# Nematocidal action of some plants extracts against root knot nematode on tomato plants

Graduation Project
Submitted to the Collage of Agricultural Engineering Sciences/ Department of Plant Protection/ Salahaddin University in partial fulfillment of the requirements for the degree of BSc. in Plant Protection

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## SUPERVISOR CERTIFICATION

This graduation research entitled (Nematocidal action of some plants extracts against root knot nematode on tomato plants), has been written under my supervision and has been submitted for the award of the degree of B.Sc. in Plant Protection Sciences with my approval as supervisor.

Signature: 

Supervisor Name: **Assist. Prof. Dr. Kamalaldeen M. F. Hawrami**

Date: / / 2022

# DECLARATION

I hereby confirm that:

This graduation project entitled: (**Nematocidal action of some plants extracts against root knot nematode on tomato plants)** is my own original work; quotations, illustrations and citations have been duly referenced; this research has not been submitted previously or concurrently for any other degree at any other institutions; intellectual property from the research and copyright of research are fully-owned by University of Salahaddin, as according to the Salahaddin University (Research) Written permission must be obtained from supervisor and the office of Deputy Vice Chancellor (Research and Innovation) before research is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular.

Signature:

Student Name: **Narmin Fkri Shekha**
Date: / / 2024

# Dedication

**To:**

Dedicated to my family, especially my parents, my supervisor, Dr. Kamalaldeen M. Fatah and finally to all those who helped me.

## ACKNOWLEDGMENTS

I would like to first thank my Lord for the ability to perform and finish this work and all the accomplishments that I have made throughout my life and over the past years.

I would also like to thank my esteemed supervisor, Dr. Kamalaldeen M. Fatah Hawrami whose advices have given me encouragement and has been a great mentor for me over the research years, he had always an open door policy and gave his time, knowledge and experience in a very sacrificial way to me**.**

I also want to thank my family for all of the support, encouragement, and most of all, for your prayers. I wouldn't have made it through this program without them.

# List of Contents

**Abstract:**
This excerpt highlights the significance of tomatoes (Solanum lycopersicum L.) in global cuisine and human health. Originating in Central and South America, tomatoes have become a versatile ingredient, consumed raw or cooked in various dishes. Rich in vitamins, minerals, and the antioxidant lycopene, they contribute significantly to dietary needs. Tomatoes are susceptible to a diverse range of destructive diseases caused by pathogens like bacteria, fungi, viruses, and nematodes. These diseases can significantly impact yield and fruit quality. Fungal diseases such as early blight, late blight, and Septoria leaf spot cause spotting, wilting, and defoliation on leaves and stems. Bacterial leaf spot manifests as water-soaked lesions that turn black and greasy. Southern blight attacks the stems and fruits, causing rot. Tomato mosaic and tomato yellow leaf curl viruses distort and discolor leaves, while tomato spotted wilt virus produces brown spots and uneven ripening. Root knot nematodes are microscopic worms that parasitize roots, forming galls and hindering nutrient and water uptake. This can lead to severe wilting, stunted growth, and substantial yield losses.

This study investigated the effectiveness of natural pesticides derived from Eucalyptus leaves, Pomegranate peel, and Better orange peel against root knot nematodes (Meloidogyne javanica) in tomato plants. The experiment was conducted in a greenhouse using a randomized complete block design. Local tomato seeds were planted in pots containing sterilized soil and peat moss. After two weeks, some pots were inoculated with nematode-infested cucumber roots.

The results showed high susceptibility of the tomato variety to M. javanica, with infected plants exhibiting stunted growth, yellowing, wilting, and numerous root galls. To assess the nematocidal activity, researchers incorporated powders made from the three test plants and three commercial pesticides into the pots before transplanting tomato seedlings.

