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The Effects of Some Bio-Nematicides on the Productivity of *Capsicum annum*

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Abstract

Biopesticides have found intrinsic application in the control of plant pests and diseases, due to their eco-friendly activities and bioavailability as opposed to synthetic pesticides. Nematode infestation has become a major problem associated with poor yield and low economic value of farm produce. The height and dry shoot weight (DSW), of *C. annum* (pepper plant), treated with some potential nematicides (*Azadirachta indica*, *Vernonia amygdalina*, *Manihot esculenta*, *Carica papaya*. and *Citrus sinensis*), were investigated *ex-situ*. The investigation was carried out in a two trials, within exposure periods of 30, 60 and 90-days, at different concentrations of 20, 30 and 40g. Results showed that, compared to the control, all treatments similarly demonstrated significant improvement in the monitored parameters of both trials ($p < 0.05$). Thus the applied treatment significantly ($p < 0.05$), improved productivity of *C. annum* induced due to their varying degrees of nematotoxicity. Based on the findings of this research, we therefore conclude that the application of the treatments (i.e. bionematicides), can improve the productivity of *Capsicum* plant.

Keywords: Biopesticide, bioavailability, nematotoxicity, treatment, bionematicide

1 Introduction

Pepper has become a globally important vegetable behind tomato [1], it is processed as vegetable, spices or condiments [2]. Unfortunately, root-knot nematodes (RKNs) have become a global problem due to their devastating effects on pepper plant (*Capsicum annum*), and several other plant hosts [3]. For instance, their synergistic effects with other microbs like bacteria and fungi are documented in literature [1]. From the foregoing, the ecology and endemicity of the phylum nematoda have already been documented in literature [4]. Thus, making them one of the most ancient and ubiquitous organisms [5].

Root knot nematode (*M. incognita*) is a major pest of pepper plant [6], causing severe damage globally [7]. It is clear from visible inspection that severely attacked plants affect the productivity of several growth parameters such as; the fruits, root (galling), leaves (chlorosis) [6, 7, 8], and the eventual death of the plant [9]. The disease is also complicated by some abiotic factors such as temperature, soil moisture and pH [10, 11]. Statistically, it is established in literature that plant parasitic nematodes are responsible for global financial agricultural loss over $100 billion [12], which has been estimated at 10% and 12.2% [13], representing one third of the total losses attributed to pests and diseases [14].

The applications of synthetic pesticides in the control of RKNs have been found to induce high toxic residual effect on the environment and particularly on non-target organisms. Some are mobile, volatile in the air and soluble in water [15]. It has become an important issue to find alternative control strategies, which are as effective as synthetic pesticides, safer to farmers, consumers, and the environment and relatively easily available at low price [16].

Furthermore, one of the possible alternatives is the utilization of pesticides from plant origin, known as botanical pesticides [17]. Several applications of many botanicals have been documented in literature for their activities in pest and disease control. Reports indicate that synthetic pesticides could be poisonous if absorbed by skin contact or swallowed [18]. Repeated exposure may cause allergic disorders, systemic effects, kidney injury, upper respiratory tract damage and central nervous system depression [19]. Additionally, the Environmental Protection Agency also has concerns of methyl bromide's potential to destroy ozone [20]. As such it has become necessary to investigate the effects of some bionematicides on the productivity of *C. annum*.

2 Materials and Methods

2.1 Study Area

The experiment was conducted 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, Abuja Campus, Choba, Port Harcourt, Rivers

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State, Nigeria. The coordinates and exact location of the Green House area was determined using GPS (global positioning system) and found to be N04° 54" 26'. E06° 55" 39'.

2.2 Soil Analysis and Sterilisation

Samples of garden soil were obtained. 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 [21]. The sterilized soil was allowed to cool and later packaged and stored in 10cm X 8cm polythene bags, which served as pots for the experiment according to [11].

