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Original Article

Performance Evaluation of Some Potential Bionematicides on Leaf and Fruit Production of *Capsicum annuum*

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Abstract

Plant-derived pesticides have become a mainstay in the control of pest, due to the fact that synthetic pesticides induce environmental degradation. The effective control of plant nematode has been found to improve the yield and economic value of farm produce. Certain growth parameters are visible in diseased plant, these in turn affects the final output of the produce. The performance evaluation of some bionematicides were investigated with five plants (*Azadirachta indica*, *Vernonia amygdalina, Manihot esculenta, Carica papaya.* and *Citrus sinensis*) using *Capsicum plant* in a two trial sessions, within exposure periods of 30, 60 and 90-days, at different concentrations (20, 30 and 40g). The growth parameters monitored were numbers of leave and fruit produced. Results showed that, compared to the control, all treatments similarly demonstrated significant improvement in leaf and fruit production, with much more improvement in the second trial ($p < 0.05$). Thus the applied treatment significantly ($p < 0.05$), induced varying degrees of nematotoxicity resulting to improved leaf and fruit production of Capsicum annum (Pepper plant. Based on the findings of this research, we therefore conclude that all treatments (i.e. tested plant), demonstrated significant improvements in the development of *Capsicum plant* and as such can be recommended as Bionematicide.

1. Introduction

The global prevalence of root-knot nematodes known as *Meloidogyne* species, has become a source of concern due to its complex host range[1]. It accounts to one third of the overall losses incurred by pests and diseases [2]. Statistical data by Abad *et al*., (2008), shows that agricultural annual losses caused by plant nematodes could be estimated to about $157 billion, which amounts to about 10% of global loss [3]. Although the hunger problem is not confined to food shortage, raising food production in the developing, tropical countries, where 60% of the arable land is situated, remains an important objective to meet the UN Millennium Development Goal to reduce by 2015 the proportion of people who suffer from hunger by half compared with the number in 1990 [4].

Nematode infestation is inevitably linked reduction in yield and economic values of farm produce. It affects vital growth parameters such as quality, quantity of fruits as well as the entire life span of the plant [5].It is clear from visible inspection that severely attacked plants have a reduced vitality, which produce less fruits, and finally will die [6].

Furthermore, Mansoor *et al* [7] reported that nematode infection impairs nodulation and nitrogen fixation and thus the overall yield. Although several pests and diseases are responsible for growth impediment and poor economic value [5], but one of the pests that inhibit the dream of African countries from achieving food sustainability is a group of plant parasitic nematodes commonly referred to as Root Knot nematodes of the Genus *Meloidogyne* [8 - 10].

The nematode has a wide range of host plants and cause economic damage to many agricultural crops [11]. Extensive data sets were available, providing nematode species occurrence and densities for the following countries: Côte d'Ivoire [12], Ghana [13], Cameroon [14], Nigeria [13], Kenya [15], Tanzania-Bukoba [13], Uganda-central [13], Uganda-western [13], Tanzania- Zanzibar [16], and Ethiopia [17]. Control of root-knot nematodes is inevitable so as to make vegetable production profitable, thus developing new and environmentally friendly control methods is required [6]. Currently, soil fumigants and nematicides are used to control nematodes. However, the need to reduce dependence on nematicides, imposed by legislation and consumers, requires the development of new management strategies. Biological control as an alternative is an important tool for plant-parasitic nematode management [18].

The modern way of nematode control is totally based on the nematicides as higher population growth demands increase crop production. But on the other hand these nematicides are not only toxic to the root-knot but also accumulate in plant. These nematicides often lead to environmental pollution and even the depletion of the stratospheric zone [19].

Hence, there is an urgent need for an eco-friendly substitute for nematode control. Plant parts/products proved to be the promising alternative means and showed toxicity to pest up to a certain extent and their application offers complete economic advantage [7]. Biocontrol of nematode has been emphasized to control the chemical means of management, as the use of nematicides are hazardous to the environment which in some cases further leads to biomagnifications [7]. Many naturally occurring compounds are known to possess nematicidal activity [20].