The findings revealed promising results for the plant-based powders. Pomegranate and Better orange peel powders at 25g/pot completely inhibited gall formation (100% efficacy). Additionally, these powders at higher concentrations (50g and 100g/pot) and Eucalyptus leaves at 100g/pot achieved impressive gall reduction rates of 90-91%. Notably, the performance of these natural products surpassed that of the commercial pesticides, which yielded a maximum decrease of 82% in gall number. In conclusion, the study suggests that Eucalyptus leaves, pomegranate peel, and Better orange peel have the potential to be developed into safe, environmentally friendly, and efficient nematicides for managing root knot disease in tomatoes. These findings

warrant further research to optimize application methods and explore the underlying mechanisms of their nematocidal action.

# 1. Introduction

The tomato (Solanum lycopersicum L., Lycopersicon lycopersicum), also known as the tomato plant, is a flowering plant of the nightshade family, Solanaceae (Ramos-Bueno et al., 2017). Native to Central and South America. Tomato has become a global culinary staple, with its versatile fruits consumed raw or cooked in a variety of dishes (Jones *et al*., 1991). Rich in vitamins and minerals, including the antioxidant lycopene, tomatoes offer a wealth of health benefits (Elbadrawy and Sello, 2016). As a popular ingredient across cultures, tomatoes continue to play a significant role in human nutrition and cuisine.

From the nutritional side, tomato is regarded as an important source of many essential vitamins, minerals and antioxidants. Tomato and products derived from it, are major food sources of carotenoids providing as much as 80% of daily intake of potassium, folate, flavonoids, ascorbic acid, and lycopene in the western diet. Over the entire world China, India, USA, Turkey, and Egypt are considered as the top producers of tomato with an estimated product of 59626900, 20708000, 12750000, 10910990, 7297108 tons respectively. According to FAO the total globally product of is 241928300 tons (Kamalaldeen, 2023 and Abdurrahman, 2018).

Culturing tomato either in green houses or in open field is highly attractive to growers in all parts of Kurdistan. The total amount of tomato produced in 2021 was 248546 tons (Unpublished data, Ministry of Agriculture and Water Resources/ Kurdistan Regional Government - Iraq). Comes in the forefront, Erbil Province with a production quantity of 105371tons; Next comes Sulaimani, Duhok, and Garmyan with a total production amount of, 94332, 46628 and 2215 tons respectively. On the other hand, Iraq is ranked tenth in the sequence of tomato producing Arab countries with annual production of 286600 tons (Kamalaldeen, 2022).

Tomato may be consumed fresh, as salads or juice or cooked and it has been used from a long time to prepare tomato paste which is widely used in the preparation soups. In Iraqi there is an old saying describes the importance of tomato in human being life says: Like tomato with every bouncy Saucepan (Jones et al., 1991).

**Figure 1. Tomato fruits can be consumed fresh or processed**

There are obligate parasites and a highly harmful plant pest that limits agricultural productivity, and most of the cultivated plant species are susceptible to infection by root-knot nematodes resulting in 30–50% reduction in tomato yield (Sasser and Carter, 1985). Using plant extracts is approved way to combat nematodes, they are cheap, easy to apply, and produce no potential to improve soil health (Sultana, V., et al., 2010).

## Objectives of the research:

1- Isolation and identification of root knot nematodes.

2- Evaluation of the effectiveness of plant extracts in nematode control compared to some pesticides.

# 2. Literature review

## 2.1. Tomato and its importance:

The tomato (Lycopersicon lycopersicum) is a bright red fleshy fruit that is commonly eaten as a vegetable. It is native to Central and South America, and was domesticated by the Aztecs, it introduced to Europe by the Spanish in the 16th century, and quickly became popular. (Østergård, 2001). Today, tomatoes are grown all over the world and are the second only after the rice, which is the most consumed food. Vitamins A and C in addition to lycopene are abandon in tomato. Lycopene is an antioxidant that has been linked to a number of health benefits, including reducing the risk of heart disease and cancer. Tomatoes can be consumed raw, cooked, or dried. They are used in a wide variety of dishes, including salads, soups, sauces (Pizza Rao, 2014).

