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Examining the Effects of *Ruta Graveolens* on the Growth of Caenorhabditis Elegans

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ABSTRACT

Ruta graveolens ("common rue") is a shrub-like plant in Mediterranean countries. Although it is consumed as a food spice in small amounts, it has also been used as an abortifacient and contraceptive by many communities worldwide. When used as an abortificient, it is shown in pregnant mice to cause embryo damage and fetal death. In this report, the abortifacient activities of *Ruta graveolens* were studied using a more straightforward and less complex method to show its adverse effect on fertility by exposing living worms to the plant extract. *C. elegans* were exposed to *Ruta graveolens*, and the nematode was counted daily to observe the retardation in the reproduction rate. Exposing the nematode to the plant extract while observing a control group showed that the reproduction rate was hindered, and multiplication was reduced. *C. elegans*, however, overcame the toxic setback in the environment and continued to multiply until the food supply was exhausted and the dead nematodes reached a toxic state. Furthermore, our observation of *Ruta graveolens* against nematodes was consistent with the previous data reported using *Ruta graveolens* against plant growth.

Introduction

Ruta graveolens is known by many names, and the most common ones are Herb-of-Grace, Herb of Repentance,

Tena Adam, or simply Common Rue. The herbs originated in the Mediterranean and have spread to the rest of Africa, South America, Asia, and the world. Many people find Ruta attractive because of its intense aroma, its bitter taste, its encapsulated seedhead, its aesthetic small yellow flowers, and the availability of the grounded root-stem-seed-leaf parts of the plant for consumption. The herb has a blueish color, can self-seed to replenish itself, requires little attention, and can tolerate rough and arid growing conditions. The dangers of *Ruta graveolens* as an abortifacient were studied in the biotechnology field using the growth rate of onion roots as a medium [1]. This study aims to reveal the toxicity of *Ruta graveolens*, which was originally intended to be enjoyed at a distance and kept for safety reasons. Traditionally, Ruta has been used as a universal self-heal and cure-all herbal medicine for many ailments and has been utilized in many countries. It has been applied to cure mental sickness, epilepsy, arthritis, ulcers, and other disorders. In the United States, *Ruta graveolens* has been recognized as one of the most poisonous herbs and is only used for ornamental purposes. The two most common uses of *Ruta graveolens* are for medical and culinary purposes and, to a lesser extent, as a pest repellant. *Ruta graveolens* was discovered to contain over one hundred listed chemicals through the medical application of biotechnology. Among the numerous compounds identified in the literature, Ruta makes rue, the essential oil used to repel insect predators. The second compound is known as Coumarin, which is a blood-thinning medicine that pharmaceutical companies use.

These biologically active chemicals are light-sensitive, and when people are exposed to sunlight, they cause skin irritations that cause rashes and bleeding. The underlying problem with using *Ruta graveolens* is that a safe amount has yet to be determined, and the toxicity effect has overshadowed the benefits [2]. The toxic effects of this plant are attributable to its characteristics as a blood thinner, and it also causes abortion [3]. Rue is known to increase blood flow among women, and many young South American women who cannot get pregnant take the herb to clean their bodies during the menstrual cycle. On the other hand, pregnant women are advised not to take rue because it is known to cause miscarriages. People who work with *Ruta graveolens* are advised to wear long sleeves and gloves to protect their skin from rashes. Countries like Ethiopia, which regularly use *Ruta graveolens* to season Shiro Wat in cooking and add the herb to tea and coffee, must be aware of these plants' dangers. The quantity used for different ailments has not been determined, and it is easy to overdose, causing organ failure and even death. Caenorhabditis elegans is a small (1 mm long) free-living nematode in the Phylum Nematoda displaying a primitive nervous, reproductive, and digestive system [4].