Plant parts possess nematostatic as well as nematicidal property [21,22]. Nonchemical methods and strategies for nematode management including cultural methods and engineered measures have been recommended as an alternative to methyl bromide (a major soil fumigant), due to its role in the depletion of the ozone layer. Hence, an international agreement has recently been reached calling for its reduced consumption and complete phasing out [23]. Most Plant-derived pesticides are eco-friendly and effective, and consequently, it has become necessary to investigate Performance Evaluation of some Bionematicides on some on Some Growth Parameters of *Capsicum annuum*.

2 Materials and Methods

2.1 Study area

This Bioassay was carried out in the Parasitology laboratory of the Department of Animal and Environmental Biology and The Green House

area of the Department of Plant Science and Biotechnology of University of Port Harcourt, Choba, Port Harcourt, Rivers State, Nigeria (N04° 54" 26'. E06° 55" 39').

2.2 Soil Sterilisation and Analysis

Samples of garden soil were first analysed to determine the physico-chemical properties and found to consist of the following: clay 16.8%, silt 12.8%, sand 70.4%, Cu 13.2 mg/g, Zn 2.76 mg/g, Fe 86.35 mg/g, Mn 35.22mg/g, Al 0.00 Cmol/kg, Na 0.351 Cmol/kg, K 0.093 Cmol/kg, Mg 0.535Cmol/kg, Ca 0.246 Cmol/kg, P 12.22 mg/g, N 0.293%. The soil was heat-sterilized at 60 °C for 45 minutes. This process raised the soil temperature to an average that is lethal to parasitic nematodes and other soil fauna and flora [24]. The sterilized soil was allowed to cool and later packaged and stored in 10cm by 8cm polythene bags, which served as pots for the experiment.

2.3 Procurement and Preparation of Biocontrol Agents -Treatments

Twigs of fresh green leaves of Neem and Bitter Leaf plants were are purchased from Swahli Market in the Metropolitan city of Yenagoa, in Bayelsa State. Meanwhile, fresh leaves of Cassava (*Manihotes culentus*), Pawpaw (*Carica papaya*) and Orange (*Citrus sinensis*) were obtained from different farms at Olodani in Okoloba Town of KOLGALGA, of Bayelsa state. The leaves were properly washed and air dried separately in a well-ventilated room at room temperature ranging from 26-30 °C for 6 weeks. It was later spread in direct sunlight for one hour before grinding them into fine powder [18]. It was later packed in black polythene bags and stored in air and watertight containers [19].

2.4 Nursery Preparation and Transplanting

An aggregate of topsoil, compost and coarse river sand was achieved in ratio 3:2:1 [24].The soil was sterilized by heating at 60°C for 45 minutes [24]. Furthermore, the sterilized soil was stored in 4 plastic baskets in order not to firm the soil during transplanting [24]. The stored and heat-treated occupy volume of about 80% containment. The planted nurseries were kept in the Green house for 3 weeks and watered regularly, in order to achieve favourable condition, before onward transplantion in the field [24]. Twenty grams of Hot pepper (*Capsicum annuum* var. bell) used for the bioassay was purchased from Agritropic Vilmorin Limited- Vegetable Seeds for Nigeria, RC: 338009, Ibadan, Nigeria. The seedlings were transplanted (using a trowel), from the nursery to the pots in the field 3 weeks after germination [24]. NPK fertilizer (15:15:15) was applied at the rate of 5 grams per plant 2 weeks after transplanting [18].

2.5 The Experimental Design

This Bioassay was conducted *ex-situ* in a completely randomized block design. Five groups of plant were potted for each treatment to make 180 plants, with 36 plants were in each group, as well as the control. All potted plants were inoculated 1000 eggs/juveniles/adults of *M. incognita*. Excluding the control, all assayed plants were treated with 20g, 30g, and 40g of the powder of the bio-nematicides for exposure of 30, 60 and 90 days.

2.6 Extraction and Sterilization of Nematodes

Capsicum plants infested with nematodes (*M. incognita*) was collected from a pepper farm in Gokana LGA of Rivers State. The eggs were extracted from the roots. About 10g of the roots was chopped into smaller bits with the aid of scissors. It was immersed in 100ml of 5% Sodium hypochlorite (NaOCl) solution and was vigorously agitated for about 3 minutes. Furthermore, the chopped pieces of roots was rapidly filtered in a 200 mesh (75µm), and over a 500 mesh (26µm).The egg masses, juveniles and adult species of nematodes that were trapped on the 500 mesh were washed in a gentle stream of tap water to remove residual Sodium hypochlorite (NaOCl) solution. The remaining roots pieces in the 200 mesh (75um) were rinsed with tap water to recover additional nematodes, egg masses and juveniles. [21, 22].