**Some of the reasons why tomatoes are so important:**

**Nutrition:**

Tomatoes are a good source of the vitamins A, C, and K, as well as lycopene which is an antioxidant linked to health benefits. Lycopene has been shown to reduce the risk of heart disease, cancer, and Alzheimer's disease. Tomatoes are also a good source of fiber and water, which helps you feel full and satisfied (Rao & Kaur, 2014).

**Versatility:**

Tomatoes can be eaten raw, cooked, or dried and can be used in a wide variety of dishes, including salads, soups, sauces, and pizzas. Tomatoes can also be used to make juice, paste, and ketchup (Sissons & Barrett, 2000).

**Taste:**

Tomatoes fruits are delicious and have a sweet, tangy flavor. They can be eaten on their own, or they can be used to add flavor to other dishes (Baldwin, *et al*., 2006).

**Cultural Significance:**

Tomatoes are an important part of many cultures around the world. They are used in a variety of traditional dishes, and they are often seen as a symbol of good luck and prosperity (Smith, 2007).

**Economic Importance:**

Tomatoes are a major agricultural product, and they are grown all over the world. They are considered as a valuable source of income for farmers and food processors, and are contributed to the economy in many countries. In addition to the benefits listed above, tomatoes are also a good source of potassium, folate, and vitamin E. Tomatoes are also low in calories and fat, which makes it suitable for consumption by all groups of people, regardless of any health problems, which makes the demand for its consumption large and continuous, and this encourages farmers to grow it annually with the aim of making money. Overall, tomatoes are a versatile, nutritious, and delicious food that can be enjoyed by people of all ages. They are a valuable part of many cultures around the world, and they have a number of health benefits (FAOSTAT, 2023).

## 2.2 Diseases affecting tomato crop:

Bacteria, fungi, viruses and nematodes all are known to cause destructive diseases to tomato plant in addition to the problems that happened as a result of insect's attack. Diseases that attack tomato field causes destructive damages to the field and in some cases may lead to the loose of 100% of the yield . The following are Some of the most common diseases of tomatoes:

### 2.2.1 Early blight:

Early blight (figure2), caused by the fungus *Alternaria solani*, is common on garden tomatoes and potatoes, and occasionally infects eggplants and peppers. This fungal disease causes brown spots on tomato plants leaves and stems. The spots can enlarge and merge causing the leaves to die (Jones, *et al.*,1991).

**Figure 2. Tomato early blight symptoms**

### 2.2.2 Late blight:

This fungal disease is caused by the fungus *Phytophthora infestans*. Late blight (figure 3) can cause severe defoliation and fruit rot (Campbell and Madden, 1990).

**Figure 3. Tomato late blight symptoms on the fruits**

## 2.2.3 Septoria leaf spot:

This fungal disease is caused by *Septoria lycopersici*, the disease causes small, circular spots on the leaves of the plants (figure 4). The spots can enlarge and merge, causing the leaves to die (Jones, *et al*.,1991).

**Figure 4. Tomato Septoria leaf spot**

## 2.2.4 Bacterial leaf spot:

A bacterial disease caused by a group of xanthomonads (*Xanthomonas spp*.), Initially, spots appear as small, water-soaked, light to dark green areas on the young infected leaves, individual lesions develop rapidly to a size of about 0.2 cm in diameter and appear to be black and greasy (figure 5). Lesions are with or without a yellowish halo and generally irregular (Jones, *et al*.,1991).

**Figure 5. Tomato bacterial leaf spot**

## 2.2.5 Southern blight:

This fungal disease is caused by *Sclerotium rolfsii* causes the stems of tomato plants to rot (figure 6). The fungus can also cause the fruit to rot (Jones, *et al*.,1991).

**Figure 6. Tomato southern blight**

## 2.2.6 Tomato mosaic:

This virus (TMV), causes the leaves of tomato plants to become mottled and distorted (figure 7). symptoms are more severe when infected at an earlier age and vary somewhat among the different cultivars, upward curling of leaves; mottling and often chlorotic leaf margins, leaves are reduced in size and plants are stunted. The virus can also cause the fruit to ripen unevenly.