2.3 Procurement and Preparation of Treatments Agents

Fresh green leaves of Neem (*Azadirachta indica*) and Bitter Leaf (*Venonia amygdalina*) were purchased at Swahli Market in Yenagoa L.G.A in Bayelsa State. Fresh leaves of Cassava (*Manihot esculentus*), Pawpaw (*Carica papaya*) and Orange (*Citrus sinensis*) were obtained from different farms in Okoloba Town in Kolokuma/Opokuma L. G. A. of Bayelsa State. After procurement, the leaves were properly washed and air-dried in a well-ventilated room at room temperature ranging from 26-30 °C for 6 weeks and were later spread in direct sunlight for one hour before blending them into fine powder [22]. These were later packed in black polythene bags and stored in air and watertight containers away from direct sunlight [23].

2.4 The Experimental Design

The experiment was conducted 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, Abuja Campus, Choba, Port Harcourt, Rivers State, Nigeria. The coordinates and exact location of the Green House area was determined using GPS (global positioning system) and found to be N04° 54" 26'. E06° 55" 39'.

2.5 Nursery Preparation and Transplanting

A good mixture of topsoil, compost and coarse river sand in ratio 3:2:1 [24], was heat-sterilized at 60ºC for 45 minutes [21]. The sterilized soil was placed in 4 plastic baskets (to avoid overcrowding) occupying about 80% of the container. Care was taken not to firm the soil too much for easy transplanting [24]. After planting, the nurseries were placed in the Green house to keep the soil moist. The plants were regularly watered and allowed to grow for 3 weeks after germination before they were transplanted to the field [21]. 20g of Hot pepper (Big Sun) the seed of the pepper cultivar (*C. annuum* var. bell) used for this experiment was purchased from AgritropicVilmorin Limited- Vegetable Seeds for Nigeria, RC: 338009, Ibadan, Nigeria. Using a hand trowel, the seedlings were transplanted from the nursery to the pots in the field 3 weeks after germination [24]. The plants were regularly watered and unwanted plants (weeds) were removed. NPK fertilizer (15:15:15) was applied at the rate of 5 g per plant 2 weeks after transplanting [22].

2.9 Statistical Analysis

Version 20 of SPSS software was used to carry out the statistical analysis of the data. The data were expressed as Mean. All graphs were plotted with Microsoft excel package, also 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.

3 Results and Discussion

Figure 1 presents the first trial determination of average

2.6 Extraction and Sterilization of Nematodes

Capsicum plants infested with root knot nematodes (*M. incognita*) were collected from a pepper farm at Gokana L.G.A in Rivers State. Eggs were extracted from the infested roots. The roots (about 10g) was chopped into smaller pieces with scissors and immersed in 100ml of 5% Sodium hypochlorite (NaOCl) solution and was vigorously shaken for about 3 minutes. The chopped pieces of roots were quickly passed through a 200 mesh (75um) over a 500 mesh (26um). 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. [25, 26].

2.7 Standardization of Innocula and Inoculation

The volume of the suspension was standardised to 50ml. Aliquot of 1ml of each suspension were taken with a pipette into a counting tray after bubbling air through the suspension for homogeneity. Counting was done with the aid of a binocular microscope and the number of eggs/ juveniles of *M. incognita* per ml were estimated. For the inoculation, 1000 eggs/juveniles of *M. incognita* were inoculated to each of the 180 plants innundatively 4 weeks after transplanting. Holes were made in a triangular form, 2cm from the pepper plant. The eggs/ juveniles of *M. incognita* in the suspension were then dispensed into the holes made around the roots of each plant and was covered with the soil [22].

2.8 Treatment and Bioassay

Forty-eight (48) hours after inoculation, each of the sets of plants (the control excluded) were treated with the 20g, 30g and 40g of the powder of the *A. indica, V. amygdalina, M. esculentus, C. papaya* and *C. sinensis*, accordingly. The bioassay of plants was carried out every 30 days after inoculation for 3 months (90 days) to determine the height and dry shoot weight of aerial portion of the plant.

