (last one)

Ethics Title Card

This research has been approved by the CEMIT animal ethics committee

Protocol

Videographer: The authors will provide footage for the protocol section, so you don't need to film it.

NOTES:

1. Authors filmed the protocol
2. Videographer filmed the interviews
3. Authors have slated the protocol sections as section 4 and then 5 (as given in their post shoot script). So protocol starts at 4.1.1 shot.

4. Parasitological Procedures Using the McMaster Egg Counting Technique

4.1. To begin, mix 30 grams of each fecal sample with 50 milliliters of saturated salt solution with a density of 1.2 grams per milliliter to prepare for coprology assays **[1]**.

4.1.1. WIDE: A shot of the prepared fecal solution and the talent holding it or standing beside it.

4.2. Homogenize 3 grams of fecal sample in 50 milliliters of saturated sodium chloride solution **[1]**. Filter the mixture through cheesecloth and fill both chambers of a McMaster slide with the filtered suspension and allow the slide to stand undisturbed for 5 minutes **[2]**.

4.2.1. Talent mixing fecal sample in 50 milliliters of sodium chloride solution.

4.2.2. ~~Talent pouring the mixture through a cheesecloth into a beaker~~. **NOTE**: Delete, VO merged with the previous shot

4.2.3. Talent pipetting the filtered sample into both chambers of a McMaster slide.

4.3. Examine the slide under a microscope at 10x *(ten-ex)* magnification **[1]** and count strongyle-type eggs **[2]**. Calculate eggs per gram by using the given formula **[2]**.

4.3.1. Talent placing the slide under a microscope.

4.3.2. SCOPE: Microscope view showing strongyle-type eggs at 10 times magnification.

4.3.3. TEXT on PLAIN BACKGROUND:

$$\text{Eggs per g or EPG} = (\text{egg count}) \times 50$$

5. Egg Hatch Assay (EHA)

5.1. Filter the feces twice using sterile gauze **[1]**.

5.1.1. Talent pouring fecal mixture through sterile gauze into a clean beaker.

5.2. Then, filter the resulting suspension **[1]** and transfer it to a sieve **[2]**.
5.2.1. Talent pouring the filtered liquid again through sterile gauze.
5.2.2. Talent transferring the filtrate into a sieve.

5.3. Wash the material retained on the sieve with distilled water **[1]** and collect the washed suspension into 15 milliliter tubes **[2].**
5.3.1. Talent using a squeeze bottle to rinse the sieve with distilled water.
5.3.2. Talent transferring the rinsed suspension into 15 milliliter centrifuge tubes.

5.4. Place the samples in a tabletop centrifuge with a swinging bucket rotor and spin at approximately 4800 *g* for 5 minutes at room temperature **[1-TXT]**.
5.4.1. Talent placing the tubes into a tabletop centrifuge and closing the lid. **TXT: Repeat centrifugation 2x with distilled H_2O; 1x with saturated saline to concentrate the eggs**

5.5. Resuspend the eggs in PBS and adjust the egg concentration to 100 eggs per milliliter for assay setup **[1]**.
5.5.1. Talent pipetting PBS into the centrifuge tube containing the sample and mixing gently.

~~5.6. Now, adjust the egg concentration to 100 eggs per milliliter for assay setup **[1].**~~
5.6.1. ~~Talent diluting the suspension to achieve 100 eggs per milliliter~~. **NOTE**: Delete, VO merged with the previous shot

5.7. Prepare the positive control by dissolving thiabendazole in PBS to obtain a 1000 micrograms per milliliter stock solution **[1-TXT]**.
5.7.1. Talent dissolving thiabendazole in PBS. **TXT: Negative control: Egg suspension in PBS**

~~5.8. Then, prepare the negative control using the egg suspension in PBS **[1].**~~
~~5.8.1. Talent adding PBS to the egg suspension in a fresh tube to prepare the negative control.~~ **NOTE**: Delete, VO moved as on screen text

~~5.9. Take a 48-well multiwell plate and prepare a final volume of 250 microliters in each well using a 1:1 *(one to one)* mixture of extract and egg suspension **[1].**~~
5.9.1. ~~Talent pipetting 125 microliters of extract and 125 microliters of egg suspension into the wells of the 48-well multiwell plate.~~ **NOTE**: Delete, VO merged with the next shot

5.10. After adding the 1:1 *(one to one)* mixture of extract and egg suspension, add approximately 100 eggs per milliliter to each well of a 48-well plate **[1-TXT]**.

5.10.1. Talent gently pipetting the egg suspension into wells. **TXT: Prepare each concentration in quadruplicate; Count the eggs under an inverted microscope at 40x**

~~5.11. Finally, count the eggs under an inverted microscope at 40x *(forty ex)* magnification **[1]**.~~

~~5.11.1. SCOPE: View of eggs at 40 times magnification through the inverted microscope~~.

Results

6. **Results**

6.1. The concentration-dependent ovicidal and larvicidal effects measured by the egg hatch assay are presented in this table **[1]**.

6.1.1. LAB MEDIA: Table 2. *Video Editor: If the whole table is too large to be displayed on screen, only include:*

Columns: *Concentration, % hatching mean, % inhibition mean, % LFE, % OE, % hatching, and % hatching* inhibition.

Rows: *Title row of the applicable columns (the row with bold words), 2400µg/ml (and rows 2, 3, 4 below this), 1200µg/ml (and rows 2, 3, 4 below this), and Control-PBS (and rows 2, 3, 4 below this).*

If possible, include the equations that are at the top of the table (%LFE, %OE, %hatching, and %inhibition).

6.2. At 2400 micrograms per milliliter, a highly significant reduction in egg hatching was observed, with a mean hatching percentage of only 0.76 percent, indicating near-complete suppression of embryonic development **[1]**.

6.2.1. LAB MEDIA: Table 2. *Video editor: Highlight the row for 2400 µg/mL and emphasize the "% hatching mean" value of 0.76*.

6.3. The inhibition percentage at this concentration reached 99.24 percent, confirming a strong ovicidal effect **[1]**.

6.3.1. LAB MEDIA: Table 2. *Video editor: Highlight the row for 2400 µg/mL and emphasize the "% inhibition mean" value of 99.24.*

6.4. The percentage of larvae failing to eclose was 66.67 percent, indicating a disruption in the hatching process of gastrointestinal nematode eggs **[1]**.

6.4.1. LAB MEDIA: Table 2. *Video editor: Highlight the row for 2400 µg/mL and emphasize the "% LFE" value of 66.67.*

6.5. The ovicidal effect, measured at 30.30 percent, suggests that a portion of eggs were arrested during early embryonic stages **[1]**.

6.5.1. LAB MEDIA: Table 2. *Video editor: Highlight the row for 2400 µg/mL and emphasize the "% OE" value of 30.30.*

6.6. The PBS control group showed over 80 percent hatching, confirming the expected baseline in the absence of inhibitory agents **[1]**.

6.6.1. LAB MEDIA: Table 2. *Video editor: Highlight the row for 2400 µg/mL and emphasize the "% hatching" value of 83.02.*