**Figure 7. Tomato mosaic disease**

## 2.2.7 Tomato yellow leaf curl:

This is a virus disease caused by ((TYLCV), The infection causes the leaves of the plants to curl and yellow (figure 8). The virus can also cause the fruit to ripen unevenly (Jones, *et al.*,199, Mustansar, et al., 2020).

**Figure 8. Tomato yellow spot leaf curl**

## 2.2.8 Tomato spotted wilt virus:

This disease is caused by the Tomato spotted wilt virus (TSWV), the disease causes the leaves to develop brown spots (figure9). Initial symptoms are leaf bronzing and wilting, followed by necrotic leaf spots and some degree of vein, stem, and petiole necrosis, at flowering through fruit set stage. Symptoms may develop in only one or two shoots, these symptoms include leaf curling, pale green to yellow discoloration, and purpling. Fruit may be bumpy, deformed, and develop ringspots, blotches, and necrosis The virus can also cause the fruit to ripen unevenly (David, 2011).

**Figure 9. Tomato spotted wilt virus**

## 2.2.9. Root knot (Gall) nematode:

Many pests and diseases damaging both the quality and quantity of tomato production. Plant parasitic nematodes are one of them. They represent an important

constraint on the delivery of global food security. Damage caused by plant-parasitic nematodes has been estimated at US$ 80 billion per year (Nicol et al., 2011). This is likely to be a significant underestimate of the actual figure as many growers in developing nations are unaware of the existence of plant-parasitic nematodes. It is generally admitted that four major species, i.e., *Meloidogyne incognita, M. javanica*, *M. arenaria* and *M. hapla*, as well as a few emerging species such as *M. enterolobii* and *M. chitwoodi*, cause the vast majority of crop damage. Root-knot nematodes can cause severe damage to the roots of tomato. Symptoms are more prevalent with tropical species compared to temperate root-knot nematodes. Yield losses of heavily infected tomato crops can reach up to 80%. Nematodes can make plants more susceptible to damage by plant pathogenic fungi, bacteria and viruses, which increases the losses of the yield Feeding activities of the root-knot nematodes in the root tissue result in the formation of massive galls of different sizes on root system (figure 10). Severally affected plants often wilt readily and may also exhibit nutrient deficiency symptoms because galled roots have limited capacity to absorb and translocate nutrient and water to other plant part (Abolusoro, et al, 2020).

**Figure 10. Tomato root knot symptoms**

## 2.2.9.1 Taxonomy of root knot nematodes

Root knot nematodes are obligate plant parasitic nematodes that belong to the genus Meloidogyne. They are among the most important and destructive plant parasites in the world, causing billions of dollars in damage to crops each year. (Siddiqi, M. R., 2013). The taxonomy of root knot nematodes is as following (Gebissa, 2021):

**Description of the organism**:

Domain: Eukaryota

Kingdom: Metazoa

Phylum: Nematoda
Class: Secernentea
Order Tylenchida
Family: Heteroderidae
Genus: *Meloidogyne*

### 2.2.9.2 Tomato root knot disease in Kurdistan:

Root knot (gall) disease is a sever destructive disease occurred in tomato fields leading to variable damaging effects on infected plants. The disease is caused by plant infection with plant parasitic nematodes known as root-knot nematode. Root-knot nematodes belong to the genus *Meloidogyne* which includes many species of plant parasitic worm-like creatures the mature female of which have pear-like shape. Meloidogyne spp. infect plant roots and causes the formation of galls in the site of infection (Sasser, J.N., and Carter 1985; Siddiqi, M. R. 2013; Jones, et al., R.E.; and Zitte et al.,1991).