height reached by *Capsicum* plants (pepper); treated within exposures of 30, 60 and 90 days at concentrations of 20, 30 and 40g respectively. The *A. indica*, bioassay indicated that average height ranged from 28.87- 31.43cm in 30 days, in 60 days it improved from 39.01 – 41.63cm, while in 90 days it increased from 54.53 - 69.41cm. The *V. amygdalina* treatment indicated height reached ranging from; 25.43 - 32.33cm, 32.47 – 36.17cm and 59.43 – 61.53cm, within exposures of 30, 60 and 90 days respectively. Furthermore, *Capsicum* plant treated with *M. esculentus* produced average height within the ranges of 21.81 -28.17cm in 30

days, 33.11 – 45.53cm in 60 days, and 56.21 – 61.47cm in 90 days. The *C. papaya* was active with heights reached ranging from 21.63 – 23.50cm, 23.27 – 41.93cm and 41.41 – 61.53cm in 30, 60 and 90 days respectively. The *C. sinensis* treatment had 18.91 – 29.43cm (30 days), 42.81 – 49.21cm (60days), and 61.83 – 74.83cm (90 days). Comparatively, average height reached in the control was lowest (15.91 – 32.43cm), showing significant difference compared to all treatments ($p < 0.05$).

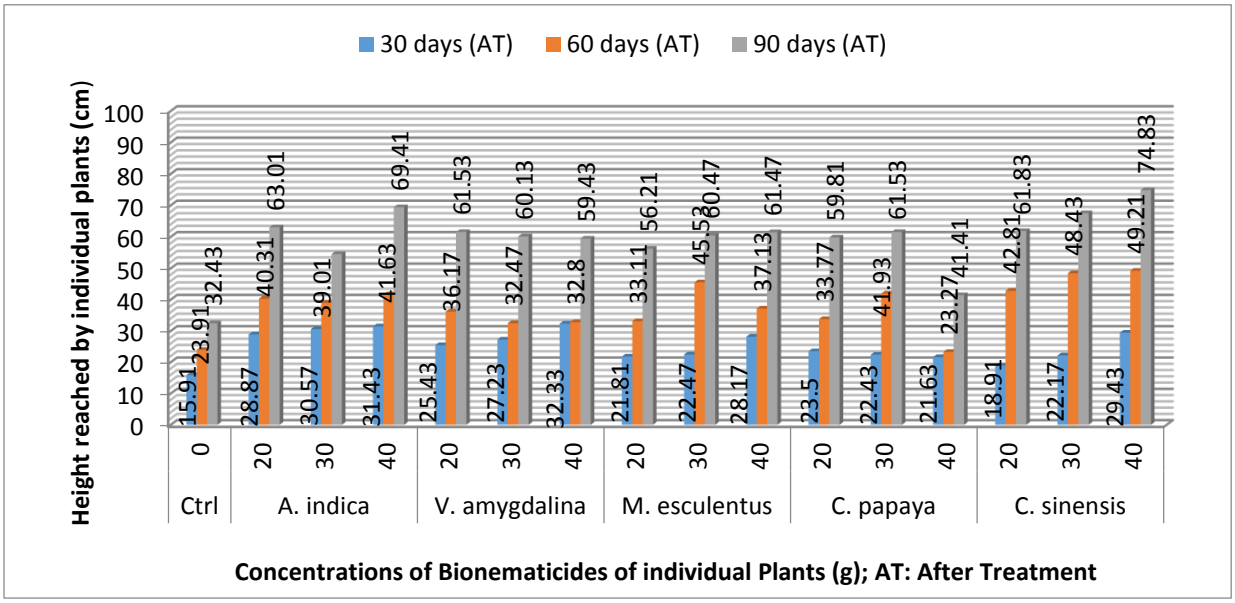

Figure 1: First trial; Determination of height reached by treated *Capsicum* plants

