GLOBAL JOURNAL OF BIOLOGY, AGRICULTURE & HEALTH SCIENCES
(*Published By*: *Global Institute for Research & Education*)
www.gifre.org

Impact of *Citrus Aurantium* and Moringa Dried-Leaf Powders or Poultry Eggs Shell or Snail Shell Powder in Comparison with Furadan against *Meloidogyne Incognita* Infecting *Citrus Aurantium* under Greenhouse Conditions

*Helal E. M. Mona & **Gad, S. B.
* Citrus Division, Orchards Inst. Res. A.R.C., Mansoura , Egypt.
**Nematology Res. Unit, Agric. Zool. Dept, Fac. of Agric., Mansoura Univ. Mansoura, DK., Egypt.

Abstract

A greenhouse experiment was conducted to evaluate efficacy of poultry egg shell powder, snail shell powder or dried-leaf powders of moringa and *Citrus aurantium* solely or in combination with furadan at half doses each in comparison with furadan at the recommended dose on growth of *C. aurantium* infected with *M. incognita* under greenhouse conditions (31±3 C◦). Results indicated that all tested materials significantly ameliorated plant growth criteria and diminished nematode parameters. It was clear that the concomitant treatments showed the maximum results in the increments of plant growth characters of *C. aurantium* and in suppressing the root-knot nematode *M. incognita* criteria. Of the single treatments, poultry egg shell powder at dose of 5 g/ plant achieved the maximum increase percentage and ranked first in diminishing number of nematode galls (75.7%), females (79.2%), and eggmasses (78.9%), followed by *Citrus aurantium* dried-leaf powder application in this respect . Among the integrated applications, treatment containing half doses of poultry egg shell powder plus furadan {½(Poultry egg shell powder + furadan)} was superior to that of *C. aurantium* dried leave power plus furadan at their half doses { ½ (*C. aurantium* + furadan)} treatment in improving tested plant growth parameters and overwhelmed other integrated application in this study in suppressing numbers of nematode galls, females and eggmasses comparing to nematode alone.

Keywords: *Meloidogyne incognita* , control, , *Citrus aurantium*, furadan, snail shell, poultry egg shell.

Introduction

Plant parasitic nematodes caused significant damage and losses to various agricultural crops in the tropical and sub-tropical (Luc *et al.,* 2005). Root-knot nematodes, *Meloidogyne* spp. are economically the most damaging nematode pests on wide range of crops in subtropical climates (Stirling and Stirling, 2003). In view of the extent of the yield losses caused by root-knot nematodes in citrus cultivation, it is necessary to minimize crop damage by adopting appropriate management methods available. Application of chemical nematicides does not always prove effective and economic (Pakeerathan *et al*., 2009). In addition, poor target specificity of chemicals pose environmental and human toxicity hazards (Barker *et al.,* 1998). Therefore, environmentally friendly alternatives are required for nematode control. However, integrated nematode management using several control techniques i.e. organic soil amendments, plant leave powder with minimal use of nematicides received great attention among the nematologists providing effective control measures against the target nematode, keep the nematode low at the safe level and avoiding environmental pollution. Therefore, the objectives of this investigation were conducted to determine the influence of poultry egg shell powder, snail shell powder, dried-leaf powders of moringa and *C. aurantium* singly or mixed with half doses of furadan each in comparison with furadan on *C. aurantium* plant infected with *Meloidogyne incognita* under greenhouse conditions.

Materials and Methods

Source of Nematodes:

Second stage juveniles (J_2) of *Meloidogyne incognita* (Kofoid & White) Chitwood, were obtained from a pure culture of *M. incognita* that was initiated by a single eggmass propagated on coleus plants, *Coleus blumei* in the greenhouse of Nematology Research Unit,(NERU) Agricultural Zoology Department, Faculty of Agriculture, Mansoura University, Egypt, where this work was carried-out.

Nematicide:

Furadan: 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate; IUPAC: 2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate.

Preparation of the tested materials.

Both leaves of moringa and *C. aurantium*, were collected, sun dried and crushed. The brown garden snail *Cornu aspersum* were collected from Faculty of Agriculture farm, Mansoura University, as well as poultry eggs peel then crushed and milled.

