

STUDIES ON THE MANAGEMENT OF PHYTOPHTHORA BLIGHT OF PIGEON PEA (*Cajanus cajan* L. Mill. sp.) THROUGH THE USE OF NEEM (*Azadirachta indica*) PLANT PARTS AQUEOUS EXTRACTS IN OWERRI, IMO STATE, NIGERIA.

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Management of Phytophthora blight of Pigeon pea (*Cajanus cajan*) using aqueous extracts of Neem (*Azadirachta indica*) plant seeds in Owerri, Imo State, Nigeria

ABSTRACT

This study is focused on the management of phytophthora blight, a fungal disease of "pigeon pea" (*Cajanus cajan* L. Mill. sp.) by the application of some aqueous extracts of "neem" plant parts. The study was conducted in the year 2021, at the Demonstration Farms (both field and laboratory) of the Department of Crop Science and Technology, School of Agriculture and Agricultural Technology, Federal University of Technology, Owerri, Imo State, Nigeria. The experiment was designed as Randomized Complete Block Design (RCBD) with five replications. Data on pigeon pea growth parameters and disease incidence and severity, were statistically analyzed by the use of Analysis of Variance (ANOVA) software as outlined by Wahua [1]. The means were separated for difference using the Fisher's Least Significant Difference (FLSD) [2]. Analysis of variance indicated that neem seed aqueous extract application significantly ($P < 0.05$) led to the production of the tallest Pigeon pea plant (103.30 cm), the highest number of pigeon pea leaves (99.40), the lowest phytophthora blight disease incidence (11.00%) and also the lowest phytophthora blight disease severity (1.00). On the basis of the outstanding performance of neem seed aqueous extract on the above parameters, it is, therefore, satisfactory to accord recommendation to the aqueous neem seed extract for the pigeon pea production and management and control of phytophthora blight of pigeon pea (*Cajanus cajan*) in the study area.

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Key words: studies, aqueous extracts, control, fungal diseases, neem, phytophthora blight, management, fungal diseases, neem, pigeon pea, aqueous extracts.

Introduction

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Pigeon pea, [*Cajanus cajan* (L.) Hutch Mill. sp.] is an important legume of the Fabaceae family grown for its edible pods and seeds. The crop is rated as the sixth most important grain legume crop the world over [3]. The major producers of pigeon pea are India, Myanmar, while in West Africa major producers are Malawi, Tanzania, Kenya and Uganda [3]. Pigeon pea plays an important role in small holder subsistence, though it is regarded a minor crop in Benin, Nigeria and Ghana [4, 5, 6, 7]. The importance of pigeon pea cannot be over emphasized. Saxen *et al.* [8] described the nutritious nature of mature seeds of pigeon pea. Several other authors have outlined the many advantages of pigeon pea as having the possibility of several harvests, enhancement of soil fertility, high tolerance of drought stresses, high biomass production, and production of the most nutrient and moisture contributions to the soil. [9, 10, 11, 12]. It has a high amount of protein, carbohydrate, B-group vitamins, and minerals. Besides its nutritional value, pigeon pea also possesses medicinal properties due to many polyphenols and flavonoids that are known to prevent and cure human ailments such as cough, soreness, bronchitis,

