# Comparative effects of effective microorganism activated solution, leaf extracts and carbofuran on the growth and yield of cucumber planted on *Meloidogyne incognita* infested soil.

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## ABSTRACT

Two field experiments were conducted between 2014 and 2016 on the effects of effective microorganism activated solution (EMAS), carbofuran and some plant leaf extracts (*Azadirachta indica* and *Tithonia diversifolia)* on the performance of cucumber planted on root-knot nematode (RKN) infested soil. The trials were carried out on two separate fields in 2014/2015 and 2015/2016, each measuring about 15 m x 10 m. The experimental designs were randomized complete block, comprising of five treatments (EMAS, *Azadirachta indica, Tithonia diversifolia* Carbofuran and control). Each treatment was randomly assigned to a plot in a block and replicated three times. After land preparation, Cucumber seed *cv.* Monalisa F1 which is nematode susceptible was planted and inoculated with 2000 J2 *M. incognita* two weeks after planting while treatments were applied one week after inoculation. The result from the experiment showed that all the cucumber treated with EMAS, plant extract and carbofuran resulted into significantly ($P \leq 0.05$) increased vegetative growth and fruit yield compared with the control. Nematode soil population and root gall index were reduced in plant extract and carbofuran treated soil while it remained high in EMAS and not significantly ($P \leq 0.05$) different from the control. Phytochemical screening of the plant extracts revealed the presence of alkaloid, tannin, flavonoid and saponin. EMAS did not bring about reduction in soil nematode population as it was not significantly ($P \leq 0.05$) different from the control but recorded the highest yield showing that it strengthened the tolerance level of cucumber to nematode attack.

Key words: *M. incognita*; nematode; cucumber; *Azadirachta indica*; *Tithonia diversifolia*; EMAS; carbofuran

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## INTRODUCTION

Cucumber (*Cucumis sativus* L.) is a member of Cucurbitaceae family. It is an important vegetable and one of the most popular member of the Cucurbitaceae family (Lower and Edwards 1986; Thoa, 1998). Cucumber have not received as much attention as other vegetables in terms of health benefits, but this widely cultivated food provides us with a unique combination of nutrients. At the top of the phytonutrients list for cucumbers are its Cucurbitacins, Lignans and Flavonoids. These three types of phytonutrients found in cucumber provides us with valuable antioxidant, anti-inflammatory and anticancer benefits. cucumbers are mostly eaten raw. Sliced cucumber with the peel contains only 8 calories per serving, with only 0.6 gram total fats and less than 1 gram sugar. Cucumber is a nutrition choice, considering that it contains almost half a gram of dietary fiber which is essential for slowing down body's digestion of food giving body enough time to absorb essential nutrients (Dannie, 2013). Cucumber is grown for its immature fruits that are used as salad vegetable and for pickles.

Cucumber, like other vegetable crops, is attacked by wide range of pests and diseases, resulting in great yield losses. Prominent among them is root-knot nematode, *Meloidogyne incognita,* which brings about a great yield reduction (Aminu-Taiwo and Fawole, 2012; Rivera and Abballay, 2008). Management of root-knot nematode with synthetic namaticides can be very effective (Sikora and Feanadeq, 2005; Adegbite and Agbaje, 2007; Dubery and Triberdi, 2010). However, scarcity, highs cost, environment safety and global restriction on importation of synthetic nematicides have spurred scientists to search for alternative control measure against nematode pests of economic food crops (Anonymous, 2004). The use of plant extract has been suggested (Hoan and David, 1979).

In the present study, extracts from the leaf of *T. diversifolia and A. indica* as well as carbofuran were compared with EMAS (Effective microorganism activated solution). EMAS comprises of a wide variety or multi-culture of effective, beneficial and non-pathogenic microorganisms coexisting together. Essentially, it is a combination of aerobic and anaerobic species commonly found in all ecosystems. EMAS is not a pesticide and contains no inorganic chemical. It is a microbial inoculant that works as bio-control measure in suppressing and or controlling pest through the introduction of beneficial microorganism to soils and plant. Pests and pathogens are suppressed or controlled through natural processes by enhancing the competitive and antagonistic activities of the microorganisms in EMAS inoculants (Higa,1998).