In Kurdistan root-knot disease is very common in greenhouses used for planting vegetable specially tomato greenhouses. According to previous studies (Hoshang et al., 2022) four *Meloidogyne* species were found to be related to root-knot disease in the northern region of Iraq, which are:

- *M. incognita*
- *M. javanica*
- *M. hapla*
- *M. arenaria*

## 2..2.9.3 Disease symptoms:

I the early infection stage there may be no symptoms appear on the plant but later on when the disease progress galls are formed on the infected roots which in some cases may be 1-2cm in diameter or even larger.
Because of galls formation nutrients transportation is prevented, this prevention affects the aerial parts of the plant causing the appearance of nutrient deficiency like, wilting, stunting, yellowing, and death.(Sasser, J.N., and Carter, C.R. (1985).

## 2.2.9.4 Disease management:

The following methods (figure 11), helps in the control of root knot nematode (Tauheed et al., 2021)

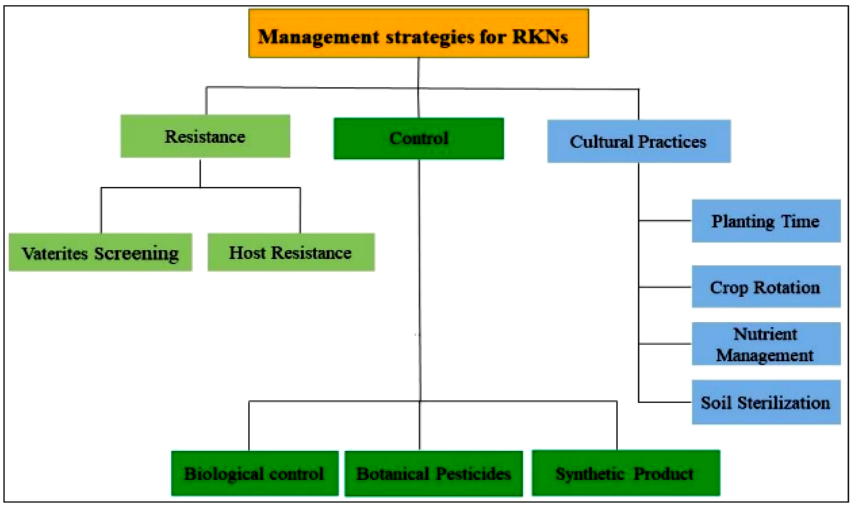


**Figure 11. Management strategies of root knot disease**

## - Crop rotation:

Crop rotation is the practice of planting different crops in the same field in successive years. This can help to reduce the population of root knot nematodes in the soil (Barkeret al.,1998; Tauheed et al., 2021).

## - Resistant cultivars:

There are a number of plant cultivars that are resistant to root knot nematodes. Planting resistant cultivars is an effective way to control the pest (Sikora and Todd, 1992; Tauheed et al., 2021).

## - Chemical control:

There are a number of different chemicals that can be used to kill root knot nematodes. However, chemical control should be used as a last resort, as it can be harmful to the environment and to beneficial insects (Castillo, et al., 1996; Tauheed et al., 2021).

## - Biological control:

There are a number of different biological control agents that can be used to control root knot nematodes. These include beneficial nematodes, fungi, and bacteria.(Lopez-Bellido, et al. 2011; Tauheed et al., 2021).

## 3. Methods and materials:

The experiment was conducted in the greenhouse of the department of Plant Protection. All the steps were performed according to İlker (2016) with minor modifications. The steps were as following.

### 3.1 Tomato seeds:

The seeds of local tomato varieties (Erbil garden). were bought from local markets:

### 3.2 Nematode inoculum:

Infected cucumber roots showing galling symptom were collected from tomato greenhouses in Qushtapa/ Hawler, Mazne/Hawler and Bazyan/ Sulymany) which are previously diagnosed by DNA- based technique in the department of plant protection to be infected with the root-knot nematode (*M. javanica*) were used as inoculum in this research.

### 3.3 Soil:

Soil, peat moss, and plastic pots used in this research were obtained from local nurseries. The soil and peat moss were sterilized by fumigation for 72 hours using 5% Formalin solution.