2.8 Standardization of Innocula and Innoculation

The volume of the suspension was standardised to 50ml. Aliquot of 1ml of each suspension was pipetted into a counting tray after bubbling air through the suspension for homogenecity. Counting was done with the aid of a microscope and the number of eggs/ juveniles/adults of *M. incognita* per ml estimated. During inoculation, 1000 eggs/juveniles/adults of *M. incognita* were inoculated to each of the 180 plants 4 weeks after transplanting. Holes were made in a triangular form, 2cm from the pepper plant. The eggs/ juveniles/adults of M. incognita in the suspension were then dispensed into the holes made around the roots of each plant and was be covered with the soil [25]. Forty-eight (48) hours after inoculation, each of the plants (the control excluded) were treated with the 20g, 30g and 40g of the powder of the *Azardirachta indica, Venonia amygdalina, Manihot esculentus, Carica papaya* and *Citrus sinensis*, accordingly.

2.11 Bioassays

Bioassay on the growth parameters monitored (number of leaves and fruit produced), was carried out within an interval of 30 days over a period of 90 days (i.e. 30, 60 and 90 days), beginning from the first month after inoculation. (90 days) to determine the nematode population and the rate of infection.

2.12 Statistical Analysis

SPSS software version 20 was used to carry out the statistical analysis of the. The data were expressed as Mean ± standard deviation. A one-way analysis of variance was carried out at P = 0.05, and Duncan Statistics was used to determine the source of the detected differences. Furthermore, charts were plotted with the mean values using Microsoft Excel Package 2013.

3. Results

Figure 1 shows the First trial Determination of average number of leaves produced by Capsicum plant within 30, 60 and 90 days after treatment with 20, 30 and 40g of all Bionematicides. For *A. indica*, the result indicated that average leaves growth increased from 18.33 - 24.67 in 30 days, in 60 days it ranged from 28.67 – 37.33, while it was 32.33 - 38.66 in 90 days. For *V. amygdalina* it was 19.00 - 31.33, 28.67 – 43.33 and 31.67 – 37.67 for 30, 60 and 90 days respectively. Capsicum plant treated with M. esculentus produced average leaves within the ranges of 16.00 -18.00 in 30 days, 32.00 – 43.33 in 60 days and 31.33 – 37.67 in 90 days. For C. papaya, it was 11.67 – 25.67, 21.66 – 51.33 and 17.66 – 57.00 in 30, 60 and 90 days respectively. The *C. sinensis* treatment had 16.00 – 21.00 (30 days), 30.00 – 33.00 (60days) and 22.67 – 51.33 (90 days). Comparatively, average leaf growth in the control was lowest (13.00 – 18.67), showing significant difference compared to all treatments ($p< 0.05$).

Figure 2 shows the Second trial determination of average number of leaves produced by Capsicum plant within 30, 60 and 90 days after treatment with 20, 30 and 40g of all Bionematicides. For *A. indica*, the result indicated that average leaves growth increased from 29.33 - 35.67 in 30 days, in 60 days it ranged from 56.67 – 64.00, while it was 68.00 - 81.00 in 90 days. For *V. amygdalina* it was 27.67 - 34.00, 42.43 – 43.67 and 55.33 – 81.00 for 30, 60 and 90 days respectively. Capsicum plant treated with *M. esculentus* produced average leaves within the ranges of 19.67 -21.67 in 30 days, 40.33 – 50.67 in 60 days and 55.33 – 81.00 in 90 days. For *C. papaya*, it was 23.67 – 33.33, 41.00 – 48.00 and 56.00 – 73.00 in 30, 60 and 90 days respectively. The *C. sinensis* treatment had 21.00 – 22.00 (30 days), 39.00 – 32.67 (60days) and 66.00 – 77.67 (90 days). Comparatively, average leaf growth in the control was lowest (18.00 – 34.00), showing significant difference compared to all treatments ($p< 0.05$).