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pneumonia, respiratory infections, dysentery, menstrual disorders, wounds, abdominal tumors, and diabetes {13}. Apart from being a food source, it is also grown as green fodder, intercrop, cover crop, etc., in many farming systems. Pigeon pea crop suffers from dreaded fungal, bacterial, and viral diseases resulting in huge losses in yield {13}. The following are the devastating diseases of pigeon pea that constitute limiting factors in the production of the crop: Phytophthora stem blight, Pigeonpea ~~sSterility~~ ~~mMosaic~~ ~~vVirus~~, ~~sSterility mosaic disease-(SMD)~~ ('green plague'), fusarium wilt, root rot, powdery mildew, bacterial leaf spot, etc. {13}. The pathogen, *Phytophthora drechsleri* f. sp. *cajani*, inciting Phytophthora blight is considered the most devastating disease-causing economical losses in pigeon pea, especially when excessive rains fall within a short period and hot and humid weather persists during the crop season {13}. The disease has also been reported in other countries. Chava Vinay Kumar {14}, described neem crop, the large tree of India, as a medicinal plant used over the world as anti fungal-plant, insecticide, and nematocide, and for many other medicinal purposes. Chava Vinay Kumar {14}, also noted the effect of aqueous extracts of neem on soil – borne pathogens: *Fusarium oxysporum* f. sp. *cicieri*, *Rhizoctonia solani*, *Sclerotium rolfsii*; the growth of these pathogens was affected by the extracts of the leaf trunk, bark, fruit pulp and leaf neem. Many other authors have pointed out the control of crop pests and diseases, like toxicity to fungal pathogens and insecticides {15}. Extracts of Neem (*Azadirachta indica* A. Juss.) plant parts extracts are biodegradable and cheaply available to farmers {14}; this when compared to synthetic pesticides employed in the control of plant pathogenic fungi, viruses, bacteria and nematodes. Lesser emphasis on traditional methods of mitigating these pathogens has promoted the increasing but destructive use of synthetic chemicals in control and management of the fungi diseases including phytophthora blight. Consistent use of these chemicals have brought about formation of more resistant fungi-strains, large amount of residuals of these persistent chemicals in the crop, posing great threat to the crop plant, man and farm animals. Furthermore, the high cost of purchasing these chemicals (fungicides) calls for the adoption of a more economical approach to contain these diseases. The aqueous extract of Neem, as a natural product, cannot leave residues on the plants, and therefore, cannot pose any danger to humans and livestock. Azadirachtin (present in neem seeds), the main constituent of neem-based products, helps in the protection of crop plants from several pests and diseases{14}. In view of the many advantages and uses of the neem plant, its inclusion in the control and management of diseases of pigeon pea is-, therefore, highly justified, hence the this study.

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MATERIALS AND METHODS

Description of the experimental site:

The experiment was carried out at the Teaching and Research Farm of Department of Crop Science and Technology, School of Agriculture and Agricultural Technology, Federal University of Technology, Owerri, Nigeria. The experimental area is a typical tropical climate, characterized by distinct dry and wet seasons; the wet season starts from March to October and the dry season falls between November and March {16}. The soil of the area is characterized by deep, porous, red soil, highly weathered and low in mineral reserves {17}. The annual rainfall varies from 1,100 mm in the northern part of the state to 1,600 mm in the southern parts. The

average temperature is 26.4°C [18]. The geographical co-ordinates of the study area are 5.4891°N and 7.025853E [19].

Experimental design and treatment combinations

The treatments comprised three neem plant parts extracts: the ~~neem seeds extract (NSE)~~, neem leaves extract (NLE), neem stem bark extract (NSBE), ~~neem seeds extract (NSE)~~, and no neem extract (NNE) as the control. The treatments were laid out in a Randomized Complete Block Design with five replications. The size of the experimental area was 132 m² (0.0132 ha²). The area was mapped out into five blocks, while each block contained four plots. The space between plots and block was 1 m. Preparation of the field was done through the use of a spade.

The seeds were sown at the spacing of 0.60 x 0.60 m, giving a total plant population of 240 stands of pigeon pea plants.

Measurement of disease parameters

1. Disease Incidence (%): this is the number of plant units that are visibly diseased, usually relative to the total number assessed [20, 21]. Cardoso *et al.* [22] further defined disease incidence mathematically, thus, $I = \sum x/N$, where x= number of diseased plants and N = total number evaluated. Thus,

$$\text{Disease Incidence \%} = \frac{\text{Number of affected plants per plot}}{\text{Total number of plants per plot}} \times \frac{100}{1}$$

2. Disease Severity: This is the proportion of the plant infected by a disease in a sample or plot. For *S*septoria leaf spot, it was estimated using the observation and scoring format described by Ford and Hewitt [23]

Table 1. Observation and scoring format for disease severity.

