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Effect of *Allium sativum* (garlic) extract on the growth and nodulation of cowpea *(Vigna unguiculata)* and groundnut *(Arachis hypogea)*

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It has been demonstrated that symbiotic N_2 fixation enhances soil fertility and productivity as well as increases carbon sequestration and nutrient conservation. Green house experiments were conducted to ascertain the effect of garlic extract on the nodulation of cowpea and groundnut plants. Different concentrations of the extract (20, 40, 60 and 80%), were applied to the plant at 2 weeks after planting. Both legumes were adversely affected by the garlic extract, but the effect was more pronounced on the groundnut plants. Before application of the garlic extract to the soil, the soil pH was 6.5, phosphorus 8.3 mol/kg, calcium 2.51 mol/kg, potassium 0.45 mol/kg, and nitrogen 0.295 mol/kg, and these make the soil fertile enough for nodulation. However, after garlic extract application, there was reduced nodulation in the roots and a marked drop in plant height, leaf area and root development in comparison to the control. This adverse effect was more with increasing concentration of the extract. It is recommended, that land for cowpea or groundnut product remains free of garlic and members of the families Allium and Cyperus plants. They should not be left in the soil or turned over in it, during land clearing and should be grown in inter-crop. This is because their exudates during decomposition or growth could have inhibitory effects on the growth and nodulation of legumes.

Keywords: Garlic extract, nodulation, legumes, nitrogen fixation.

INTRODUCTION

Legumes (Fabaceae) constitute the third largest family of flowering plants comprising more than 650 genera and 18000 species (Dadi and Bekele, 2006). Economically, legumes represent the second most important family of crop plants after Poaceae (grass family), accounting for approximately 27% of the world's crop production (Dadi and Bekele, 2006). The Leguminosae family has enormous diversity as it include every morphophylogical types of plants- viz. herbs, shrubs, small and big tree, climbers, creepers, lianes, annual, perennial etc. One of the most important attributes of legumes is their unique capacity for symbiotic nitrogen fixation, underlying their importance as a source of nitrogen in both natural and agricultural ecosystems.

Accordingly the family is subdivided into three subfamilies, Mimosoideae, Caesalpinioideae, and

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Papilionoideae. Papilionoideae subfamily contains nearly all economically important crop legumes, including soybean (*Glycine max*), peanut (*Arachis hypogaea*), mungbean (*Vigna radiata*), chickpea (*Cicer arietinum* L.*)*, lentil (*Lens culinaris)*, common bean (*Phaseolus vulgaris*), pea (*Pisum sativum*), and alfalfa (*Medicago sativa*). Humans have used legumes in agriculture for thousands of years either as a food crop for humans and livestock, or as a rotational crop to supply N to non-legume crops. The shift from biological to industrial sources of N fertilizer in agricultural systems did not occur until the 20th century (Smil, 2001; Crews and Peoples, 2004). Symbiotic N_2 fixation enhances soil fertility and productivity as well as increases carbon sequestration and nutrient conservation (Cromwell and Winpenny, 1993).

A biochemical dialogue is initiated between the host plant and the rhizobia, causing nodulation to occur (Lum and Hirsch, 2003). A variety of molecular and genetic mechanisms have evolved between the legume and the rhizobia to regulate the nodulation and N2 fixation processes (Lum and Hirsch, 2003). Nod genes direct the various stages of nodulation; the initial interaction between the host plant and free-living rhizobia is the release of a variety of chemicals by the root cells into the soil. Some of these encourage the growth of the bacterial population in the area around the roots (the rhizosphere).

Reactions between certain compounds in the bacterial cell wall and the root surface are responsible for the rhizobia recognizing their correct host plant and attaching to the root hairs. Flavonoids secreted by the root cells activate the nod genes in the bacteria which then induce nodule formation. The whole nodulation process is regulated by highly complex chemical communications between the plant and the bacteria, once bound to the root hair the bacteria excrete nod factors (Madigan et al., 2000). These stimulate the hair to curl. Rhizobia then invade the root through the hair tip where they induce the formation of an infection thread; this thread is constructed by the root cells and not the bacteria and is formed only in response to infection.