Figure 3 shows the First trial Determination of average number of fruits produced by Capsicum plant within 30, 60 and 90 days after treatment with 20, 30 and 40g of all Bionematicides. Generally, there was no fruit within 30 days in all treatments as well as the control. Notwithstanding, *A. indica* result showed that average highest fruit was 4.00 in 60 days and 4.67 in 90 days. For *V. amygdalina* it was 2.67 in 60 days and 4.00 in 90 days, while *M. esculentus* produced average highest fruit of 2.33 and 4.00 within 60 and 90 days respectively. C. *papaya* and *C. sinensis* produced 2.33 and 4.67 as well as 2.00 and 4.00 fruits within 60 and 90 days respectively. Compared to the treatment, the control produced only one fruits in 60 days but no fruit in 90 days ($p< 0.05$).

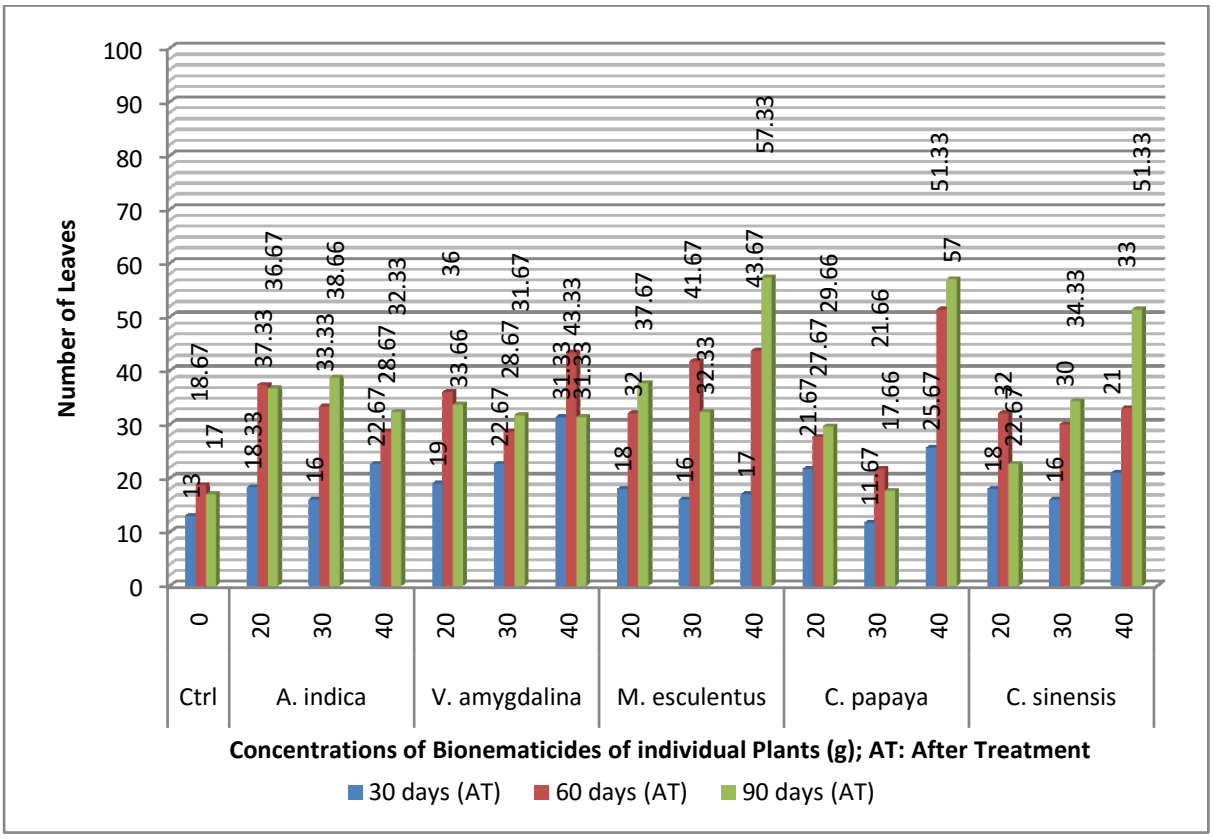

Figure 1: First trial Determination of number of leaves produced by individual Treated Capsicum plant