Influence of poultry egg shell powder, snail shell powder, moringa leave powder and *Citrus aurantium* leave powder singly or mixed with half doses of furadan each in comparison with furadan on *Citrus aurantium* plant infected with *Meloidogyne incognita* under greenhouse conditions.

A greenhouse experiment was carried-out in order to evaluate the efffect of poultry egg shell powder or snail shell powder or moringa and *C. aurantium* dried-leaf powders singly or mixed with half doses of furadan each in comparison with furadan on *Citrus aurantium* plant infected with *Meloidogyne incognita* under greenhouse conditions (31±3 C◦). Forty four *C. aurantium* plants (one years old) growing in plastic bags (15 cm-d) filled with 1 kg steam loamy sandy soil

(1:1) (v:v) were chosen to conducted this experiment. Forty seedlings were separately inoculated with 2000 juveniles of *M. incognita* and left four seedlings (bags) without nematode to serve as check. The tested materials were separately added to four seedlings each and mixed with soil one week after nematode inoculation, while four seedlings (bags) with nematode only were left without any treatment. Each treatment was replicated four times. Treatments were as follows:

1- N+ poultry egg shell powder (5g / plant),
2- N+ snail shell powder (5g / plant),
3- N+ moringa leave powder (5g / plant),
4- N+ *Citrus aurantium* leave powder(5g / plant),
5- ½ (Poultry egg shell powder+ furadan),
6- ½ (Snail shell powder + furadan),
7- ½ (Moringa dried-leaf powder + furadan),
8- ½ (*Citrus aurantium* dried-leaf powder + furadan),
9- N+ furadan (5 g/plant),
10- N alone and
11- Plant free of N and any treatment.

Plastic bags were then arranged in a randomized complete block design on a bench of greenhouse at 31±3°C and irrigated with tap water as needed. Plants were harvested after 45 days from nematode inoculation. Data dealing with plant length and weights of fresh shoot and root; and shoot dry weight of each plant / pot were measured and recorded. Infected *C. aurantium* roots were washed in tap water and examined for the numbers of galls and egg-masses and recorded. Number of juveniles (J_2) per soil of each plant were counted and recorded. The root gall index (RGI) and egg mass index (EI) were estimated according to the scale given by Taylor and Sasser (1978) as follows: 0= no galls or egg-masses, 1= 1-2 galls or egg-masses, 2= 3-10 galls or egg-masses, 3= 11-30 galls or egg-masses, 4= 31-100 galls or egg-masses and 5= more than 100 galls or egg-masses. *M. incognita* (J_2s) were separately extracted from 250 g. soil of each treatment / replicate by sieving and modified Baermann technique (Goodey, 1957), counted , recorded and then determined for the soil of each plastic bag. Statistically, the obtained data were subjected to analysis of variance (ANOVA) (Gomez and Gomez, 1984) followed by Duncan's multiple ranges to compare means (Duncan, 1955).