Apart from the obvious potential for increased agricultural productivity, a more comprehensive understanding of nodulation will enhance our knowledge of plant development and plant–microorganism interactions. For example, the development of root nodules has parallels with lateral root formation (Krusell et al., 2002). Therefore, nodulation research will help provide insight on this general developmental process in plants. In addition, some of the steps in nodule formation are common to, and likely derived from the pathway leading to symbiosis between mycorrhiza and their respective plant hosts (Endre et al., 2002) similarly, *Meloidogyne incognita*, is proposed to infect susceptible plants via a process that shares similarities with *Rhizobium* infection (Weerasinghe et al., 2005).

Allelopathy is the suppression of growth of one plant species by another due to the release of toxic substances (Portales-Reyes et al., 2015; Cipollini et al., 2012). One of the studied aspects of allelopathy is the role of allelopathy in agriculture. Recent researches have focused on the effect of weeds on crop, crops on weeds and crops on crops. This research furthers the possibility of using allelochemicals as growth regulators and natural herbicides, to promote sustainable agriculture. For example the effect of yellow nutsedge (*Cyperus esculentus*) on cowpea has also been studied. When yellow nutsedge wad intercropped with cowpea, the yellow nutsedge root exudation inhibited the cowpea growth (Kadioglu and Yanar, 2005). Cocks comb (*Celosia argentea* L.) is one of the most famous allelopathic plants. It has effect on the seed germination, seeding growth and flowering of legumes (Kadioglu and Yanar, 2005).

Garlic (*Allium sativum L)*. Is a member of the Alliaceae family, has been widely recognized as a valuable spice and a popular remedy for various ailments and physiological disorders. The name garlic may have originated from the Celtic word 'all' meaning pungent. As one of the earliest cultivated plants, garlic is mentioned in the Bible and in the literature of Ancient Israel (The Talmud), Egypt (Codex Ebers) and India (Vedas and Purans, Charak Sanghita). As and Purans, garlic is reported to have medicinal as well as nutritive value in food items. It is a remarkable plant, which has multiple beneficial effects such as antimicrobial, antithrombotic, hypolipidemic, antiarthritic, hypoglycemic and antitumor activity. Allicin has antibacterial and antioxidant activity (Karin et al., 2008). This study therefore seeks to find out the effect of having garlic exudates around the roots of some legumes namely, cowpea and groundnut.

Intercropping of garlic with other crops could be advantageous in overcoming continuous cropping problems but could also harmful due to the allochemicals released into the environment which are considered to alter a variety of physiological and biochemical processes. There are increasing evidences that the allelochemicals have significant effect on cell division, ion and water uptake, phytohormone metabolism, respiration, photosynthesis, enzyme function, as well as gene expression (Sadaqa et al., 2016; Portales-Reyes, 2015; Ian et al., 2014; Hale and Kalisz, 2012; Singh and Thapar 2003). This study was conducted to ascertain the effect of garlic extracts on the growth and nodulation of the legumes - cowpea and groundnut.

MATERIALS AND METHODS

Plant materials

Green house experiment were conducted at the Department of Applied and Environmental Biology, Rivers State University of Science of Technology, Port Harcourt, Nigeria to determine the effect of garlic extracts on the nodulation of legumes (cowpea and groundnut). The groundnut and cowpea seeds were also collected from the mile 3 market in Port Harcourt Rivers State. Fresh garlic

Table 1. Plant height of groundnut plants following treatment with varying concentrations of garlic extract after 7 to 31 days.

Treatment applied	Plant height (cm)						
	DAP 7	DAP 9	DAP 11	DAP 13	DAP 27	DAP 29	DAP 31
Control	5	10	15	20	40	60	80
20% conc Trmt 1	5	12	17	22	46	50	60
40% conc Trmt 2	5	10	15	22	30	35	35
60% conc Trmt 3	4	8	13	18	26	30	35
80% conc Trmt 4	7	10	15	20	25	25	30

Table 2. Leaf area of cowpea plants following treatment with varying concentrations of garlic extract after 7 -31 days.