Severity (%)	Scale	Interpretation
1 – 20	1	slight infection
21 – 40	2	moderate infection
41 – 60	3	severe infection
61 - 80	4	very severe infection
81 – 100	5	leaves completely infected

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$$\text{Disease Severity (DS)} = \frac{\text{Number of infected leaves}}{\text{Total number of leaves assessed}} \times 100$$

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Another manner to write this:
(number of infected leaves/total number of leaves assessed) x 100

Preparation of planting materials and application of treatments

The seeds of late maturing variety of *Cajanus cajan* were sourced from Terminus Local Market, Jos-, Plateau state, Nigeria. The treatments consisted of three aqueous extracts of different parts of *A. indica*: ~~neem seeds (NS)~~, neem leaves (NLE), neem stem bark (NSBE), ~~neem seeds (NSE)~~, no neem extract (NNE), as the control for experiment. A grinder, distilled water, 5-ml syringes, three (-250 ml) conical flasks labeled: NLE, NSBE and NSE, weighing devices were used for the measurement and application of the treatments {24}. The treatments (NLE, NSBE, NSE) were sourced from neem trees in the premises of Federal University of Technology, Owerri. Water extracts of neem plant parts (leaves, stem, and seeds) were obtained by washing them in running tap water respectively, then ~~later~~ with distilled water. Each was homogenized using a grinder (Corona, Landers ~~and~~ Cia. IA, S. A.). To prepare the extracts, 800_g samples of each ~~of the~~ treatments ~~were~~ weighed into a 250_ml conical flask and 400_ml distilled water into the three labeled 250_ml conical flasks to give 50% weight by volume (W/V) {24}. This quantity was doubled for the sufficient application on the plants. The infusions were allowed to stand for 24 hours after which they were filtered using a double layer cheese cloth into another three 250_ml conical flasks respectively. The aqueous neem plant parts extracts (treatments) were applied one week after emergence, by spreading them aerially with the aid of a calibrated syringe (5ml/plant).

RESULTS AND DISCUSSION

Table 2+ shows the effect of neem plant parts extracts on the height (cm) of pigeon pea. Analysis of variance showed absence of significant ($P > 0.05$) difference among treatment means in weeks 2, 4 and 6 of planting. On the other hand, significant ($P < 0.05$) difference occurred among the treatment means in week 8 of planting. This means that the different pigeon pea plant parts extracts showed variation on the plant heights. The result evidenced a positive relationship between the height of pigeon pea and the increase in the weeks of planting. The shortest pigeon pea plants were observed in week 1 after planting (15.03 cm, 15.51cm). On the other hand, the tallest plant was witnessed in the last week of planting (103.30 cm) and produced by neem seed extract. This was followed by ~~NSBE and NLE with~~ 86.00 cm and ~~76.00~~ cm respectively. This result corroborates a similar result of the work done by Kankam and Adomako {25}, where neem seed powder yielded the highest plant height. Neem seed extract yielded the highest plant height in week 4; ~~(28.89 cm), in week 6 (63.60 cm)-in week 6,~~ and the highest plant height in week 8 of planting (103.30 cm). The difference between the performance of ~~NLE and NSBE neem leaf extract and neem stem bark extract~~ was marginal, as ~~at week 8~~ the plant heights were 84.90 cm and 86.00 cm, ~~produced by neem leaf extract and neem stem bark extract~~ respectively.

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The results of the control treatment, where no neem extract was applied, showed no much difference between the rest of the treatments, except between 4th to 8th weeks with increasing difference with the neem seed extract.