In the second trial determination (Figure 2), the average height of the *Capsicum* plants was similarly assayed within 30, 60 and 90 days after treatment with 20, 30 and 40g of all bionematicides. The *A. indica*, result showed that average height reached ranged from 31.33 - 32.43cm in 30 days, 37.73 – 46.03cm in 60 days and 65.81 - 71.77cm in 90 days. The *V. amygdalina* treatment was 27.63 - 31.51cm, 46.53 – 49.53cm and 64.47 – 68.43cm in 30, 60 and 90 days respectively. *Capsicum* treated with *M. esculentus* had height ranging from 30.41 - 31.13cm in 30 days, 45.81 – 53.11cm in 60 days and 67.53 – 74.47cm in 90 days. For *C. papaya*, it was 22.91 – 26.33cm, 45.07 – 56.07cm and 65.21 – 72.67cm in 30, 60 and 90 days respectively. The *C. sinensis* treatment had 20.77 – 31.03cm (30 days), 50.07 – 52.91cm (60days) and 65.81 – 70.11cm (90 days). Comparatively, average height reached in the control was lowest (17.51 – 44.01cm), showing significant difference compared to all treatments ($p < 0.05$).

Figure 3 below, presents results of the first trial determination of average dry shoot weight (i.e. aerial portion), of *Capsicum* plants treated with the bionematicides within 30, 60 and 90 days' exposures at concentrations of 20, 30 and 40g. While the control significantly ($p < 0.05$), exhibited the lowest dry shoot weight (16.31 – 25.24g). Notwithstanding, *Capsicum* treated with *A. indica* indicated dry shoot weight (DSW), ranging from 20.72 – 22.69g in 30 days, 25.93 – 28.23g in 60 days and 32.71 - 36.35g in 90 days. The *V. amygdalina* treatment had 19.31 – 20.73g in 30 days, 25.33 – 29.11g in 60 days and 31.39 – 36.35g in 90 days, while *M. esculentus* produced average DSW of 18.24 – 20.41, 24.41 – 25.33 and 29.53 – 31.71g within 30, 60 and 90 days respectively. Furthermore, the *C. papaya* bioassay had DSW of 18.41 – 20.41, 22.61 – 24.67 and 26.21 – 28.07g in 30, 60 and 90 days respectively; while the *C. sinensis* had DSW of 16.21 – 18.31, 21.71 – 23.21 and 26.21 – 27.71g in 30, 60 and 90 days respectively.

Figure 4 below, shows the second trial determination of average DSW produced by treated *Capsicum*. Similarly, there was significant difference ($p < 0.05$) between the control and all treatments. Notwithstanding, the *A. indica* treatment recorded DSW ranging from 21.31 – 23.01g in 30 days, 24.21 – 27.81g in 60 days and increasing to 33.11 - 38.51g in 90 days. The *V. amygdalina* had DWS of 20.41 – 22.51g, 26.21 – 27.51g and 30.71 – 35.31g within 30, 60 and 90 days respectively. Meanwhile *M. esculentus* produced had average DSW of 19.27 – 23.27g in 30 days, 25.07 – 27.03g in 60 days and 31.43 – 34.21g in 90 days. The *C. papaya* treatment was active with values ranging from 19.51 – 21.11g, 23.37 – 24.31g and 27.07 – 28.77g within 30, 60 and 90 days respectively. The *C. sinensis* treatment was active with average DSW ranging from 17.77 – 18.81cm, 23.07 – 24.53cm and 25.13 – 29.32cm in 30, 60 and 90 days respectively