Treatment applied	Leaf area (cm^2)						
	DAP 7	DAP 9	DAP 11	DAP 13	DAP 27	DAP 29	DAP 31
Control	30	30	30	30	90	90	90
20% conc Trmt 1	30	36	36	42	60	66	72
40% conc Trmt 2	30	30	36	36	48	54	60
60% conc Trmt 3	30	30	36	48	48	60	60
80% conc Trmt 4	30	30	48	54	60	60	60

bulbs (2 kg) purchased from mile 3 market in Port Harcourt were thoroughly washed to remove soil and other debris. About (1 kg) of Garlic bulbs were sliced into small pieces and put in a clean blender with 500 ml of water and blended into a fine paste. About (1 L) of crude liquid extract was obtained by filtering the garlic paste through a piece of sterile muslin cloth. The extract was collected into a sterile bottle and kept in a refrigerator (at 4°) until used. The same thing was done with the second set of (1 kg) garlic bulbs.

Greenhouse experiment

About 3 kg capacity polyethylene bags were filled with 2 kg of air dried loamy soil. The soil used for the planting of the crop and for the analysis was collected from the university school research farm. Three replications were made for each treatment, for both cowpea and groundnut, and the control. So a total of 30 bags were half filled with soil, 15 bags for cowpea and 15 bags for groundnut. Cowpea and groundnut seeds were sown 2 cm deep in each bag. The pots were watered after every 2 days with 100 ml distilled water till after two weeks of germination and were kept weed-free by hand weeding. The garlic extract was retrieved from the refrigerator, allowed to warm up to room temperature and administered to the plants. About 100 ml of the extract solution of was added at the dilution of 20, 40, 60 and 80% treatment for each treatment the three replica respectively while distilled water was used on the control. Germination counts were made and measurements were taken of plant height and leaf area before and after treatment application. All the chemicals used in the experiment were of the analytical grade and were obtained from the department and from the soil science department in the faculty of agriculture.

RESULTS

Effect of garlic extract on cowpea nodulation and growth

After the application of the treatment (garlic extract) it was observed that the cowpea plant with the 20, 40, 60 and 80% concentration showed adverse response to all treatments after 2 days of application by wilting but recovered after 5 days (Tables 1 and 2). The data of higher the concentration of garlic extracts having an effect on the growth and leaf area of the plant is represented graphically in Figures 1 and 2. The plants with the highest concentration of garlic (80%) had the slowest growth rate followed by that of the 60 and 40% concentration. Pictures of reduced growth and nodulation are shown in Figures 3 and 4.

Effect of garlic extract on groundnut nodulation and growth

Similar to cowpea, groundnut plants treated with 20, 40, 60 and 80% garlic extracts showed wilting three days after application but recovered seven days after (Tables 3 and 4; Figures 5 and 6). The plants with the highest concentration (80%) all died, the plant with the 60 and 40% concentration recovered after seven days but showed very slow growth while the plants with the lowest concentration (20%) fully recovered but did not compare favorably with the control plants.

Tables 3 and 4, and Figures 7 to 11, showed that plants with the lowest concentration (20%) grew well but not as the control; while the plants with the highest concentration (80%) of the treatment were most affected that they all died.

A graph showing the relationship between the plant height and the date of planting before and after treatment application. This shows that the higher the treatment concentration the slower the growth of the plant.