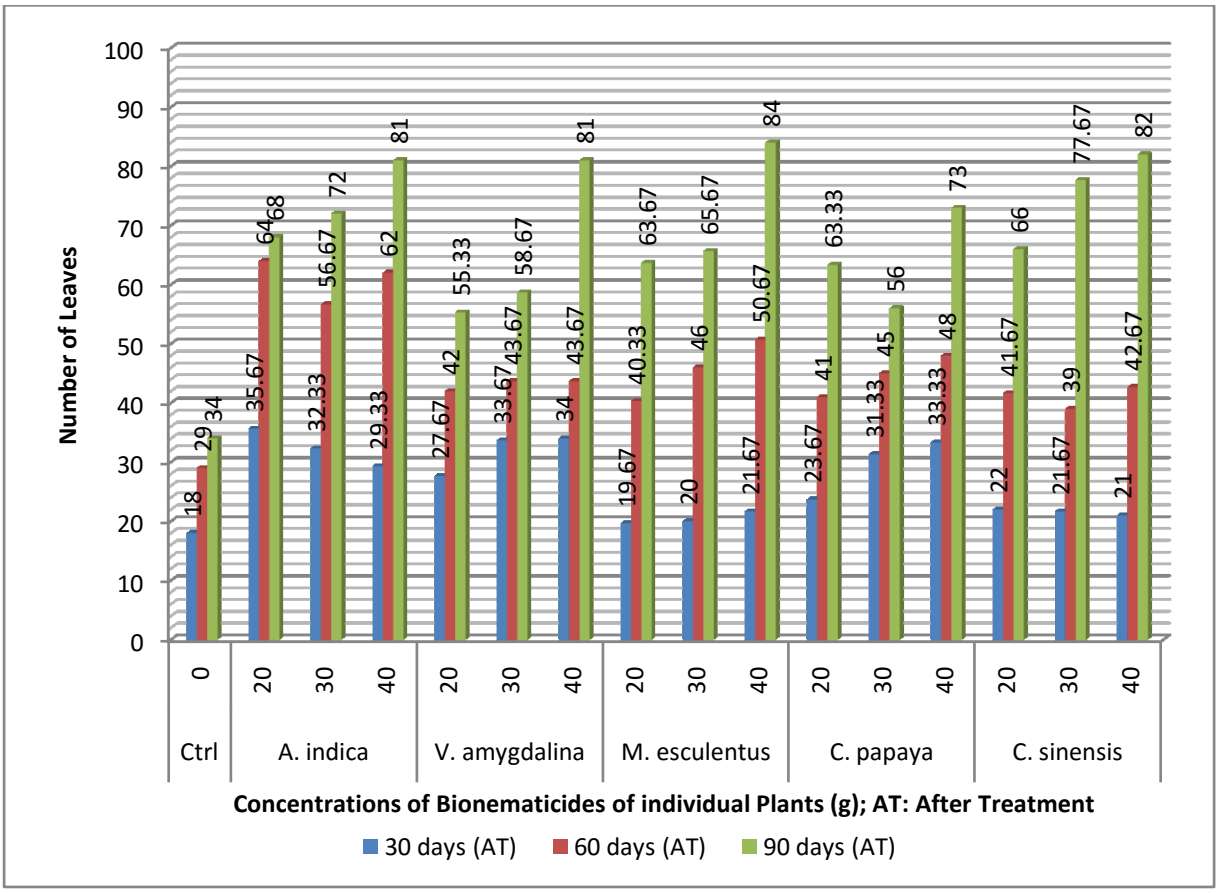

Figure 2: Second trial Determination of number of leaves produced by individual Treated Capsicum plant.