The research on the use of EMAS for nematode control in nematode infested crop is yet to be exploited, if at all is available. It is against this background that this research work is conducted with the following objectives:

- To compare the performance of root-knot nematode infested cucumber treated with EMAS, *T. diversifolia* (wild sunflower) *A. indica* (neem) extracts and carbofuran
- To compare the soil nematode population and level of root damage on cucumber due to treatment with EMAS, *T. diversifolia, A. indica* extracts and Carbofuran
- To access if treatments have effect on the nutrient value of cucumber
- To investigate the bio-active chemical components present in the leaf extracts of *T. diversifolia* and *A. indica*.

## MATERIALS AND METHODS

**Sources of Seeds and Control Agents:** Seed of cucumber (*Cucumis sativus*) Manolisa F1 which is susceptible to *M. incognita* infection were obtained from Shongai Centre Benin Republic, Cotonou. The leaves of *T. diversifolia* (Mexcan sunflower) and *A. indica* (neem) were obtained from Omu-Aran town, Kwara State. Furadan (3G), a brand of Carbofuran, was purchased from agrochemical store in Ilorin, kwara State, Nigeria.

**Experimental Site:** Two field trials were carried out at Landmark University, Teaching and Research farm, Omu-Aran, Kwara State, Nigeria. Two separate plots, 15 m x 10 m each, were prepared for the experiment between the months of September, 2014 to January, 2015 and at the same period in 2015/ 2016.

**Experimental Design and Treatment Preparation:** The experimental designs were randomized complete block, comprising of five treatments (*Tithonia, Azadirachta*, EMAS, Carbofuran and Control) and each treatment was replicated three times. Plant extracts were from *T. diversification and A. indica* leaf. The leaves were collected from each plant, air-dried for 3 – 4 weeks on laboratory benches and then ground into powder using blender, and soaked in an aspirator separately over a period of 24 hours. This was done by pouring 1 kg each of the powder plant material into one litre of distilled water separately and it remains in the solution for 24 hours. The solution obtained thereafter was 100% concentrated or 1,000,000 ppm (Stock solution) which was diluted to 500,000 ppm (50%). EMAS was applied at 20 mls into knapsack sprayer load (15 litres) and Carbofuran was applied at the rate of 3kg ai/ha (Recommended rates).

**Crop Establishment:** The seeds of cucumber *cv* Nomalisa F1 which had been confirmed to be susceptible to nematode attack were planted into each of the experimental plots (Aminu-Taiwo and Fawole, 2012). Two seeds were planted per hole, at a depth of 5-6 cm and 60 cm spacing between and within rows. Two weeks after planting, seedlings were thinned to one healthy plant per stand.

**Extraction and Inoculation of Cucumber:** Plant parasitic nematodes were extracted from previously infested tomato grown in pure culture of *M. incognita* in previous experiment using standard method (Whitehead and Hemmings, 1965). *M. incognita,* (2000 J2) were introduced into the root of each cucumber. Meanwhile the initial soil nematode population was recorded.

**Treatment Application:** A week after inoculation, *Tithonia, Azadirachta* and EMAS, carbofuran were applied. *Tithonia and Azadirachta* extracts were applied at the rate of 500,000 ppm (50%), this is by pouring 100ml of the solution within the plant rhizosphere. EMAS was applied at the rate of 1,000 ppm (0.1%) by direct spray on the plant biomass (recommended application rate and method) but it was applied four times over a period of four weeks at weekly interval while carbofuran was applied at the rate of 3kg ai/ha. Untreated plot served as the control.

**Assessment of Crop Growth and Yield:** Four weeks after planting, data on growth parameters (numbers of leaves, average vine length, stem girth) and yield components (number of fruits and fruit weight) were recorded.

**Assessment of fruit for mineral nutrient composition:** Fruits were harvested at maturity, and samples were analysed for total mineral matter. Analysis was carried out according to the method described by Kacar (1995).

**Phytochemical screening of test plants *(Azadirachta indica* and *Tithonia diversifolia)***

**Test for alkaloids**
To the extracts, dilute hydrochloric acid was added, shaken well and filtered. With the filtrate, the following test was performed:
*Mayer's reagent test*
To 3ml of filtrate, few drop of Mayer's reagent were added along sides of tube. Formation of creamy precipitate indicates the presence of alkaloids.

