NEMATICIDAL EFFECTS OF DIFFERENT PARTS OF BITTER LEAF *(Vernonia amygdylina)* AQUEOUS EXTRACTS ON ROOT KNOT NEMATODE INFECTIONS ON SOYBEAN *(Glycine max).*

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Abstract
Pot experiment was used to evaluate the nematicidal effects of different parts of bitter leaf *(Vernonia amygdylina)* aqueous extracts on root knot nematode infections on soybean *(Glycine max)* in Federal College of Agriculture Ishiagu Ebonyi State, Nigeria. Edible soybean seeds were planted in pots containing sterilized soil. The plants were inoculated with 3000eggs of *Meloidogyne incognita* four weeks after emergence. The inoculated pots were treated with 30mls of aqueous extracts of leaf, stem and root of bitter leaf at 1kg/l concentration 24 hours after inoculation. Water was applied as control. The experiment was arranged in Completely Randomized Design with four treatments replicated five times. Data were collected on plant height (cm) and number of functional leaves at 10 weeks after planting, number of pods/plant, and weight(g) of 100 seeds at harvest, Number of galled roots and number of juvenile nematodes per 100g soil at harvest. The collected data were subjected to analysis of variance (ANOVA). Significant treatment means were separated using Least Significant Difference (LSD) at 5% level of probability. Results showed that the plant height, number of pods and 100 seeds weight were significantly ($P<0.05$) enhanced in the pots treated with the aqueous extracts of the different parts of bitter leaf. The number of galled roots and number of juvenile nematodes were also significantly ($P<0.05$) reduced by the extracts. In all, the leaf extract proved better than the other extracts and could be employed in the field.
Key words: Nematicidal; *Meloidogyne incognita*; aqueous extract; evaluate; different parts; concentration.

Introduction
Soybean *(Glycine max)* belongs to the leguminous family and a sub family Fabaceae. It is a tropical and subtropical plant, a native to South-Eastern Asia. Soybean *(Glycine max)* is one of the most important vegetable crops grown throughout the world.
The crop can be successfully grown in many states in Nigeria using low agricultural inputs. Soybean cultivation in Nigeria has expanded as a result of its nutritive and economic importance and diverse domestic usage (Kumar, and Kamara, 2009).
Nematodes are among the major pests of soybean globally especially in the tropical and sub-tropical regions. The production of soybean in Nigeria is limited as a result of nematodes infestation, and this results to the low yield and acute shortage of its yield in certain periods of the year (Adegbite, 2005).
In Nigeria, nematode problem is commonly controlled with synthetic nematicides. These nematicides are very costly, scarce, and have adverse residual effects on the environment. This underscores the need for alternative control measures which are affordable, readily available, economical and environmentally friendly.
The objective of the study is to evaluate the nematicidal influence of different parts of bitter leaf *(Vernonia amygdylina)* aqueous extract on root knot nematode infections on soybean *(Glycine max).*

Materials and methods
The experiment was carried out at the Research and Teaching Farm of Federal College of Agriculture Ishiagu, Ebonyi State, Nigeria, located on latitude 5^{o}N and longitude 7^{o}E with average annual rainfall

of about 1,665mm. Seeds of edible soybean obtained from Federal College of Agriculture Ishiagu seeds store were used.

Preparation of inoculum

Already identified and maintained infested roots of Indian spinach were collected from the inoculum buckets and thoroughly washed under a running tap water. The roots were cut into pieces, put into 100ml measuring cylinder, mixed with 0.5% sodium hypo-chlorite solution, poured into the measuring cylinder, covered tightly and agitated vigorously for 5 minutes to free the eggs mass from the gelatinous matrix. The liquid was poured and back washed through a 200-mesh sieve nested upon a 500-mesh sieve. Eggs suspended in the agitated solution passed through the 200-mesh which removed the root debris and collected on the 500-mesh sieve (Hussey and Barker, 1973). The eggs were washed off the NaOCl solution using a wash bottle with distilled sterile water. The concentration of the root knot nematode eggs suspension was diluted with water to 500ml to facilitate counting.

Root knot nematode eggs population estimation

Egg suspension was stirred and with the aid of a graduated syringe, 1ml was introduced into a counting dish and placed under a high stereo microscope. The eggs were counted using a tally counter. The counting was repeated three times and a mean number of eggs per 1ml was estimated at 300 eggs/ ml.

Soil Sterilization and Potting

Top soil of 30cm depth collected from the experimental site was sterilized at 50^{o}C for 3 hours to ensure that all micro organisms were all eliminated.

Black polythene bags of 30cm x 30cm x 30cm were filled with 5kg of sterilized soil for the planting of the soybean. Each pot was sown with two seeds and later thinned down to one, seven days after emergence. The pots were laid out on a platform in the farm.

The design for the micro plot experiment was Completely Randomized Design (CRD) with four (4) treatments replicated five (5) times.

Inoculation

The soil around each soybean stand was slightly opened in a ring form of about 2cm deep and 3cm from the base of the plant and a graduated syringe was used to collect 10mls of the inoculum containing estimated 3,000 eggs and inoculated to each plant stand.

The pots were treated with 30mls of the various extracts as soil drench around the roots of the plants 24 hours after inoculation with water as the control.