C. elegans is a soil-dwelling worm that naturally consumes E. coli and other bacteria. *C. elegans* commonly exist as self-fertilizing hermaphrodites, although a small percentage (<1%) of the microscopic worms are males due to altered X chromosome-autosomal ratios. The metazoan roundworm has a long history as a model organism in biology and genetics and is easily cultivated in the laboratory [5]. Adult worms complete their life cycle in 3-4 days and contain approximately 1000 cells, each of which can be tracked developmentally using molecular and cell biological procedures. Moreover, *C. elegans* has roughly the same number of genes found in humans and shares a high degree of homology with many mammalian genes. Many research tools are available to assign putative genetic functions in response to experimental treatments. We found *C. elegans* to be a great model system to study the reproduction problems caused by *Ruta graveolens*. It is known that one female *C. elegans* can produce hundreds of fertilized eggs during its life cycle. The effects of *Ruta graveolens* on the reproductive processes of *C. elegans* have not been studied before, and we chose the nematode for this experiment because of its high reproduction rate. The biological properties of *C. elegans*, such as its high reproduction rate and its tolerance to stressful conditions, have made it helpful as a bioindicator. The result in lab animals has been organ failures when meaningful concentrations of *Ruta graveolens* are increased to significant levels of effectiveness. The results can be used for further confirmation in human toxicity screening [6]

Experimental Procedure

Caenorhabditis elegans (N2 strain), Escherichia coli (OP50), and nematode growth agar were purchased from the Carolina Biological Company. The worms were cultured on solid media as previously described [7]. Cold extraction of the ground powder of *Ruta graveolens* (stem, leaves, roots, and flowers) was performed in the laboratory [1]. The concentrated solution was used to test its abortifacient properties. *Ruta graveolens* was added incrementally (1-10 drops) to *C. elegans* growing on a nematode growth agar plate. Furthermore, 10, 20, 30, and 40% by weight of *Ruta graveolens* were transferred to another set of plates, and the toxicity was observed for two weeks.

Results

The experimental results examing the effect of *Ruta graveolens* on the reproduction of *C. elegans* are shown in Figure 1. A small agar sample was cut from the original *C. elegans* culture plate, and the agar cube was placed in a petri dish. Populations of *C. elegans* were counted under the compound light microscope. The nematode population increased significantly during the first 24 hours of transmission. *C. elegans* were counted periodically, and the results are shown in Figure 1. The curve is shaped like a bell curve, with the population of *C. elegans* starting small and rapidly increasing once they are removed from the transport sample.The growth curve of *C. elegans* imitates the bacterial growth curve, which has an initial lag phase, log phase, stationary phase, and death phase. The factors that affect microbial growth, like pH, temperature, moisture, and nutrient availability, are held constant for all samples. Days 1-6 are a high-energy period of metabolic activity during which *C. elegans* begin to grow in their new environment. Days 6-8 was the growth phase when the nematode rapidly divided, hatching several eggs and resulting in exponential growth. Days 8-10 were the stationary phase, where the birth and death of the *C. elegans* have reached equilibrium. The population continued to increase until the nutrients were exhausted, there was an accumulation of waste products, and changes in the pH of the growth medium. During days 12-14 the population of *C. elegans* decreased as the number of animals that died exceeded the number of new animals formed. On day fourteen, the number of *C. elegans* decreased to a minimal number, but the toxicity of the waste products may have reduced the number of worms that survived.