**Test of flavonoids**
*Alkaline reagent test*
The extract was treated with few drop of sodium hydroxide solution separately in a test tube. Formation of intense yellow colour, which becomes colourless on addition of few, drops of dilute acid indicates the presence of flavonoids.

**Test for Saponin**
Froth test
The extract was diluted with diluted water and shaken in a graduated cylinder for 15 minutes. The formation of layer of foam indicates the presence of saponins.

**Data collection and analysis**
In both experiments, data were collected on the growth and yield components of cucumber. Data was also collected on the soil nematode population and gall index. The data were pooled together, means were subjected to analysis of variance (ANOVA) and means separated using Duncan's multiple range text at 5% probability level.

## RESULTS

**Effect of leaf extracts of wild sunflower leaf, neem leaf, EMAS and carbofuran on the growth and yield of cucumber:** The effects of different leaf extracts (wild sunflower and neem), EMAS and Carbofuran on the leaf number of Cucumber was presented in Table 1. Cucumber treated with leaf extract (wild sunflower and neem), EMAS and Carbofuran produces more leaves than the control. The number of leaves produced by the plants that were treated with leaf extract, EMAS and carbofuran recoded significantly greater number of leaves from week 6 of the experiment. EMAS and carbofuran treatments were significantly better than the leaf extract treatment at week 8.

**Table 1: Effect of EMAS, leaf extracts and carbofuran on the mean number of cucumber infected with *M. incognita***

| Treatments | 4WAP | 5WAP | 6WAP | 7WAP | 8WAP |
|---|---|---|---|---|---|
| Neem | 7.67[a] | 13.43[ab] | 17.67[ab] | 24.43 [b] | 26.10 [b] |
| Mexican sunflower | 9.66 [a] | 14.17 [bb] | 17.43[ab] | 24.60 [b] | 26.19 [b] |
| EMAS | 9.455[a] | 16.20 [a] | 27.43[ab] | 29.30 [a] | 35.90 [a] |
| Carbofuran | 9.47 [a] | 15.53 [a] | 27.70 [a] | 28.43 [a] | 33.53 [a] |
| Control | 8.43 [a] | 13.43[ab] | 15.10 | 18.43 [c] | 19.90 [c] |

Means within the same column followed by the same letters(s) are not significantly different ($P \leq 0.05$)

**Table 2: Effects of EMAS, leaf extracts of plant and carbofuran on the vine length of cucumber infected with *M. incognita***

| Treatments | 4WAP | 5WAP | 6WAP | 7WAP | 8WAP |
|---|---|---|---|---|---|
| Neem | 23.50 [a] | 46.00[ab] | 69.77[ab] | 87.13[ab] | 88.00 [b] |
| Mexican sunflower | 32.20 [a] | 50.67[ab] | 67.10[ab] | 89.90 [b] | 89.97 [b] |
| EMAS | 35.70 [a] | 58.63[ab] | 80.23 [a] | 111.60 [a] | 127.77 [a] |
| Carbofuran | 38.20 [a] | 61.10[ab] | 80.03 [a] | 113.67 [a] | 123.33 [a] |
| Control | 27.20 [a] | 34.00 [a] | 64.30 [b] | 80.23 [c] | 82.23 [b] |

Means within the same column followed by the same letters(s) are not significantly different ($P \leq 0.05$)

**Table 3: Effects of EMAS, leaf extracts of plant and Carbofuran on the stem girth of cucumber infected with *M. incognita***

| Treatments | 4WAP | 5WAP | 6WAP | 7WAP | 8WAP |
|---|---|---|---|---|---|
| Neem | 0.47 [a] | 0.67 [a] | 0.70 [a] | 0.80 [a] | 0.80 [a] |
| Mexican sunflower | 0.50 [a] | 0.70 [a] | 0.70 [a] | 0.76 [a] | 0.76 [a] |
| EMAS | 0.53 [a] | 0.73 [a] | 0.76 [a] | 0.80 [a] | 0.83 [a] |
| Carbofuran | 0.47 [a] | 0.68 [a] | 0.75 [a] | 0.80 [a] | 0.82 [a] |
| Control | 0.40 [a] | 0.60 [a] | 0.66 [a] | 0.76 [a] | 0.76 [a] |