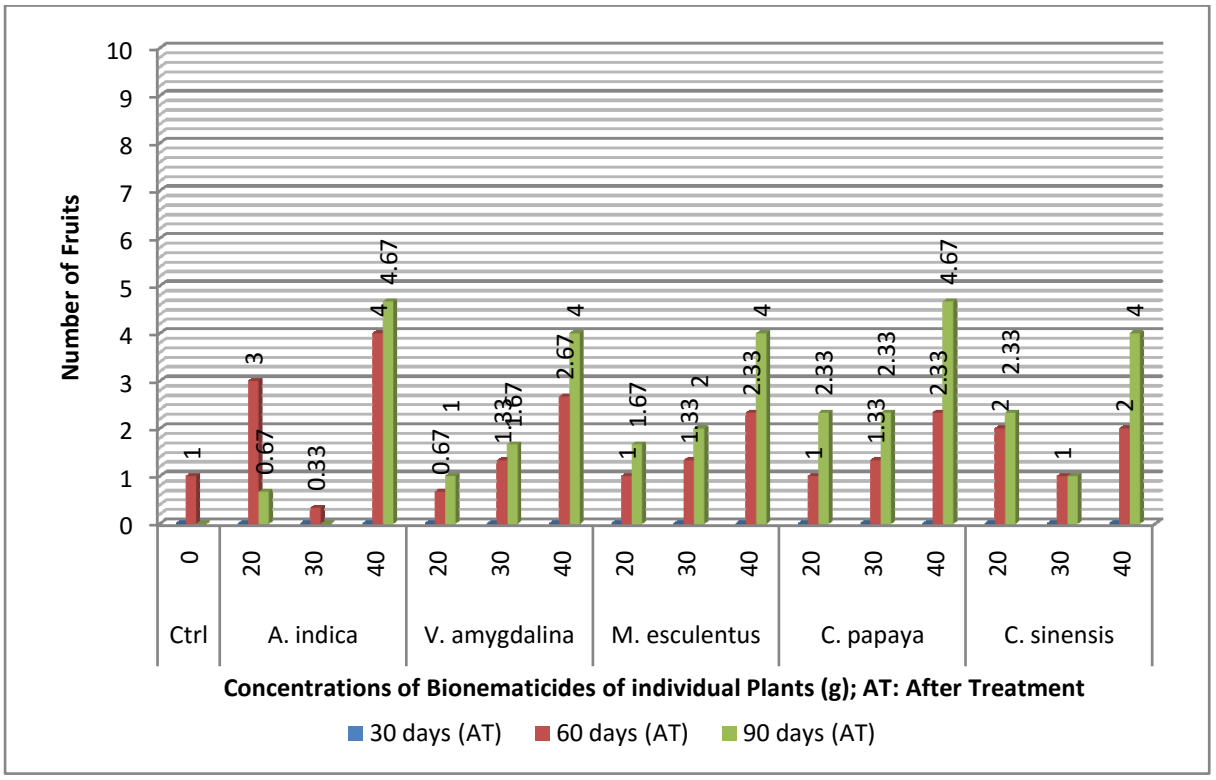

Figure 3: First trial Determination of number of fruits produced by individual Treated Capsicum plant.

Figure 4 shows the Second trial Determination of average number of fruits produced by Capsicum plant within 30, 60 and 90 days after treatment with 20, 30 and 40g of all Bionematicides. Similarly, there was no fruit recorded within 30 days in all treatments as well as the control. Notwithstanding, the *A. indica* treatment showed that average highest fruit was 5.33 in 60 days and 7.67 in 90 days. For *V. amygdalina* it was 3.67 in 60 days and 5.33 in 90 days, meanwhile *M. esculentus* produced average highest fruit of 3.00 and 5.00 within 60 and 90 days respectively. The C. *papaya* and *C. sinensis* treatment produced 4.67 and 7.67 as well as 4.00 and 5.33 fruits within 60 and 90 days respectively. Compared to the treatments, the control produced an average highest fruits of 2.33 in 60 days and unfortunately produced no fruit in 90 days ($p < 0.05$).