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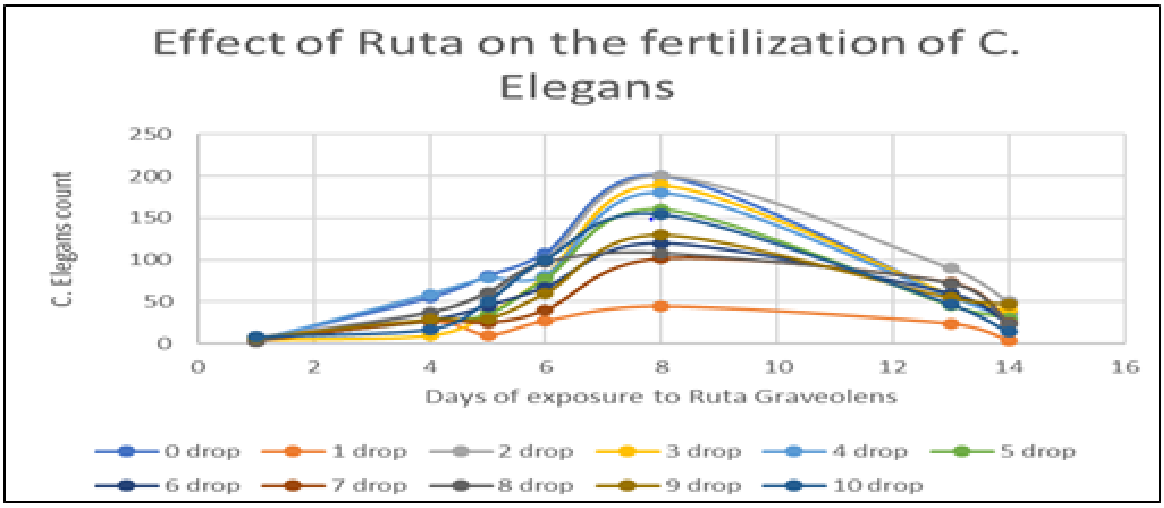

Figure 1: The number of *C. elegans* counted during the 14-day exposure period to *Ruta graveolen*.

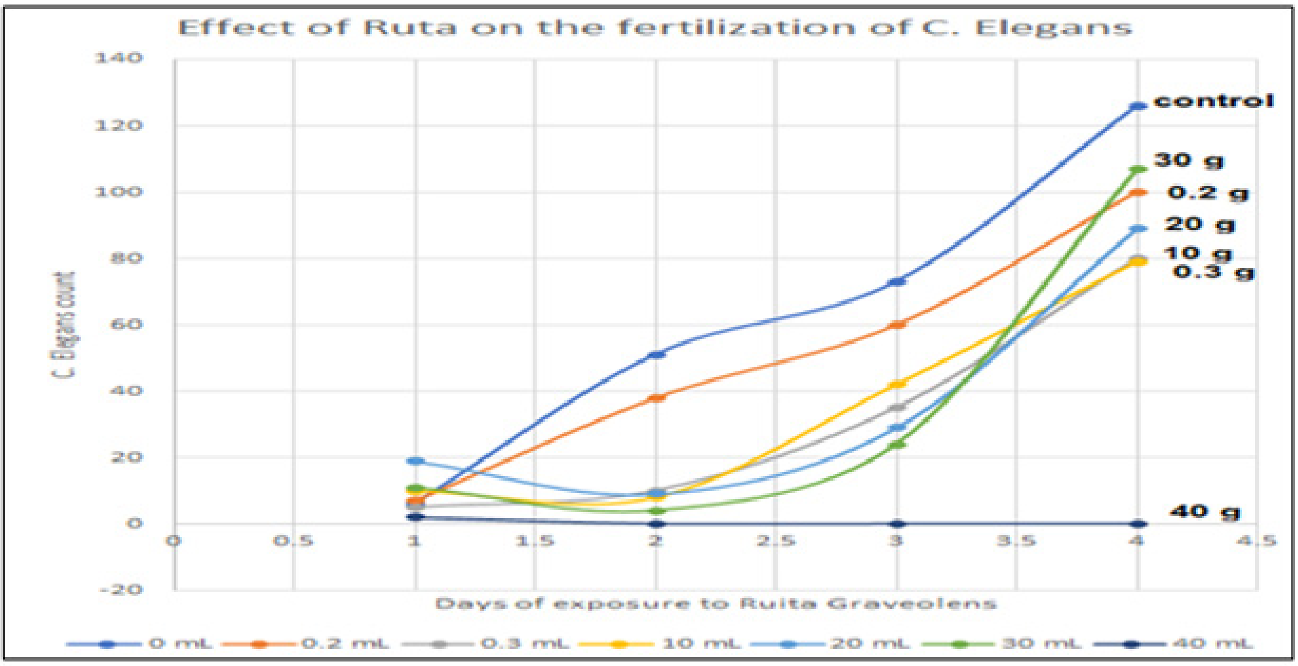

Figure 2: Cumulative effect on the reproduction of C. elegans versus Ruta graveolens (grams).