Table 24: Effect of different neem plant parts extracts on the height of Pigeon pea (*Cajanus cajan*, Fabaceae). ~~(103.30 em).~~

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<u>Extracts of Neem pPlant</u> <u>pParts-Extracts</u>	2WAP	4WAP	6WAP	8WAP
Neem leaf extract <u>(NLE)</u>	16.03	26.60	52.30	84.90
Neem seed extract <u>(NSE)</u>	15.03	28.89	63.60	103.30
Neem stem bark extract <u>(NSBE)</u>	15.51	25.37	62.70	86.00
No neem extract <u>(NNE)</u>	16.80	27.30	61.90	76.00
LSD _{0.05}	ns	ns	ns	8.68

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Key: WAP = Week after planting.

Table 32 shows the growth pattern of pigeon pea as measured by the number of leaves. The table indicates the effect of different neem plant parts extracts on the number of leaves of pigeon pea. Statistical analysis showed no significant ($P > 0.05$) difference among the treatment means in week 2 of planting. On the contrary, analysis of variance indicated significant ($P < 0.05$) difference among the treatment means in weeks 4, 6 and 8 of planting. The increase in the number of leaves is positively related with the increase in the weeks of planting. The least number of pigeon pea leaves was 11.50, treated with neem seed extract (NSE) at week 2 of planting, while the highest number ~~of~~ was 99.40, treated with (NSE)neem seed extract at week 8 of planting. With the exception in week 2, where neem seed extract resulted to the least number of leaves, neem seed extract yielded the highest number of leaves in weeks 4, 6, and 8, respectively compared with the control. This shows that neem plant parts extracts are needed for production of pigeon pea biomass, especially the extract from the seed. Kankam and Adomako{25} attributed the increase in the number of leaves to the reduction of nematode activities by neem seed treatment, which resulted to improvement of soil nutrient absorption by the roots of pigeon pea plant. This was also

confirmed by Kumar and Kanna {26} who reported the effect of a formulation of neem product which led to an increase of the number of tomato leaves per plant.

Table 32: Effect of different Neem pPlant pParts extracts on the number of leaves of pigeon pea (*Cajanus cajan*, Fabaceae)-

Extracts of Neem pPlant pPart-Extracts	2WAP	4WAP	6WAP	8WAP
Neem leaf extract (NLE)	12.27	30.53	58.00	87.90
Neem seed extract (NSE)	11.50	35.57	64.20	99.40
Neem stem bark extract (NSBE)	13.03	23.31	48.80	83.70
No neem extract (NNE)	11.84	21.87	38.40	57.50
LSD _{0.05}	Ns	4.97	7.49	8.08

Key: WAP = Week after planting.

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Table 43 shows the effect of different neem plant parts extracts on phytophthora blight of pigeon pea disease incidence. Result of the analysis of variance showed a positive relationship between the treatments and the phytophthora blight incidence of pigeon pea in all the weeks of planting. Significant ($P < 0.05$) difference, therefore, existed among the treatments means in all the weeks of planting. Neem seed extract recorded the lowest phytophthora blight incidence in all the weeks of planting. In the 8th week of planting, neem seed extract depressed phytophthora blight incidence by 11.00 %, which is the single lowest disease incidence among all the treatments. This showed a significant reduction by neem seed extract and that it has strong bio - controlling influence in reducing the disease incidence. This was followed by 13.10 % disease incidence at week 6 at the application of neem seed extract. These results showed that seed extract of neem

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was highly effective in controlling phytophthora blight of pigeon pea. This also proves that neem seed extract can be used as an alternative to synthetic fungicides in the control of phytophthora blight of pigeon pea plant. The ability of neem extracts to control disease pathogens is attributable to the disease – inhibiting compounds present in neem. These include azadirachtin, nimbin, nimbidin, nimbinene, azadirone, which are antifungal, antibacterial, and insecticidal in their actions {27}. Up to 35 biologically active agents have been reported in neem tree, and especially, triterpenoides, nimbin, azadirachtin, present more in the seeds, leaves and other parts of the tree, they are the most potent for the control of diseases {28, 29, 30, 31}. The results in the control treatment, where no neem extract (NNE) was used, showed the highest disease incidence, compared with the rest of the treatments. This evidenced the reality of the bio - efficiency of the neem plant parts extracts against the phytophthora blight disease of pigeon pea.