The expermint was performed by randomized complete distribution (PCD)

### 3.4 Planting the seeds:

The soil and peat moss were mixed in equal volumes and the transferred into one-kilogram size pots. four tomato seeds from the locally cultured golden variety were planted in each pot. For each treatment 3 pots were prepared to be tested in this research in addition to two controls (Health Untreated Control & Inoculated Untreated Control). The pots were kept in suitable place in the laboratory of plant pathology/ Department of plant protection.

### 3.5 Inoculation:

Ten grams of infected roots showing heavy galling symptoms were used to inoculate each test pot after two weeks of seeds germination by mixing them with the soil/peat moss mixture in the rhizosphere zone. All pots then were irrigated with equal volumes of water when required (İlker et al., 2016).

### 3.6 Pathogenicity test:

In this test tomato seeds were planted in previously prepared pots containing (soil/peatmoss) mixture inoculated with tomato roots showing root knot symptoms. All pots then were irrigated with equal volumes of water when required (İlker et al., 2016). The results were collected after two months and the number of galls/ each plant root were measured.

### 3.7 Testing nematocidal action of plant powders:

In this test the powder of three plants (Eucalyptus leaves, sore pomegranate peel, and *Citrus aurantium,* "Bitter orange" peel) in addition to three pesticides (Nimbecidine, Velum 400SC, and Vapcomic) were used by mixing them with the pots contents before planting tomato seedling at the 4 leaves stage. The plant powders were used in three ratios (100, 50, manufacturer. The following table (1) shows the experiment design.

Table 1: Description of the experiment design.

| Treatment (Pesticide/ powders) | Ratio g/pot | Abbreviation | Description of pot content |
|---|---|---|---|
| Nimbecidine | 10ml | Nim | 10ml Nim. solution, soil mixture and 10g. galled roots |
| Velum 400SC | 10ml | Vel | 10ml Nim. solution, soil mixture and 10g. galled roots |
| Vapcomic | 10ml | Vap | 10ml Nim. solution, soil mixture and 10g. galled roots |
| Eucalyptus leaves | 100g | Euc100 | 100g. powder, soil mixture and 10g. galled roots |
| | 50g | Euc50 | 50g. powder, soil mixture and 10g. galled roots |
| | 25g | Euc25 | 25g. powder, soil mixture and 10g. galled roots |
| Sore pomegranate peel | 100g | pom100 | 100g. powder, soil mixture and 10g. galled roots |
| | 50g | pom50 | 50g. powder, soil mixture and 10g. galled roots |
| | 25g | pom25 | 25g. powder, soil mixture and 10g. galled roots |
| Bitter orange peel | 100g | Bor100 | 100g. powder, soil mixture and 10g. galled roots |
| | 50g | Bor50 | 50g. powder, soil mixture and 10g. galled root |
| | 25g | Bor25 | 25g. powder, soil mixture and 10g. galled roots |
| Health untreated control | / | HUC | Only soil mixture |
| Inoculated Untreated Control | / | IUC | Soil mixture and 10g. galled roots |

### 3.7 Statistical analysis:

The research data were analyzed using STATGRAPHICS Centurion XV.I software.

# 4. Results:

## 4.1 Pathogenicity test:

Results of this experiment showed high infectivity of the *M. javanica* nematodes to the locally cultured tomato variety (Golden), examining the roots of the plant showed formation of large number of root galls some of which were 0.4-1cm in diameter. On some of the infected roots we counted as much as 10 galls (4-10). The total count of the galls on some of the plant was as much as 30 galls of variable sizes. The overall size of the infected plants shows symptom of stunting compared with the healthy plant. There were also symptoms of yellowing and wilting appear on them (figure12).