The expected curve was an S-shaped model (Figure 2) where the nematode shows exponential growth; however, there is a kink in the lag phase on days four to six before the nematode gets off to a good start. After the nematodes have moved to their new environment, the graph shows that they reached their highest population growth on day eight, which is represented by the highest point on the curve. The drop after day ten was caused by a lack of food and the toxicity caused by many dead nematodes. Overall, the result of exposure of *C. elegans* to different amounts of Ruta and the cumulative effect on the reproduction of *C. elegans* versus Ruta is presented in the graphs.

Discussion

Ruta graveolens is a shrub-like plant found in Mediterranean countries. Many countries, including England, France, and Brazil, used the plant to stimulate menstruation and induce abortion. One of the proposed mechanisms of Ruta as an abortifacient is that the plant caused embryo damage and fetal death when given by mouth to pregnant mice. Similar effects are also observed in humans. For example, it is reported to cause abortion within a day of consumption when given to humans. Besides its abortifacient effect, the plant can produce severe maternal toxicity, including vomiting, liver damage, and anemia. Moreover, other severe cases have been reported, including tremors, respiratory distress, and even death. Despite its reported toxic effects, the plant remains a common herbal medication used for pregnancy termination, and the plant, if consumed in a small amount as an herb to flavor food, is generally safe.Our study also confirms its abortifacient effect using a less complex procedure in a resource-limited laboratory. As shown in Figure 2 the worm population increased when first exposed to *Ruta graveolens*. *C. elegans* were counted on the first day of the experiment. The control group showed healthy growth while in the treatment group *C. elegans* fed with E. coli and *Ruta graveolens* (e.g., 10, 20, and 30 grams) demonstrated a slowdown in growth during the second and third days of the experiment. Nematode growth, however, picked up momentum between the third and the fourth days. The decline in the growth rate shown in Figure 2 can be explained by *C. elegans*' desire to survive and recover. The graph also shows that *C. elegans* must have an instinct for survival to monitor their environment and adapt to survive harsh conditions. The University of Rochester Medical Center's paper titled "Underlying instincts: An appetite for survival" [8] describes a molecular mechanism that *C. elegans* employs to survive the lack of food and toxicity of the growth environment.

Conclusion

This article describes the toxicity of *Ruta graveolens* in *C. elegans*, a soil nematode, grown on solid growth media. The selection of *C. elegans* as the biological system in the current study is rooted in empirical evidence from several reports that have explored the use of *C. elegans* as a beneficial model system in the study of the toxicity of environmental pollutants, potential nutritional sources, and synthetic materials [9-11]. We used three different experimental conditions in which the concentration of *Ruta graveolens* was varied. The data indicated that pretreatment of *C. elegans* during the early growth stage was sufficient to retard growth. The researchers concluded that treatment of *C. elegans* irreversibly induced reproductive toxicity in *C. elegans*. The results obtained in this report provide sufficient evidence of the toxicity of *Ruta graveolens*. Plant-based research in previous publications and the current evidence from the non-mammalian nematode provided here could serve as a warning regarding the dangers of *Ruta graveolens* to reproduction and to expecting mothers. *C. elegans* has given us a platform to use inexpensive, fast, and easily manageable experimentation to evaluate various chemicals found in Ruta.

Future studies must elucidate critical metabolic pathways, molecular mechanisms, and physiologic functions that promote nematode survival due to exposure to *Ruta graveolens* to facilitate our comprehension of the molecule's antiproliferative properties. Comparison of toxicological responses when *C. elegans* is cultured on solid and liquid media may yield vital insight into the properties and mechanism of action of *Ruta graveolens*. Further, since *C. elegans* is a transparent organism, subsequent studies could involve using fluorescent probes conjugated to *Ruta graveolens* to track the molecular localization of the putative toxin following consumption.

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