Means within the same column followed by the same letters(s) are not significantly different ($P \leq 0.05$)

**Table 4: Effects of EMAS, leaf extracts of plant and Carbofuran on the flowering of cucumber infected with *M. incognita***

| | Average number of days to flowering | | |
|---|---|---|---|
| **Treatments** | Flower initiation | 50% flowering | 100% flowering |
| **Neem** | 20.66 [a] | 24.66 [a] | 29.60 [a] |
| **Mexican sunflower** | 20.67 [a] | 24.50 [a] | 29.32 [a] |
| **EMAS** | 19.95 [a] | 24.50 [a] | 28.30 [a] |
| **Carbofuran** | 19.60 [a] | 24.33 [a] | 28.60 [a] |
| **Control** | 20.60 [a] | 24.56 [a] | 29.68 [a] |

Means within the same column followed by the same letters(s) are not significantly different ($P \leq 0.05$)

**Table 5: Effects of EMAS, leaf extracts of plant and carbofuran on the yield of cucumber infected with *M. incognita***

| | Yield Component | | |
|---|---|---|---|
| **Treatments** | Average fruit no / plot | Average fruit weight (kg)/plot | Yield (kg/.ha) |
| **Neem** | 19.30[b] | 5.17[b] | 7274.60[bc] |
| **Mexican sunflower** | 20.00 [b] | 4.99 [b] | 6991.20[bc] |
| **EMAS** | 34.30 [a] | 7.00 [a] | 9362.00 [a] |
| **Carbofuran** | 33.21 [a] | 6.90 [a] | 8641.20 [a] |
| **Control** | 16.90[c] | 3.20 [c] | 5291.40 [b] |

Means within the same column followed by the same letters(s) are not significantly different ($P \leq 0.05$)

**Table 6: Effects of EMAS, leaf extracts of plant and carbofuran on the nematode Population and Root gall index of cucumber infected with *M. incognita***

| | Nematode population | | |
|---|---|---|---|
| **Treatments** | Initial nematode population | Final nematode population | Root gall index |
| **Neem** | 2005 | 562[b] | 3[b] |
| **Mexican sunflower** | 2007 | 537[b] | 3[b] |
| **EMAS** | 2011 | 2239[c] | 4[c] |
| **Carbofuran** | 2068 | 409[a] | 2[a] |
| **Control** | 2009 | 2540[c] | 4[c] |

Means within the same column followed by the same letters(s) are not significantly different ($P \leq 0.05$)

Table 2 shows the leaf extract, EMAS and Carbofuran treatments brought about variation in the length of cucumber vine. There was no significant difference in the vine length in the week 4 and 5 of the experiment. Significant differences were observed between week 6 and 8. EMAS and Cabofuran treatments recorded longer vine and significantly longer than other treatments in week 8. Table 3 shows that there was no significant difference in the stem girth of all the treatments tested. Table 4 shows that the leaf extract, EMAS and Carbofuran treatments do not affect the flowering time of cucumber. There was no significant difference in the number of days to first flower initiation, number of days to 50 and 100 % flowering respectively.

Table 5 showed that different treatments brought about variation in the yield component of cucumber plant. EMAS and carbofuran treatments recorded significantly greater yield among the various treatments. The number of fruit and average fruit weight was higher in EMAS and Carbofuran treatments compared to the leaf extract as well as control treatments and they were significantly higher than the other treatments in both the fruit weight and fruit number. EMAS treated plot recoded over 9 t/ha followed by Carbofuran with 8t/ha. Neem and wild sunflower extracts recoded over 5 and 7 t/ha respectively while the control treatment recorded about 5 t/ha.

Table 6 shows that initial and final nematode population and the gall index for all the treatments. There was a significant reduction in the soil final nematode population in plant extracts and Carbofuran treatments. There was no significant reduction in the soil final nematode population and root gall index for EMAS and control treatments indicating that the EMAS treatment has no effect on soil nematode, there were multiplication of soil nematode and high gall index which is not different from the control.

Table 7 shows that the nutrients composition of treated cucumber fruits was not affected by the various treatments. Table 8 shows that the plant extracts contained various bioactive chemical components which are capable of controlling the root-knot nematode. The bioactive chemical components observed in the plant extracts were: Alkaloid, Tannin, Flavonoid and saponin.