Results and Discussion

Data in Tables (1) represented the impact of poultry egg shell powder or snail shell powder or moringa and *C. aurantium* dried-leaf powders solely or in combination with furadan at half doses each in comparison with furadan at the recommended dose on growth of *C. aurantium* infected with *M. incognita* as well as nematode parameters on root system i.e. galls, females and eggmasses numbers under greenhouse conditions (31±3 C◦). Results indicated that all tested materials significantly ameliorated plant growth criteria and diminished nematode parameters as well, comparing to nematode alone. In general, it was evident that the concomitant treatments showed the maximum results in the increments of growth characters of *C. aurantium* plant and in suppressing *M. incognita* criteria (Tables 1&2). Of the single treatments, Poultry egg shell powder at dose of 5 g/ plant achieved the maximum increase percentage in total plant fresh weight (47.0%), plant length (43.7%) shoot dry weight (133.3%) and number of leaves (90.9%), followed by ***C.*** *aurantium* dried-leaf powder application with values of 41.0, 40.6, 66.6 and 81.8, then dried-leaf powder of moringa (38.5, 39.1, 36.7 and 51.5%), whereas plants received snail shell powder gave the least values for the same plant growth parameters, since their values averaged 25.6, 29.4 and 30.7; and 51.5% even though the latter character i.e. number of leaves value was on par (51.5%) with that of moringa dried-leaf powder treatment as compared to nematode alone, (Table 1). Among the integrated applications, treatment containing half doses of poultry egg shell powder plus furadan {½(Poultry egg shell powder + furadan)} was superior to that of ***C.*** *aurantium* dried-leaf power plus furadan at their half doses { ½ (***C.*** *aurantium* + furadan)} treatment in improving total plant fresh weight, plant length, shoot dry weight and number of leaves / plant that averaged 68.4, 90.6 , 140.0 and 187.8% for the former and 62.4, 78.6 , 73.3 and 112.1% for the latter, respectively. Meanwhile, plants receiving dose of dried moringa leaf powder at 5 g/ plastic bag plus furadan at their half doses { ½ (Moringa dried-leaf powder + furadan)} recorded clearly the improvement in plant growth characters with values of 60.7, 75.9, 60.0 and 81.8% for total plant fresh weight, plant length, shoot dry weight and number of leaves / plant , respectively, however, snail shell powder plus furadan at their half doses {½ (snail shell powder + furadan)} gave the least values for the same plant characters, since their values averaged 32.5, 40.6, 53.3 and 63.6% respectively. Moreover, furadan as a nematicide overwhelmed all integrated treatments in this investigation in ameliorating plant growth criteria i.e. total plant fresh weight (79.4%), plant length (94.1%) and shoot dry weight (160.0%) except that of leaves number (127.3%) which was lesser than that of treatment containing half doses of poultry egg shell powder plus furadan (187.8%). In the meantime, plant free of nematode that receiving non of the tested materials gave remarkable increase percentage in total plant fresh weight, plant length, shoot dry weight and number of leaves / plant with values of 11.1, 5.4, 20.0 and 21.2% , respectively, (Table 1).

Table (1) Plant growth response of *Citrus aurantium* infected with *Meloidogyne incognita* as influenced by poultry egg shell powder or snail shell powder or moringa and *Citrus aurantium* dried-leaf powder singly or mixed with half doses of furadan each in comparison with furadan under greenhouse conditions (31±3 C◦).

Treatments	* Plant growth responce											
	Length (cm)		Total plant length (cm)	** Inc %	No. of leaves /plant	** Inc %	Fresh wt. (g)		Total plant f wt (g)	** Inc %	**Shoot dry weight (g)**	** Inc %
	shoot	Root					**shoot**	**Root**				
Poultry egg shell powder	**79.2 d**	**23.0 f**	**102.2 c**	**43.7**	**6.3 ef**	**90.9**	**11.0e**	**6.2c**	**17.2 d**	**47.0**	**3.50ab**	**133.3**
Snail shell powder	**63.0g**	**29.0e**	**92.0f**	**29.4**	**5.0f**	**51..5**	**9.5 g**	**5.2e**	**14.7 g**	**25.6**	**1.96f**	**30.7**
Moringa leaf powder	**65.6f**	**33.3c**	**98.9e**	**39.1**	**5.0f**	**51.5**	**10.0f**	**6.2c**	**16.2 ef**	**38.5**	**2.05c**	**36.7**
***C aurantium* leaf powder**	**69.0e**	**31.0d**	**100.0 cd**	**40.6**	**6.0d**	**81.8**	**9.5 g**	**7.0a**	**16.5 e**	**41.0**	**2.50cd**	**66.6**
½ (Poultry egg shell powder+ox)	**93.5b**	**42.0b**	**135.5 ab**	**90.6**	**9.5a**	**187.8**	**13.2b**	**6.5 b**	**19.7 b**	**68.4**	**3.6 b**	**140.0**
½ (Snail shell powder +fu)	**69.0e**	**31.0d**	**100.0 cd**	**40.6**	**5.4e**	**63.6**	**9.5 g**	**6.0 d**	**15.5f**	**32.5**	**2.3 d**	**53.3**
½ (Moringa leaf powder + fu)	**83.1c**	**42.0b**	**125.1 bc**	**75.9**	**6.0d**	**81.8**	**11.9d**	**6.9ab**	**18.8 cd**	**60.7**	**2.4cd**	**60.0**
½ (*C. aurantium* + fu)	**82.0cd**	**45.0a**	**127.0 b**	**78.6**	**7.0c**	**112.1**	**12.0c**	**7.0a**	**19.0 bc**	**62.4**	**2.6c**	**73.3**
Furadan (fu)	**98.0a**	**40.0b**	**138.0 a**	**94.1**	**7.5b**	**127.3**	**14.0a**	**7.0a**	**21.0 a**	**79.4**	**3.9 a**	**160**
N alone	**59.6h**	**11.5g**	**71.1h**	**-**	**3.3h**	**--**	**6.4i**	**5.3f**	**11.7i**	**--**	**1.5 h**	**--**
Plant free of any treatments and N	**52.0i**	**23.0f**	**75.0g**	**5.4**	**4.0g**	**21.2**	**7.7 h**	**5.3f**	**13.0 h**	**11.1**	**1.8 g**	**20.0**