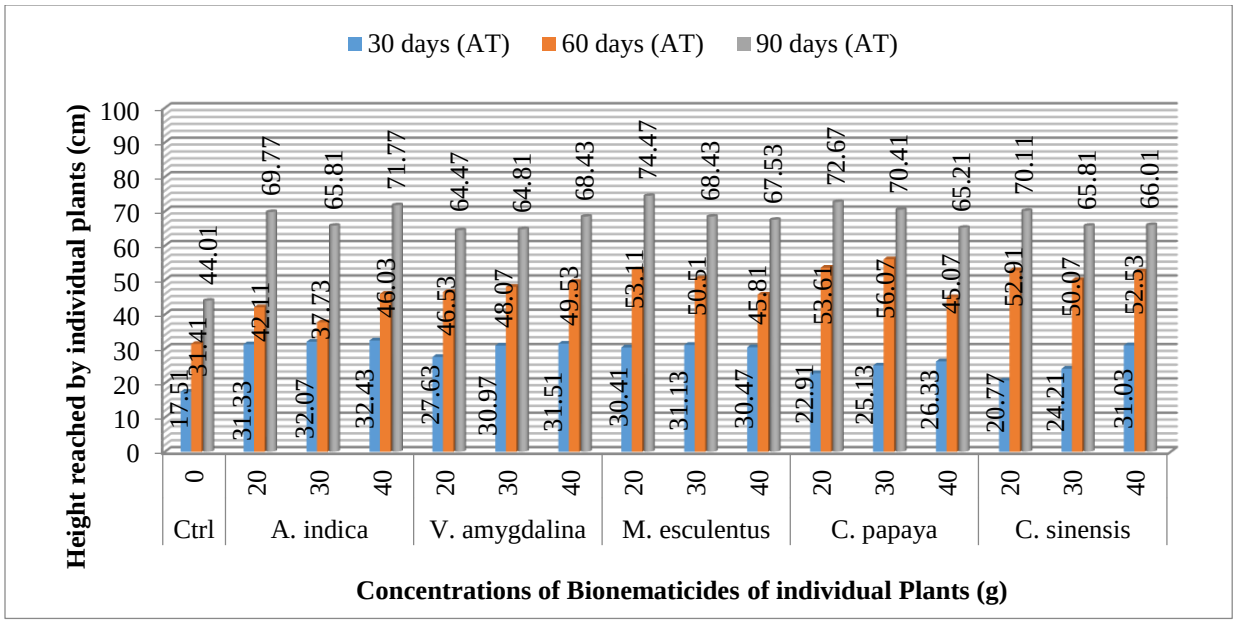

Figure 2: Second trial; determination of height reached by treated *Capsicum* plants

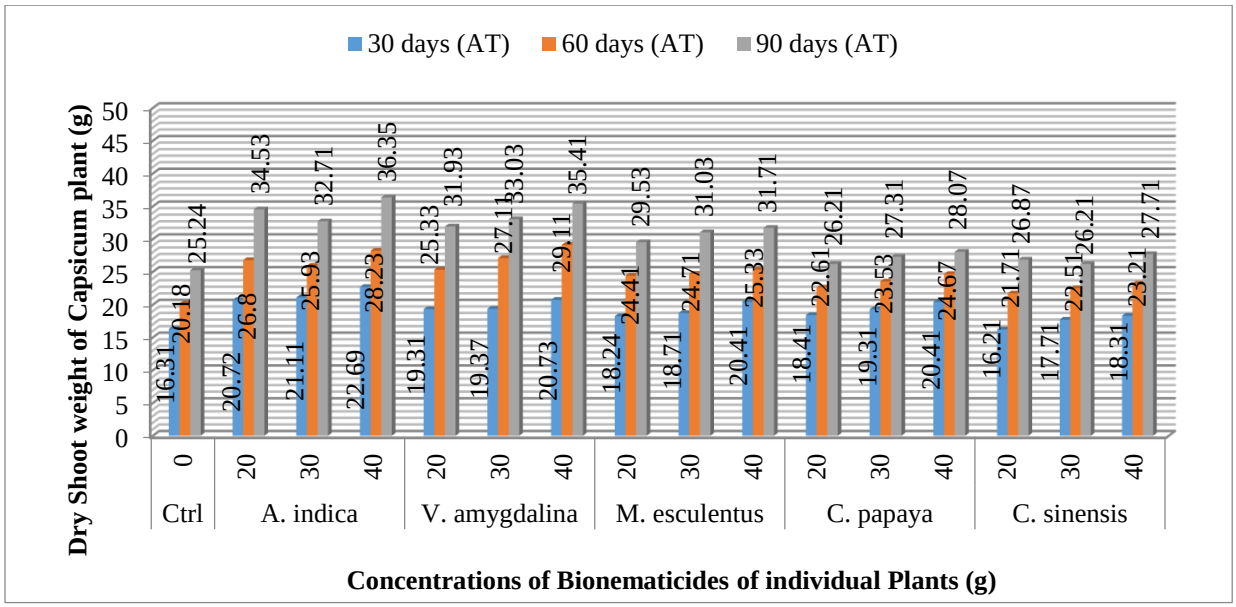

Figure 3: First trial; determination of dry shoot weight of Treated Capsicum plant