Preparation of the extracts

Fresh leaves, stems, and roots of bitter-leaf *(Vernonia amygdylina)* were thoroughly washed and rinsed with distilled water, chopped into tiny pieces with sharp knife and weighed with digital weighing scale. One kilogram of each part was measured out and soaked in one liter of water for twenty-four hours. Each mixture was filtered using a clean white cheesecloth. These mixtures served as 1kg/l of leaf, stem and root aqueous extracts respectively. They were used immediately after extraction as treatments.

Estimation of juveniles of *Meloidogyne* population in the soil

Soil samples from each treatment were collected from each pot after harvest homogenized to form a composite sample of each treatment and taken to the laboratory. 100g of each soil sample was wrapped with a tissue paper, placed on a 200 mesh sieve and placed over a 2000mls white container containing distilled water. The water in the container had contact with mesh containing the wrapped soil and allowed for forty-eight (48) hours for nematodes to crawl out of the soil into the water. The water in the container was reduced to 100mls after removing the wrapped soil. With the aid of a pipette, 5mls of the mixture were

introduced into a counting dish and the number of juvenile nematodes was estimated under the microscope.

Statistical analysis

Data were collected on plant height (cm) and number of functional leaves at 10 weeks after planting, number of pods/plant, and weight (g) of 100 seeds at harvest, number galled roots and number of juvenile nematodes per 100g soil at harvest.

The data collected were subjected to analysis of variance (ANOVA). Significant treatment means were separated using Least Significant Difference (LSD) at 5% level of probability using F-LSD = LSD as outlined by Obi (2002).

Results

The extracts significantly ($P<0.05$) affected the plant heights at 10 weeks after planting (WAP). Higher plant heights were obtained from the plants treated with the various extracts than the control. The highest plant height of 30.51cm was obtained from the plants treated with the leaf extract with the control producing plants with lowest plant height of 23.68cm (Table 1). There was no significant ($P>0.05$) effect of the extracts on the number of leaves produced by the plants at 10WAP(Table 1). Though statistically similar, it was observed that the highest number of leaves of 52.80 was produced by the plants treated with the leaf extract.

The results in Table 2 indicated that the extracts significantly ($P<0.05$) increased the number of pods produced by the treated plants at harvest. The plants treated with the leaf extracts produced the highest number of pods (13.20) with the control plants producing the lowest number of pods (7.45). The extracts equally affected significantly ($P<0.05$) the weight of 100 seeds at harvest. The highest seed weight of 6.05g was obtained in the plants treated with the leaf extracts with the lowest seed weight (0.95g) produced by the control plants.

The number of galled roots was significantly ($P<0.05$) affected by the extracts at harvest. The highest number of galled roots (20.90) was obtained from the control plants while the lowest number of galled roots (5.00) was obtained from the plants treated with leaf extract (Table 3).

The results also in Table 3 indicated that the number of juvenile nematodes in the soil was significantly ($P<0.05$) affected by the various extracts. The highest number of juvenile nematodes (9.75) was obtained from the control soil while the lowest number of juvenile nematodes (1.75) was obtained from the soil treated with the leaf extract.

Discussion

Nematode attack on susceptible plants damages the vascular tissues of the plants resulting in the reduced physiological processes and functions of the affected plants. The growth and yield parameters of the plants vary inversely with the population and virulence of the phtyo-nematode especially the root knot nematodes. This is symptomized by the formation of galls in the roots of the affected plants. Ploeg (2001) reported that the galls formed as a result of the attack by the nematode block water and nutrient flow to the plant, stunting growth, impairing fruit production and causing foliage to yellow and wilt.

The reduction in the number of galled roots and juvenile nematodes in the treated plants and soil respectively proved the nematicidal properties of the aqueous extracts of bitter leaf. Other researchers have observed nematicidal effects of bitter leaf (Ogwulumba *et al*., 2009,Oyedunmade and Olabiyi, 2006). The nematicidal qualities of the extract were attributed to the presence of alkaloids and saponins which are anti-microbial.

The nematicidal properties of the extracts were observed to affect the fecundity of the nematode negatively and the mortality positively. These resulted in the reduced population and number of galls produced by the treated plants. The nematotoxicity of the extracts resulted in the enhancement of the growth and yield parameters in the

treated plants than the untreated. This agrees with Fasahat (1990) who reported that due to reduction in nematode population due to nematicidal effect of bitter leaf extract reduced damage to plants resulting in better growth and boosted fruit production.

It was observed that the leaf extract was the most efficacious amongst the three parts. This could be due to the presence of more anti-microbial nutrients at the leaf and/or the ease with which the leaves dissolve in the water given the time for the extraction. The growth and yield parameters were more enhanced in the plants treated with the leaf extracts. The nematicidal effects exerted by the leaf extract provided more conducive environment for the plants to thrive. This was also attributed to the presence of more plant nutrients in the leaf apart from alkaloids and saponins which are anti-microbial. This is in line with the findings of Oboh (2006) that Na, K, Ca, Mg, Zn and Fe are in high amount in the leaf of bitter leaf.

In conclusion, the extracts of different parts of bitter leaf showed that they contain nematicidal properties as well as plant nutrients. The leaf extract proved more efficacious in all these qualities and the leaf could also be used to make meals after extraction. Both the leaf and its extracts could be used in human nutrition and plant protection respectively.

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