N= 2000 of *M. incognita* (J_2)

*Each value is the mean of four replicates.

Means in each column followed by the same letter (s) didn't differ at P<0.05 according to Duncan's multiple-range test.

** Increase % = $\frac{\text{Treatment - N alone (Untreated)}}{\text{N alone (Untreated)}} \times 100$

Data presented in Table (2) revealed that tested components showed protection performance in *C. aurantium* against *M. incognita* infection in terms of reduction percentage of galls, females and eggmasses numbers that significantly affected by all tested applications. It was also remarkably that the concomitant treatments gave better results more than single ones did. Of the single applications, poultry egg shell powder at the dose of 5g/ plant ranked first in diminishing number of nematode galls (75.7%), females (79.2%), and eggmasses (78.9%), followed by that of *Citrus aurantium* dried leaf powder with values of 67.9, 68.6 and 71.9%, whereas applications of snail shell powder and moringa leaf powder showed almost equal values for the same nematode criteria that averaged 63.7, 67.6 and 67.6% for the former, and 66.4, 66.3 and 69.4% for the latter which were considered to be lesser than that of other single applications in this study, respectively comparing to nematode alone. (Table, 2) Among the concomitant treatment, plant receiving half doses of poultry egg shell powder plus furadan overwhelmed other integrated application in this study in suppressing numbers of nematode galls, females and eggmasses with values of 79.4, 83.0 and 85.0%, followed by that of *Citrus aurantium* dried-leaf powder + furadan at their half doses with values of 73.4, 74.6 and 80.4%, respectively and then that of moringa dried-leaf powder plus furadan at their half doses, since their values averaged 72.7, 76.8 and 77.8%, respectively. However, the least values of these nematode criteria achieved by plant receiving the snail shell powder plus furadan at their half doses that were amounted to 64.7, 68.2 and 69.2%, respectively, comparing to nematode alone (Table, 2). Moreover, furadan gave a considerable percentage reduction of nematode galls (79.3%), females (82.5%) and eggmasses number (83.9%) that exceeded those single as well as mixed applications, except that of poultry egg shell powder + furadan at their half doses, respectively. Likewise, significant results were also observed between eggmasses and root galls indices of all tested applications and nematode alone where their values ranged between 4 for nematode galls and eggmasses each except that of furadan as well as {½ (poultry egg shell powder + fu)} that was 3 vs 5 of nematode alone. As a whole, it can be concluded from the previous results that all tested materials used either singly or integrated with furadan at their half doses improved plant growth of *C. aurantium* and suppressed nematode development, especially poultry egg shell powder product alone or mixed with furadan at their half dose which considered to be the best treatment in this investigation. Table (2).

Table (2) Percent reduction of root galls, eggmasses and females numbers of *Meloidogyne incognita* infecting *Citrus aurantium* as influenced by poultry egg shell powder or snail shell powder or Moringa and *Citrus aurantium* dried-leaf powder singly or mixed with half dose of furadan each in comparison with furadan under greenhouse conditions (31±3 C◦).