The study indicated that treated plants showed more apical competence than the control however, apical competence was significantly related to degree of organic amendment administered, (Figures 1 and 2). The extracts influenced height reached by individual plants, attributable to the nematotoxicity of the treatments. This agrees with earlier findings by Pattison [27] that the increase in height in treated plants could be due to the reduction in the activities of the root knot nematode juveniles and less galls formed on the roots. Heavily infested plants exhibit stunted growth. Plants in the control group were smaller (i.e lesser height), compared to the treatment group, this agrees with the findings of Couch and Van Staden [28], who recorded significant increase in plant height and a corresponding reduction in *M. incognita* infestation when plant material was applied as soil drench.

All applied bionematicides (*A. indica*, *V. amygdalina*, *C. sinensis*, *C. papaya*, *M. esculentus*), played a significant role in the heights reached by individual *Capsicum* plant, compared to the control. This indicated that the treatment played an important role in the heights reached by the plants (Figures 1 and 2).

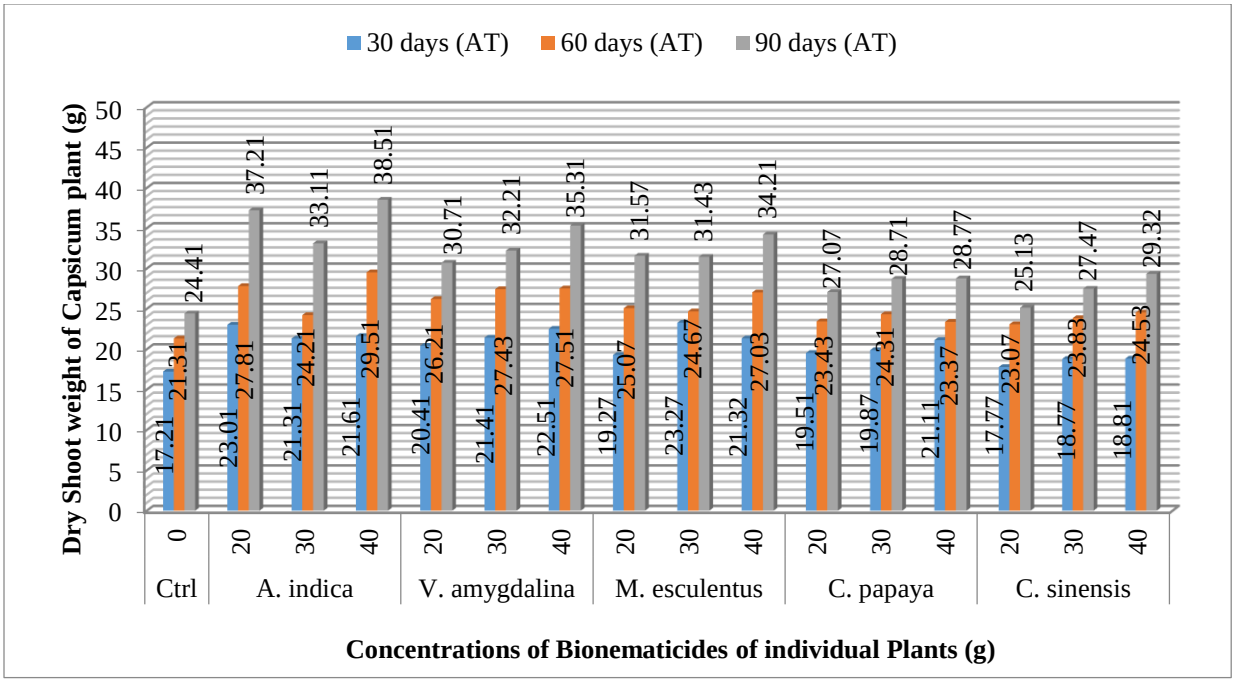

Figure 4: Second trial, determination of dry shoot weight of Treated Capsicum plant

This agrees with Gommmers *et al*. [29], Yasim *et al*. [30] and Kumar and Khanna [31], who stated that the application of plants materials as organic amendment will go a long way to reduce the nematode load, if the plant materials are bio-nematicidal and also improve healthy plant growth, generally influencing apical competence of plants.