Figure 12: Infected plant show stunting and yellowing symptoms

## 4.2 Testing plants nematocidal activity:

Results of testing the nematocidal action of the powder of the three used plants (figure13) showed excellent effective action of the plants against tomato root knot disease caused by the root knot nematode *M. javanica*. This was evident through a significant decrease in the number of root galls produced on the treated plants compared to the control plant. The best effect was shown by pomegranate peel and Better orange peel powders (25g/pot), which completely (100%) inhibited the infection (0 galls/plant), coming next are each of Eucalyptus leaves (1oog/pot), Pomegranate peel (100/pot), and Pomegranate peel (50g/pot) which induce 91% (1 gall/plant), 91% (1gall/plant) and 90% ((1 gall/plat) increase the infection respectively. Eucalyptus leaves (50g/pot) causes 84% decrease in galls number (2

galls/pot). All these results were even better than the results recorded by the used pesticides (82%, 80%, and 64% decrease in gall number). Better orange peel powder (100g/pot) and Eucalyptus leaves powder (25g/pot) reduces the infection by 72% and 67% respectively while the least effect was showed by the Better orange peel powder (50g/pot) which causes 20% decrease in the number of root galls (8galls/root). These results are highly promising and are a good indicator of the efficiency of the powder of each of the three used plants as a source of environmentally friendly plant-derived nematicides.

| name | gall | % Decrease |
|---|---|---|
| pom25 | 0 | 100 |
| Man25 | 0 | 100 |
| HUC | 0 | 100 |
| Euc100 | 1 | 91 |
| pom100 | 1 | 91 |
| pom50 | 1 | 90 |
| Euc50 | 2 | 84 |
| Vap | 2 | 82 |
| Nim | 2 | 80 |
| Man100 | 3 | 72 |
| Euc25 | 3 | 67 |
| Vel | 4 | 64 |
| Man50 | 8 | 20 |
| IUC | 10 | 0 |

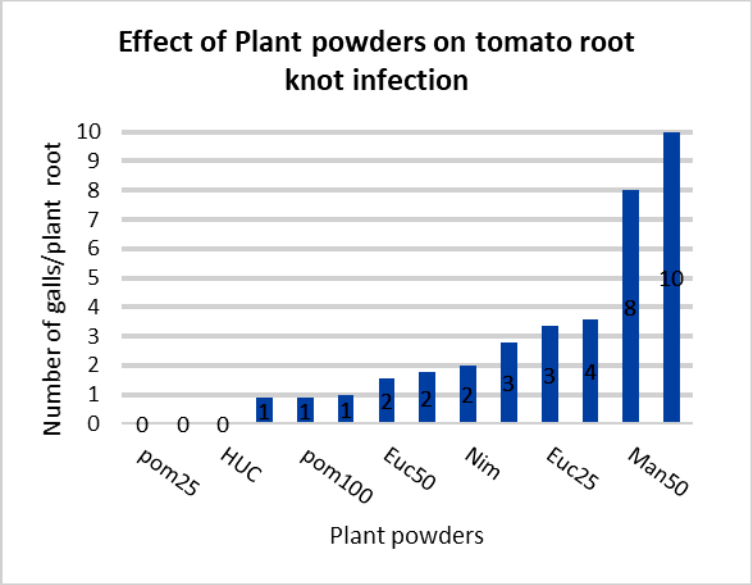


Figure 13. Effects of different treatment on root knot

These results come in consistancey with Archana U Singh, Prasad D (2014) how approved the nematocidal activity of Eucalyptus against root knot nematode *Meloidogyne incognita*, and with El-Nagdi, and Youssef, (2015), how approved the nematocidal activity of Pomegranate against this pathogen. Results also com in consistancey with Padhi, and Behera, (2000), who approved an excellent nematocidal action of sour orange by affecting egg hatching and killing nematodes.

## 5. Conclusion:

From the results of this experiment we can conclude that Eucalyptus leaves, Pomegranate peel, and Better orange peel can be used for the production of cheap, easy to use and effective nematode controlling pesticides which are safe and cause no harm to the environment or human and animal health.

## 6. Recommendations:

1- More studies are required about the action of these plants against plant diseases caused by other pathogens like fungi or bacteria.

2- Also we recommend studying the ability of formulating products derived from these plants into commercial products.

3- We recommend the evaluation of other plants for their antimicrobial action.

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