Treatments	* Nematode parameters on root system							
	No.of galls	R %	RGI	No. of **females**	R %	**No. of eggmasses**	R %	E. I
Poultry egg shell powder	**46.6h**	**75.7**	**4**	**39.3g**	**79.2**	**38.0 f**	**78.9**	**4**
Snail shell powder	**69.7b**	**63.7**	**4**	**61.0c**	**67.6**	**58.3bc**	**67.6**	**4**
Moringa leaf powder	**64.6d**	**66.4**	**4**	**63.6b**	**66.3**	**55.0c**	**69.4**	**4**
Citrus aurantium	**61.5e**	**67.9**	**4**	**59.2d**	**68.6**	**50.6d**	**71.9**	**4**
½ (Poultry egg shell powder+ox)	**39.6i**	**79.4**	**4**	**32.0 i**	**83.0**	**27.0i**	**85.0**	**3**
½ (Snail shell powder +fu)	**67.6c**	**64.7**	**4**	**60.0cd**	**68.2**	**55.5b**	**69.2**	**4**
½ (Moringa leaf powder + fu)	**52.3f**	**72.7**	**4**	**43.7f**	**76.8**	**40.0e**	**77.8**	**4**
½ (*Citrus aurantium* + fu)	**51.0g**	**73.4**	**4**	**48.0e**	**74.6**	**35.0g**	**80.6**	**4**
Furadan (fu)	**39.7i**	**79.3**	**4**	**33.0h**	**82.5**	**29.0h**	**83.9**	**3**
N alone	**192.0a**	**--**	**5**	**188.7a**	**---**	**180.3a**	**--**	**5**

N= 2000 of *M. incognita* (J_2)
*Each value is the mean of three replicates.
*Means in each column followed by the same letters(s) did not differ at p< 0.05 according to Duncan's multiple range tests.

Root gall index (RGI) or eggmasses index (EI) was determined according to Taylor and Sasser (1978) as follows:0= no galls or eggmasses, 1= 1-2 galls or eggmasses, 2= 3-10 galls or eggmasses, 3= 11-30 galls or eggmasses, 4= 31-100 galls or eggmasses and 5= more than100 galls or eggmasses.

Apparently, the importance of utilizing non chemical materials was essential for searching of alternative cheap and environmentally friendly ways for the management of phytonematodes in various agricultural cultivation. However, results of the present work proved this phenomenon in suppressing *M. incognita* development associated with ameliorating plant growth parameters infecting *C. aurantium* using poultry egg shell powder or snail shell powder or moringa and *C. aurantium* died-leaf powders singly or mixed with half dose of furadan each in comparison with furadan under greenhouse conditions, a situation which supported by the findings of El-Sherif *et al*.,(2015) who reported that using calcium sulphat or potassium silicate or moringa dry leaf powder either alone or mixed as dual or triple or tetra treatments along with oxamyl at ½ or 1/3 or ¼ doses compared to oxamyl at the recommended dose on reproduction and development of *M. incognita* play an important role in diminishing root knot nematode on tomato plants and its reproduction factors (RF), where the double, triple and tetra treatments tested gave better results than single ones did. In the meantime, among the single applications, moringa dry leaf powder accomplished the highest percentage reduction of nematode parameters. The application of poultry egg shell powder alone or mixed with furadan at half dose each ranked first in enhancing plant growth parameters and reduce nematode criteria. The nematicidal effect of The chicken eggshell may possibly be attributed to their high contents (95-97%) of calcium carbonate crystals (Burley and Vadehra,1989). Scott (1993) revealed that soil amendments of powdered calcium carbonate resulting in excellent control of *Fusarium* crown rot of tomato.

Ouedrhiria et al (2015) revealed that essential oil of *C. aurantium* leaves exhibited high single antibacterial effect against bacterial strains tested, except *P. aeruginosa* which resist to both essential oils studied. This remarkable activity is due to several majors components particularly linalool and α-terpineol. Moreover, the combined effect of the essential oil obtained from leaves of *C. aurantium* against *Bacillus subtilis* has demonstrated a remarkable synergistic effect by the combination (1/4 MIC CaL + 1/128 MIC CaZ). This optimal combination offer huge potential as alternative phytotherapy against bacteria at suitably low concentrations and lead to new research on the morphological and ultrastructural analysis of strains treated.