Compared to the DSWs of the control, the application of bionematicides in all the treatment sets considerably improved the DSWs in *Capsicum* with corresponding increase in concentration and exposures. Notwithstanding, as observed from the results of both trials (Figures 3 and 4); *A. indica* recorded the highest dry weight, followed by *V. amygdalina* and *M. esculentus*, then *C. papaya* and *C. sinensis*. This showed that the bio-nematicides applied played an important role in reducing the rate of infection in *Capsicum*, leading to a better growth and an increase in DSWs. This agrees with the assertion made by Caveness and Ogunforowa [32] that root-knot nematode-infested plants were seriously affected by their reduced uptake and transportation of water and nutrients and this in turn could affect their shoot weight. The larger DSWs were recorded in the treated plants in relation to the control a scenario that may be attributed to the inhibitory effect of the of the extracts on nematodes' ability to secure feeding sites and inability to induce pathogenicity [11].

The results of the study showed that shoot weight increased when soils are treated with plant extracts concentrations. The increased shoot weight in the extract-treated plants was due to the ability of the roots to absorb more nutrients as compared to the control that manifested prominent symptoms of nematodiasis in form of heavy galls. Heavily infested roots according to Hussey [33] reduce the uptake and transportation of nutrients leading to lower shoot weights because of high number of galls on the roots. The widespread evidence to support the effectiveness of bionematicides in bringing the nematode population below the threshold level cannot be overemphasized [34; 35]. This will provide an alternative, sustainable and inexpensive means of managing plant parasitic nematodes.

Furthermore, the bioactivity of these materials against nematodes is attributed to the presence of an array of complex compounds agreeing with Alam, [36] and Kraus, [37]. The above ground and below ground symptoms (Figures 1 - 4) were more prominent in the control where no treatments were applied thereby allowing for the endophytic proliferation of plant parasitic nematodes. This agrees with Caveness and Ogunforowa [32] who stated that in heavily infested soils, the root system of plants are usually reduced and feeder roots do not appear.

Generally, the nematicidal activities of the extracts were concentration dependent. This relationship made it possible for plants with higher concentration of extracts manifesting better responses to growth parameters such as height, weight and girth. It was also deduced that the enhance overall growth due its ability to supply nutrients to the plants making act as organic amendments. This also agrees with Khan, [38] who observed that leaves of *A.indica*, have strong nematicidal properties and their addition to soil adversely affected the development of *M. incognita* and also improved plant growth in plants. In general, the height of *Capsicum* increased with the increase in concentration of the nematicides agreeing with Kuman and Khanna [31]. Plant extracts act by producing compounds that stimulate production of oxygen radicals which block the metabolic pathways of the nematodes [29]. The applied treatment serves as organic residue with strong impact on the physical and biological properties of soils,

which promotes favourable condition, allowing for better growth (Yasmin *et al*., 2003). In a recent study we also observed significant ($p<0.05$), nematotoxicity of the assayed plants in 10g of soil and 5g of root; with recorded order of activities as; *A. indica >V. amygdalina > C. sininsis > C. papaya > M. esculentus* [39].

4 Conclusion

This research established that all the applied treatments/bionematicides exhibited significant improvement in the monitored growth parameters. As a result of their nematotoxicity as well as other compounding factors. Capsicum plants adjusted with the treatment grew taller and bigger with better dry shoot weight (DSW), as concentration the organic extracts increased. Although, there were varying degrees of activities amongst the various treatments. Notwithstanding, we therefore suffice to say that the assayed bionematicides can be regarded as potential nematicides. Since synthetic nematicides are relatively expensive and toxic to non-targeted species. It has become necessary to apply plant derivatives in nematicides, since they are bio-available and eco-friendly.

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