Table 43: Effect of different neem plant parts extracts on phytophthora blight disease incidence (%) data on pigeon pea (*Cajanus cajan*, Fabaceae).

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<u>Extracts of Neem pPlant</u> <u>pParts-Extracts</u>	2WAP	4WAP	6WAP	8WAP
Neem leaf extract <u>(NLE)</u>	22.70	20.90	16.80	17.40
Neem seed extract <u>(NSE)</u>	18.30	15.85	13.10	11.00
Neem stem bark extract <u>(NSBE)</u>	22.30	30.54	24.45	20.50
No neem extract <u>(NNE)</u>	36.30	44.99	47.60	61.90
LSD _{0.05}	9.58	5.98	8.82	7.09

Key: WAP = Week after planting.

Table 54 shows the effect of different neem plant parts extracts on the phytophthora blight disease severity of pigeon pea. Significant ($P > 0.05$) difference did not exist among the treatment means in week 2 of planting. However, in weeks 4, 6 and 8 of planting, results showed significant ($P < 0.05$) difference among all the treatments means. This shows positive relationship between the treatments and the phytophthora blight disease severity of pigeon pea. In this result, neem seed aqueous extract (NSE) significantly ($P < 0.05$) depressed the phytophthora blight disease severity of pigeon pea, especially in weeks 6 and 8 of planting (1.00) respectively. The Neem leaf extract (NLE) had an equal effect on the blight severity in week 4, 6 and 8 (1.40 respectively). The NSE neem seed extract portrayed a significant reduction in the phytophthora blight severity in week 4, 6 and 8 (1.40, 1.00 and 1.00) respectively. This showed a slight infection, according to disease severity rating by Ford and Hewitt {23}. Neem stem bark extract (NSBE) represented a moderate infection in all the weeks of planting respectively (2.20, 2.80, 1.80 and 1.60) respectively {23}. This control treatment (NNE) depicted an increasing phytophthora blight severity throughout the weeks of planting (2.20, 2.80, 2.80 and 3.80) respectively. In the whole result, neem seed extract (NSE) had the list blight severity in contrast to the control treatment that had the highest severity of 3.80. This means the severe infection occurred in the plots that were not treated with neem plant parts extracts. This is a proof that in the absence of synthetic fungicide, phytophthora blight of pigeon pea could conveniently be managed with the application of neem plant parts aqueous extracts with particular emphasis on the neem seed extract. In this way, all the problems emanating from the use of the synthetic chemicals would be avoided. Gajalakshmi and Abbas (2004) stated the effect of neem plant extracts on plant diseases in a similar experiment.

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Table 54: Effect of different neem plant parts extracts on phytophthora blight disease severity of pigeon pea (*Cajanus cajan*, Fabaceae).

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<u>Extract of Neem pPlant pPart</u>	<u>2WAP</u>	<u>4WAP</u>	<u>6WAP</u>	<u>8WAP</u>
Neem leaf extract (NLE)	1.60	1.40	1.40	1.40
Neem seed extract (NSE)	1.40	1.40	1.00	1.00
Neem stem bark extract (NSBE)	1.60	2.00	1.80	1.60
No neem extract (NNE)	2.20	2.80	2.80	3.80

LSD_{0.05}

Ns

0.55

0.60

0.56

Key: WAP = Week after planting.