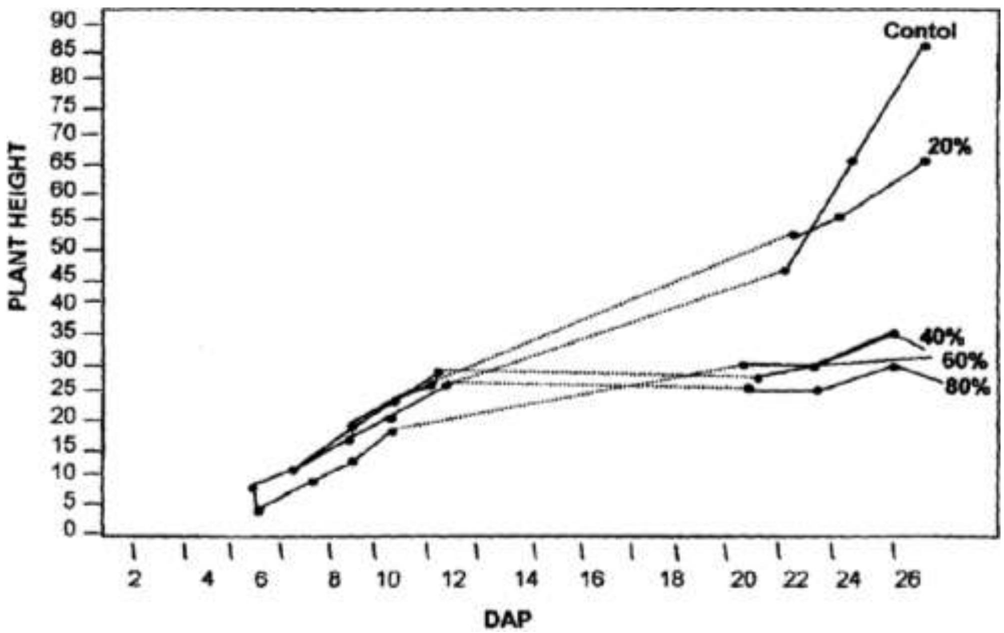

Figure 1. Relationship between plant height of cowpea days after planting (DAP) following treatment with different levels of garlic extract.

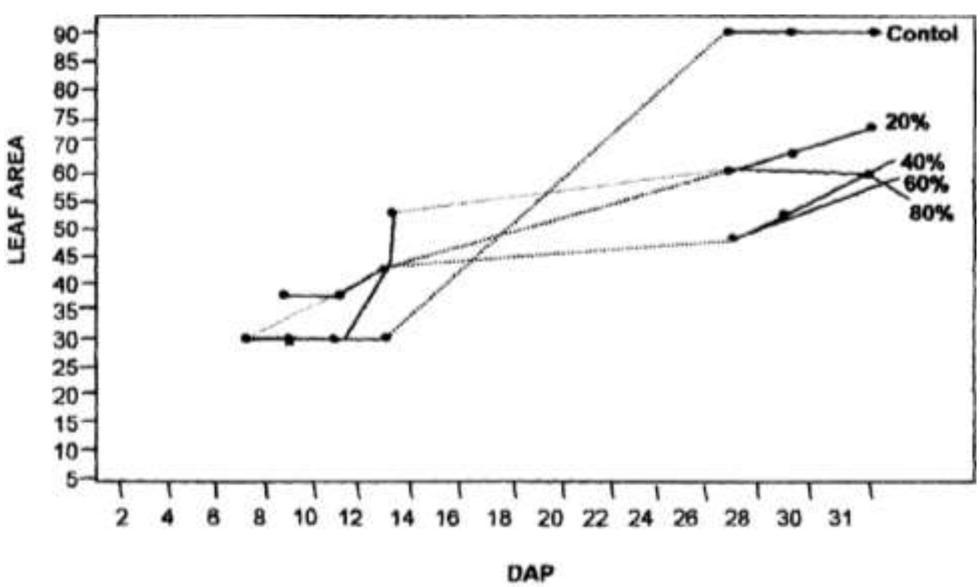

Figure 2. Relationship between leaf area of cowpea days after planting (DAP) following treatment with different levels of garlic extract.

DISCUSSION

The results of this research show that the plants treated with the lower concentration of garlic extract compared favorably with the ones in the control, while those given higher concentrations decreased in leaf area and plant height. The data shows that the effect of the garlic extract on cowpea and groundnut nodulation and growth is significant, with the effect being more pronounced on groundnut compared to cowpea. The degree of inhibition increased as the concentration of garlic extract increased for both groundnut and cowpea. A similar observation was made by Laosinwattana et al. (2009) in their study of the effect of garlic on the growth of pepper.

Figure 3. Cowpea plants before treatment with garlic extract.

Figure 4. Cowpea plants after treatment with garlic extracts at 20, 40, 60 and 80% concentration.

Table 3. Plant height of groundnut plants following treatment with varying concentrations of garlic extract after 7 to 31 days after planting (DAP).

Treatment applied	Plant height (cm)						
	DAP 7	**DAP 9**	**DAP 11**	**DAP 13**	**DAP 27**	**DAP 29**	**DAP 31**
Control	10	12	17	22	30	35	38
20% conc Trmt 1	5	10	15	20	24	26	28
40% conc Trmt 2	7	10	15	20	21	22	24
60% conc Trmt 3	6	8	13	22	22	X	23
80% conc Trmt 4	4	8	15	20	X	X	X

Table 4. Plant area of groundnut plants following treatment with varying concentrations of garlic extract after 7 -31 days after planting (DAP).