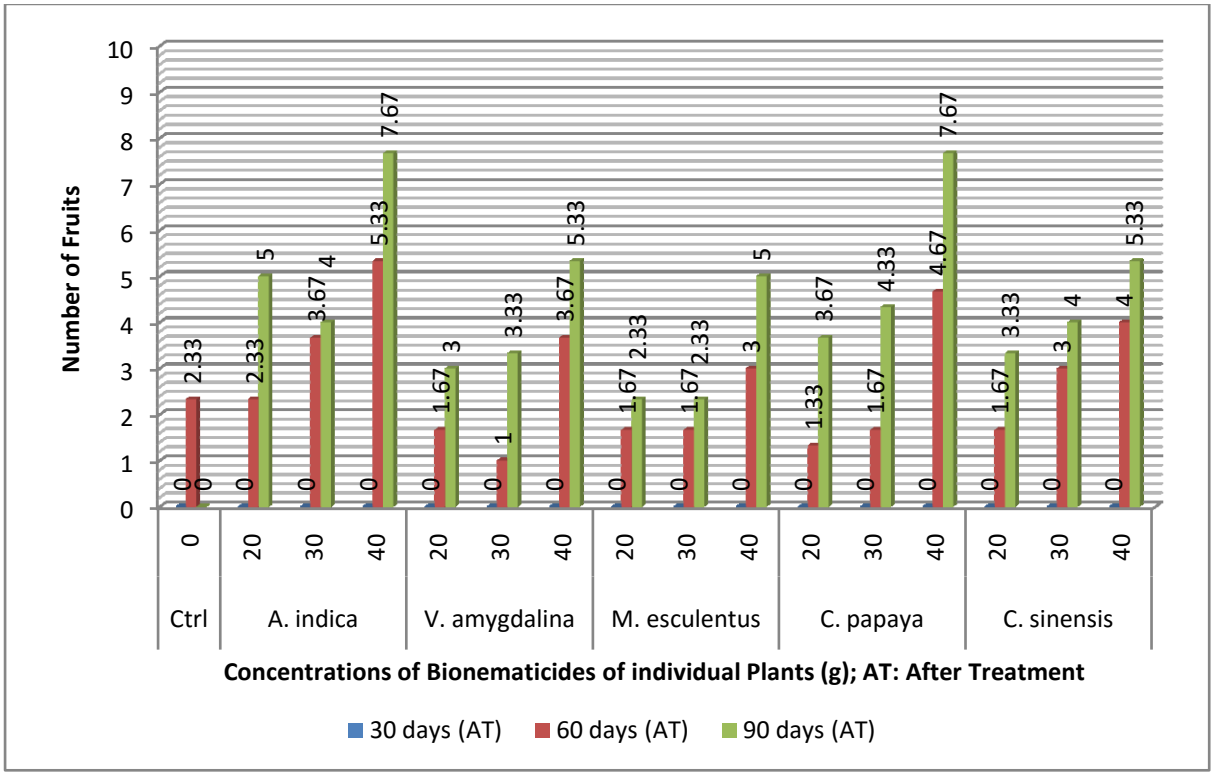

Figure 4: Second trial Determination of number of fruits produced by individual Treated Capsicum plant.

4. Discussion

The number of leaves produced by the plants treated was higher than that produced by the control group on the average as can be observed in Figures 1 and 2. However, the leaf production pattern was haphazard, not really influenced by the treatments concentration in terms of leaf numbers. For instance, some of the lower concentration produced more leaves than higher concentrations (Figures, 1 and 2). In the group treated with *A. indica*, the highest number of leaves produced in 90 days was observed the group treated with 30g (Figures 1 and 2). The lowest was produced by the group treated with 40g, the highest concentration. But in the 2nd Trial (Figure 2), the group with the highest concentration produced the highest number of leaves. The control group, in both Trials produced the lowest number of leaves. From these we can infer that the treatment applied had effects on the number of leaves produced.

In the *V. amygdalina* treatment, the highest average number of leaves was produced by the group treated with the highest concentration and the lowest was produced by the control group both in the 1st Trial and in the 2nd Trial (Figures 1 and 2). This showed that the treatment with *V. amygdalina* had a significant effect on number of leaves produced during the growth trials. The higher the concentration, the greater the number of leaves. This trend continues with the *M. esculentus* treatment, with exception of the first 30 days, where the highest number of leaves was produced by a plant treated with 20g, this could be circumstantial. On the average, the higher concentration produced the highest number of leaves and the control produced the lowest number of leaves. This was also seen in the group treated with *C. papaya* in the 1st and in the 2nd trials (Figures 1 and 2) and also in the group treated with *C. sinensis* in the both trials.

The treatments applied increased the number of leaves generally and on the average, surprisingly *C. papaya* produced the highest number of leaves, but did not produce the highest number of fruits both in the 1st or 2nd Trials. The highest number of fruits was produced by the group treated with *A. indica*. Since leaves play an important role in photosynthesis (1, 6), it was expected that the group with more leaves will produce more fruits, but such was not seen with the group of *Capsicum* treated with *C. papaya* powder. Other factor could come into play to explain this, such as the size of the leaves and their position on the branches relative to sun light [6], or even the effect of the phytochemical of the treatment on the plant hormone [27]. Even though the group treated with *A. indica* had fewer leaves on the average than the group treated with *C. papaya*, they were generally broader and fresher with more spread. These increased their surface area for sun absorption and hence produce more photosynthate that in turn led to the production of more fruits when compared with *C. papaya* treated plants, other treatments and the control.