Conclusion

This study gave an insight into the management and control of phytophthora blight of pigeon pea through the application of three aqueous neem plant parts extracts. The effects of the extracts were examined and data were taken on growth parameters (plant height and number of leaves) and phytophthora disease incidence and severity. The tallest *Cajanus cajan* plant (103.30 cm) was produced at the application of neem seed extract. This was followed by pigeon pea plant treated with neem stem bark extract (86.00 cm). Further studies could be recommended on the influence of plant height on the other growth parameters and yield of pigeon pea. In the case of foliage production, neem seed extract yielded the highest number of leaves in the last three weeks of the study when compared with the control plots. This shows that the aqueous neem extracts could be recommended for the production of pigeon pea biomass, as this plays important role in the increase of yield and yield components, and for the production of fodder for livestock. The significant effect of neem seed extract was evident in its drastic reduction of both phytophthora blight in incidence and severity. In both cases, neem seed extract depressed the disease incidence (11.00%) and severity to 1.00, in comparison to the control, where results indicated increasing disease incidence and severity. These results are proofs of the efficacy of the aqueous neem seed extract against the blight infection, and could be recommended for the control the disease. This will further eliminate the hazards of using synthetic fungicides in the management of phytophthora blight of pigeon pea.

REFERENCES

1. Wahua, T.A.T. (1999). Applied Statistics for Scientific Studies. 347p.
2. Obi, I.U. (1986). Methods of detecting difference between treatment means. Snapp Press Limited, Enugu:- 2- 28. <https://www.scirp.org>
3. FAOSTAT (2015):- Food and Agriculture Organization Corporate Statistical Database. (<http://faostat.fao.org/faostat/collections>), 2015.
4. Dansi, A., Vodouhe, R., Azokpota, P., Yedomonhan, H., Assogba, P., Adjatan A., Yoko, YL., Dossou-Aminon, I., Akpagana, K. (2012). Diversity of neglected and underutilized crop species of importance in Benin. Sci. World J. 2012: 19.
5. Ayanan, M.A.T., Dangua A., Ahoton, L.E. and Ofori, K. (2017). Utilization and farmers' knowledge on pigeon pea diversity in Benin, West Africa. J. Ethnobiol. Ethnomed. 2017; 13: 37.

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6. Adjei-Nsiah, S. (2012). Role of pigeon pea cultivation on soil fertility and farming system sustainability in Ghana. *Int. J. Agron*; ~~2012~~; ~~8~~.
7. Egbe, O.M., Vange, T. (2008). Yield and agronomic characteristics of 30 pigeon pea genotypes at Otobi in Southern Guinea Sananna of Nigeria. *Life Sci. J.* ~~2008~~; 5: 70-80.
8. Saxena, K.B., R., Sreejith, K.P., Srivastava, R.K. (2010). Vegetable pigeon pea – a high protein food for all ages; Information building no. 83. Patancheru 502-324. Andhra Pradesh, India: International Crops Research Institute for the Semi Arid Tropics; ~~2010~~.
9. Saxena, K.B., Kumar, R.V. (2010). Quality nutrition through pigeon pea – a review. *Health.* ~~2010~~; 2: 1335-44.
10. Høgh – Jensen, H. (2011). To meet future food demand, we need to change from annual grain legumes to multipurpose semi-perennial legumes. In: Aladjadjiyan A, editor. *Food production – approaches, and tasks.* Rijeka: Inlech; ~~2011~~. P.1-24 pp.
11. Lose, S, J., Hilger, T.H., Leihner, D.E., Krosche, I.J. (2003). Cassava, maize and tree root development as affected by various agroforestry and cropping systems in Benin, West Africa, ~~2003~~; 100: 137-51.
12. Odeny, D.A. (2007). The potentials of pigeon pea [*Cajanus cajan* (L) Mill sp.] in Africa. *Nat. Resour. Forum.* ~~2007~~; 31: 297-305.
13. Prince, Kumar Gupta; Manpreen Kaur, Bishal, R., Sarthak, Joshi. (2022). Pigeon pea Diseases and their Recent Approaches for Sustainable Management in India: Current Status and Future Prospective, ~~Pp.~~ 1- 34 pp.
14. Chava, V. K. (2020). Use of neem extracts (*Azadirachta indica*) in control of plant diseases: A Review. *In*; ~~J. Curr. Microbiology and Applied Sciences.~~ ISSN: 2319-7706. Special issue. 11pp. ~~3481-3487~~. Journal home page. <http://www.ijcmas.com>
15. Chaturvedi, R., Razdan, M.K., Bhojwani S.S.(2003). Production haploids of neem (*Azadirachta indica* A.Juss), by another culture plant cell. Source: TNAU Agritech Portal. In (www.agritech.tnau.ac.in/org)
16. FDALR. (Federal Department of Agriculture and Land Resources) (1985). The Reconnaissance Survey of Imo State Soil Report. 133p.
17. Ononiwu, N. (1990). The physic-chemical properties of soils and associated properties of soils and associated cropping/land use systems in Eziobodo village, Owerri LGA, Nigeria. B. Agric. Tech. Project. SAAT, FUT0.
18. Acholonu, D. W. (2009) Water quality studies of Nworie River in Owerri, Nigeria. Mississippi Academy of Science, Retrieved, 2009, 10 – 14.