Treatment applied	Plant area (cm^2)						
	DAP 7	DAP 9	DAP 11	DAP 13	DAP 27	DAP 29	DAP 31
Control	29	29	31	32	40	60	65
20% conc Trmt 1	20	28	28	28	32	36	40
40% conc Trmt 2	24	24	28	30	27	30	30
60% conc Trmt 3	21	28	28	30	28	X	X
80% conc Trmt 4	28	28	28	30	X	X	X

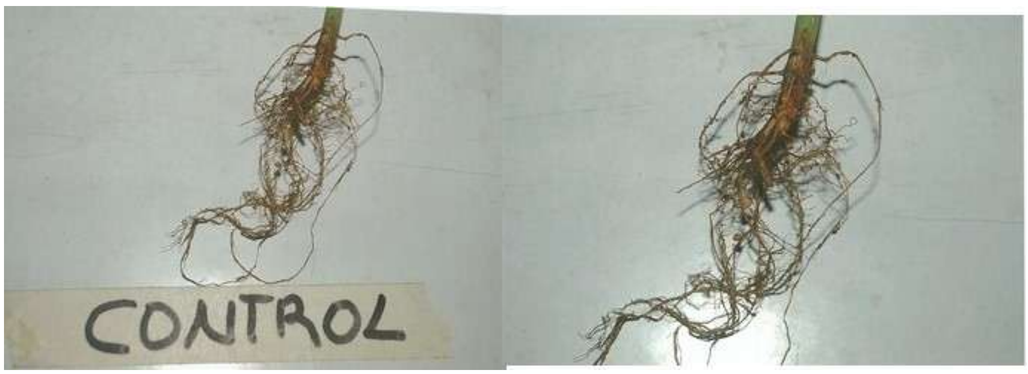

Figure 5. Roots of cowpea plants without treatment with garlic extract showing nodulation.

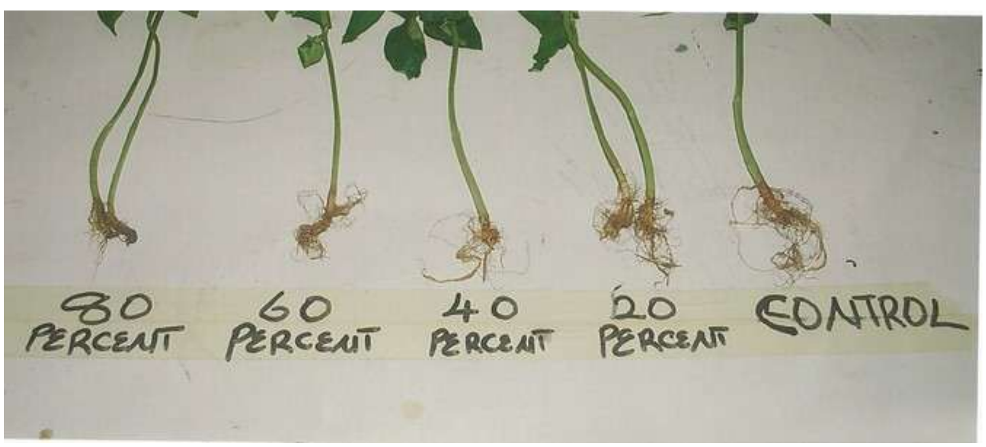

Figure 6. Roots of cowpea plants after treatment with 20, 40, 60 and 80% of garlic extract.

In addition, allelopathic effects of weed extract on crops have been observed earlier, for example the effect of yellow nutsedge (*Cyperus esculentus*) on cowpea has also been studied (Kadioglu and Yanar, 2005). When intercropped with cowpea, the yellow nutsedge root exudate inhibited the growth of the cowpea plants (Kadioglu and Yanar, 2005). Interaction effect indicated that higher concentrations produced lower vigour index. The increased inhibitory effect at higher concentration of weed extract may be due to increase in the concentration of allelo chemicals like phenolic acids namely, P-hydroxy benzoic acid, p-coumaric acid, caffeic acid, o-coumaric acid and ferulic acid (Portales-Reyes, 2015). Similar results were noticed by Singh and Thapar (2003).