Generally, the 2nd session produced more fruits than the 1st session (Figures 4). Environmental factors, such as better management of the plants by the researcher, having learned the ropes in the 1st session, and variation in plants, since seeds and not vegetative parts were used in propagation. Gene flow through cross pollination can lead to the production of better and/or different results. A more favourable weather conditions could also contribute to this difference. However, the groups treated with the bio-nematicides, in both sessions had fewer nematode attacks leading to the production of more fruits and fruits with quality better than the fruits produced in the control group. This agrees with the assertion of Sasser [11], that nematode infection can act as energy sink which absorbs photosynthate needed by plants for growth and fruit production, crop yields are reduced and harvested produce are of poor quality with reduced shelf life, as was seen in the control group.

Earlier investigation indicated the root bioassay of *Capsicum* treated with the leaf powder of *A. indica* indicated a 100% nematicidal effect in all the different concentrations[27 - 30]. As such it is believed that the nematotoxicity induced by the plant must have been responsible for improved yield. Similarly, the 2nd Trial (Figures 3 and 4) followed the same trend, but with higher yield. This could attributed to various seasonal influence or environmental factors, since the trials were carried out in a different seasons [1]. A recent study by Angaye *et al*., [6] confirmed the bionematicidal activities of *Azadirachta indica*, *Vernonia amygdalina, Manihot esculenta, Carica papaya* and *Citrus sinensis* in a two trials. He reported the degrees of nematotoxicity were reported as: *A. indica>V. amygdalina>C. sinensis> C. papaya> M. esculentus*.

The high yield of leaves and fruits amongst the *V. amygdalina* was not farfetched as earlier studies had established *V. amygdalina* as a bio-nematicidal agent [31,32]. Also the *M. esculentus* the lower concentration showed the presence of nematode in the later part of the research. This could be attributed to the fact that the length of exposure reduced the efficacy of the extract because of the lower concentration used. This result corresponds with the findings of Joymatti *et al*., [33] and Madhusudanan *et al*., [34] who reported that juvenile mortality was concentration dependent. Roots of lower concentration of extract and the control were found to be more favourable to the activities of root knot nematode. More eggs were found to be deposited on their roots compared with the higher concentrations. In the present study the powdered leaves of *M. esculentus* was found to have a nematotoxic effect on root knot nematodes. This agrees with works of earlier researchers who worked with other parts of *M. esculentus* such as the root extracts and the dry peel. Previous research confirmed the presence of cyanogenic compounds in dry peel, of *M. esculentus* [35, 36].

C. papaya also exhibited significant level of leaves and fruit production due to its nematotoxicity, as applied to other extracts. The treatment with highest concentration yielded better results due to the nematotoxicity [6]. This corroborates previous findings that dose and time are significant in nematotoxity[25, 33, 34], and thus yield improvement. Similarly *C sinensis* indicated significant levels of leaves and fruit production when compared with the control. These improvements is not farfetched as earlier studies showed that fresh peels of lemon, orange, and grapefruit induced significant nematotoxicity against *M. incognita* after 48 h treatment [1, 37]. Generally, tested plants used exhibited significant levels of yield improvements as a result of their nematotoxicity against the parasitic nematodes. Bio-derived nematicides have been found to reduce nematode populations at greater soil depths compared to most soil fumigants [38].

5. Conclusion

This research revealed all applied treatments demonstrated significant improvement in the monitored growth parameters (leaves and fruit production). This was largely due to their nematotoxicity as well as other compounding factors. Fruit production was much better in Capsicum plants treated with a higher concentration compared to the control. Synthetic nematicides are relatively expensive and unavailable; it has become necessary to apply eco-friendly strategies aim at improving yield of plants. This make remedial application plants material lucid and potentially bio-available. In order to effectively and appropriately incorporate these tested plant as bionematicides, integrated management program should be established in further study to see their extensive field application as possibly their synergistic effects.

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