Jatto,(2010) determined proximate and mineral composition of four different species of snail shells. *Achatina achatina* has: protein 0.12%, fiber 4.06%, fat 0.78%, ash content 2.00%, Nitrogen Free Extract (NFE) 93.04%, zinc 9.83mg/l, manganese 4.31mg/l, copper 6.47mg/l, and iron 251.23mg/l; *Achatina maginata* has: protein 0.42%, fiber 3.37%, fat 0.75%, ash content 10.00%, NFE, 85.46%, zinc 2.50mg/l, manganese 6.71mg/l, copper 5.33mg/l, and iron 57.45mg/l; *Achatina fulica* has: protein 0.30%, fiber 3.93%, fat 0.38%, ash content 10.00mg/l, NFE 82.36%, zinc 8.02mg/l, manganese,16.98mg/l, copper 5.51mg/l, and iron 37.04mg/l; Limicoleria species has: protein 0.23%, fiber

4.14%, fat 0.48%, ash content 13.00%, NFE 82.15%, zinc 6.30mg/l, manganese 1.99mg/l, copper 4.46mg/l, and iron 208.58mg/l.

However, more research is needed to be done in this direction under field conditions before drawing such recommendations for new trend safe and effective integrated nematode management alternative (s) based on the combined use of natural and synthetic compounds.

References

Barker, K. R.; G. A. Pederson and G. L. Windham (1998). Soybean. Plant and nematode interactions, 317-333.

Burley, R.W., D.V. Vadehra (1989). The Avian Egg: Chemistry and Biology. New York, Wiley.

Duncan, D.B. (1955). Multiple range and multiple, F-test. Biometrics, 11: 1-42.

El-Sherif, A.G.; S.B. Gad and S.M. Saadoon (2015). Evaluation of calcium sulphate, potassium silicate and moringa dry leaf powder on *Meloidogyne incognita* infecting tomato plant with reference to N, P, K, total phenol and cholorophyl status under greenhouse condition. Journal of Entomology and Nematology 7 (4), 30-38.

Gomez, K. A. and A.A. Gomez (1984). Statistical procedures for Agricultural Research. 2nd Ed., John Wiley &Sons: Inc., New York.

Goodey, J.B. (1957). Laboratory methods for work with plant and soil nematodes. Tech. Bull. No. 2. Min. Agric. Fish Ed. London, 47 pp.

Jatto, O.E.; I.O. Asia, and W.E. Medjor. (2010). "Proximate and Mineral Composition of Different Species of Snail Shell". *Pacific Journal of Science and Technology*. 11(1):416-419.

Luc, M.; R. A. Sikora and J. Bridge (2005). Plant parasitic nematodes in subtropical and tropical Agriculture. 2nd edn. CAB international , Wallingford, Uk.

Ouedrhiri, W; S. Bouhdid ; M. Balouiri; L. El Ouali; A. Mojac; F.Ouazzani and H. Greche (2015) Chemical composition of *Citrus aurantium* L. leaves and zest essential oils, their antioxidant, antibacterial single and combined effects. Journal of Chemical and Pharmaceutical Research, 2015, 7(1):78-84.

Pakeerathan K.; G. Mountain and N. Thrashing, (2009). Eco-friendly management of root-knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood using different green leaf manures on tomato under field conditions. *American Eurasian J. Agric. . Environ. Sci.* 6, 494–497.

Scott, J. W. (1993). Breeding tomatoes for resistance to high temperature, biotic and abiotic diseases. Hort Bras. 11(2): 167-170.

Stirling G.R. and A. M. Stirling (2003). The potential of *Brassica* green manure crops for controlling root-knot nematode (*Meloidogyne javanica*) on horticultural crops in a subtropical environment. Australian Journal of Experimental Agriculture.; 43:623–630.

Taylor, A. L., and J. N. Sasser (1978). Biology, identification and control of root-knot nematodes (*Meloidogyne* species). Raleigh, NC: North Carolina State University Graphics.