It was also observed that all concentrations of garlic extracts in this study markedly did not promote root development of both cowpea and groundnut plants which suggests that root development is sensitive to allelopathic

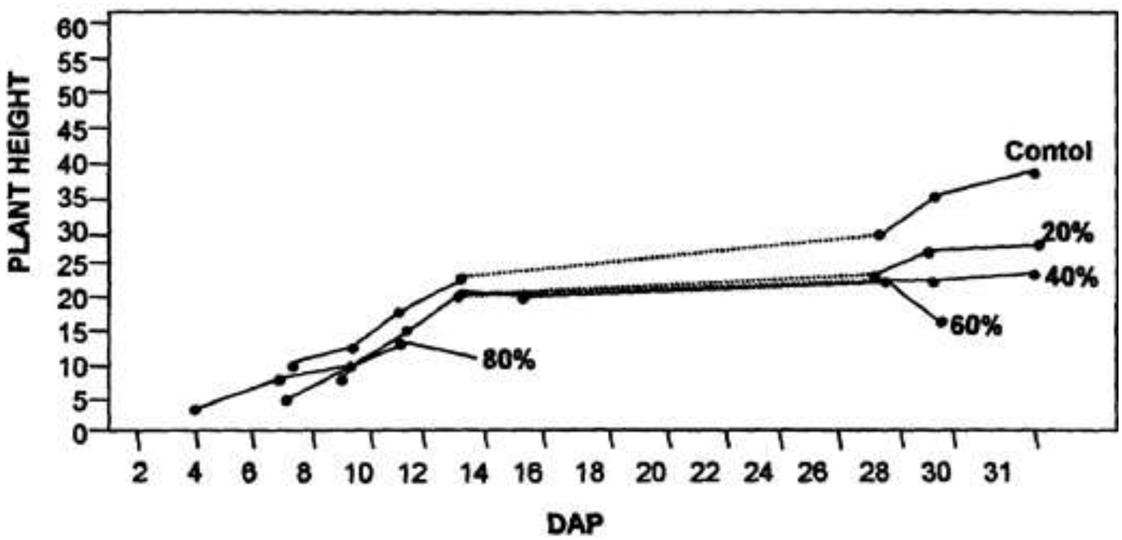

Figure 7. Relationship between plant height of groundnuts days after planting (DAP) following treatment with different levels of garlic extract.

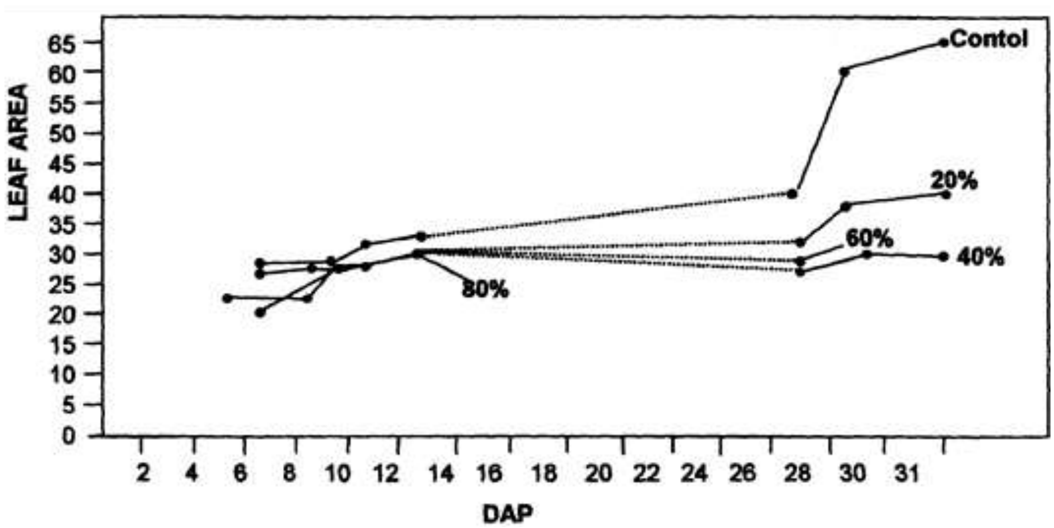

Figure 8. Relationship between leaf area of groundnuts days after planting (DAP) following treatment with different levels of garlic extract.

inhibition of extracts (Plate 4 and 7). This observation agrees with some of the earlier studies which indicate that water extracts of allelopathic plants causes more pronounced effects on root development (Batish et al., 2006). This could be due to the fact that roots are first to come in contact with the allelochemicals in the environment (Turk and Tawaha, 2002).