**Table 7: Effects of EMAS, leaf extracts of plant and Cabofuran on the mineral nutrient composition of cucumber infected with *M. incognita***

| | Mineral nutrient composition of M.incognita infected cucumber | | | | | |
|---|---|---|---|---|---|---|
| **Treatments** | Na | Ca | K | P(ppm) | N(%) | CP(%) |
| **Neem** | 0.221 | 0.1 | 1.03 | 53.2 | 2.69 | 16.81 |
| **Mexican sunflower** | 0.22 | 0.1 | 1.03 | 52.8 | 2.67 | 16.68 |
| **EMAS** | 0.44 | 0.28 | 1.28 | 53.2 | 2.71 | 16.93 |
| **Carbofuran** | 0.22 | 0.53 | 1.54 | 53.0 | 2.73 | 17.06 |
| **Control** | 2.22 | 1.02 | 1.03 | 52.8 | 2.66 | 16.63 |

Means within the same column followed by the same letters(s) are not significantly different ($P \leq 0.05$)

**Table 8:** Phyto active Chemical components present in neem and Mexican sunflower

| **Treatments** | ***Azadirachta indica*** | ***Tithonia diversifolia*** |
|---|---|---|
| **Alkaloid** | + | + |
| **Tannin** | + | + |
| **Flavonoid** | + | + |
| **Saponin** | + | + |

+ Chemical components detected

- Chemical components not detected

## DISCUSSION

Application of neem and wild sunflower extracts as well as EMAS and Carbofuran brought about significant improvement in the growth and yield of nematode infected cucumber compared with untreated control. Plant extracts (neem and wild sunflower leaf) and carbofuran brought about significant reduction in the soil population and root gall index of *M. incognita* infested cucumber leading to improved growth and yield compared with the untreated control. The observed improvement on the growth and yield of *M. incognita* infected cucumber treated with plant extracts and carbofuran may be due the negative effects of the extracts and carbofuran on *M. incognita*. This negative effect of extract and carbofuran on nematode brought about positive growth and yield index on *M. incognita* infected cucumber. These type of control effects of Carbofuran and plant extracts on nematode pest of plants have been reported by many workers including Oyedunmade (2004); Abolusoro (2006); Olabiyi *et al* (2008); Oyedunmade *et al* (2009); Abolusoro *el al* (2010).

EMAS did not reduce the soil population and root gall index of *M. incognita* infected cucumber but performed better than other treatments with respect to growth and yield parameters. This observation is a clear indication that EMAS has no toxic effect on nematode yet it recorded better growth and yield compare to other treatments. The reason for this paradox might be due to the fact that EMAS is capable of strengthening the tolerance level of *M. incognita* infected cucumber to nematode attack, hence promoting growth and yield of such plant. This observation is similar to that of Afolami and Adigbo 1999 who observed heavily galled roots of okra by *M. incognita* did not negatively correlate with yield as a result of the tolerance conferred by the okra plant to nematode attack.

The result of this study indicated that the leaf extracts of neem and wild sunflower contained alkaloid, tannin, flavonoid, and saponin. This might suggest the presence of nematicidal components in the plants. Neem leaf extracts was found to possess nematidical property (Abid and Magbool, 1991; Siddiqqu and Alam, 1989; 1999; Abolusoro 2006). Olabiyi *et al* 2013 reported the presence of tannins, saponin, flavonoids, alkaloids as some of the bioactive chemical components of wild sun flower. There was a marginal rise in some of the mineral nutrient elements of Cucumber fruits in all the treatments (extracts, EMAS and carbofuran) compare with the untreated control. This observation was in agreement with Shwetha (2008) who, reported similar trends.

## CONCLUSION AND RECOMMENDATION

The result of these experiments showed that plant extracts are capable of reducing the soil population as well as root gall index of *M. incognita* infected plant and thereby promoting growth and yield. EMAS does not reduce soil population on nematode and root gall index as it was not significantly different from the untreated control, yet it brought about better growth and yield. It therefore means that EMAS strengthens tolerance level of *M. incognita* infected plant and can be used in synergy with other control or bio-control agents so as to enhance the growth and yield of crop. The concentration of the plant extracts utilized should be increase in another experiment to monitor the level of toxicity of the extract to nematode on the crop under investigation.

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