Root nodulation was observed only in the control. None of the treatment concentrations showed root nodulation and this may have been due to the fact that root development is adversely affected by garlic extract treatment. Moreover, the extent of nodulation and amount of nitrogen fixed by legumes is largely related to the plant size and root mass (Tlusty et al., 2004). The chemical constituent of garlic extract shows that it has antimicrobial properties (Allicin), and this could have further contributed to the hindering root nodulation in the legumes (Karin et al., 2008) since nodulation is a symbiotic relationship between Rhizobium and root. Legumes will not grow well or fix nitrogen in acidic soil with pH above 6.5. These findings provide evidence that the garlic extract contains some allelochemicals and causes allelopathy through releasing the allelochemicals to environment. So, it is certainly inferred that garlic extract may have allelopathic

Figure 9. Groundnut plants before treatment with garlic extract.

Figure 10. Groundnut plants after treatment with garlic extracts at 20, 40, 60 and 80% concentration.

effect on a great majority of legume plants from this present study.

The result of the experiment reveals that the garlic extract has a negative effect on both cowpea and groundnut plants at all concentrations; the effect increasing with concentration. The effect was however more pronounced in groundnut than in cowpea. Leaf area, plant height and root development were all inhibited, hence, there was no nodulation observed. It is suggested therefore, while weeding or clearing a piece of land for cowpea or groundnut product remains of garlic and members of the families Allium and Cyperus plants

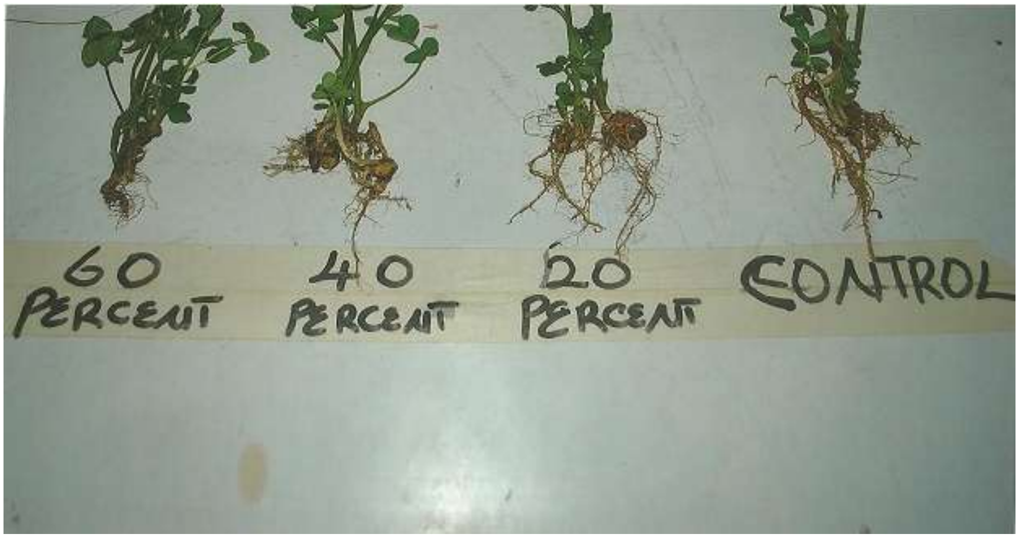

Figure 11. Roots of groundnut plants after treatment with 20, 40, 60 and 80% of garlic extract.

should not be left in the soil or turned over in it. This is because their exudates during decomposition could have inhibitory effects on the growth and nodulation of legumes. Alternatively, the land should be tilled to remove its rhizomes and root remains, and left for a while for proper decomposition of any remnants and for sufficient rains to wash away the exudates before sowing on it. Completely avoiding land that has been previously used for members of these families, for legume production would be best, where possible.

Conflict of Interests

The authors have not declared any conflict of interests.

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