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19. Nwokeji, E .M., Ogwudire, V. E., Peter – Onoh, C. A., Onwubiko, N. C., Ogbedeh, K.O., Anyanwu, P.C., Nwufo, M. I., Duruigbo, C. I., Agu, C.M., Ihejirika, G.O. and Ngwuta, A.A. (2020). Effect of some plant extracts and tomato cultivars on the growth of tomato (*Lycopersicon esculentum* Mill) in Owerri, Imo state, southeast Nigeria
20. Campbell, C. L. and Madden, L.v. (1990). Introduction to plant disease epidemiology. Wiley Interscience, New York.
21. Madden, L.V., and Hughes, G. (1995). Plant diseases: distributions, heterogeneity, and temporal analysis. Annual Rev. Phytopathol. 33: 529- 569.
22. Cardoso, J. E. Santos, A.A., Rossetti, A.G., Vidal, J. C. (2004). Relationship between incidence and severity of cashew gummosis in semi-arid north –eastern Brazil. Plant Pathology. 2004; 53(3) 363-367.
23. Ford, J.E. and Hewitt, D. (1980) . Feeding Values, Processing and Viruses. The Hage Martus Nijhoff. pp. 125 – 139.
24. Onekutu, A., Oluoma, H. O. A. and T. A. Tor-anyin (2001). Investigation into anti fungal - properties of *Ocimum gratissimum* L. West Africa Journal of Biological Science. 12:35-42.
25. Kankam, F. and J. Adomaka (2014). Efficacy of neem seed powder on root knot nematode (*Meloidogyne* spp.) infecting bambara groundnut (*Vigna subterranean*). Asian Journal of Agriculture and Food Science (ISSN: 2321-1571). Volume 02- Issue 02, April 2014.
26. Kumar, S. and Kanna, A. S. (2006). Effect of neem-based products on the root knot nematodes (*Meloidogyne incognita*) growth of tomato. Nematologia Mediterranea, 34: 141-146, 2006.
27. Bohra, B., B.N. Vyas, and K.B. Mistry (2006). Eco-friendly management of damping - off in winter vegetables and tobacco using microbial agents and neem for mutations. J. Mycol. Plant Pathol., 36: 178-181.
28. Mondall, N, K., A. Mojumdar, S, K. Chatterji, A. Banerjee, J.K. Datta and S. Guta, (2009). Antifungal activities and chemical characterization of neem leaf extracts on the growth of some selected fungal species in vitro culture medium. J. Applied Sci. Environ. Sahu, Manage., 13: 49-53.
29. Nahak, G. and R. K. Sahu, (2010). Antioxidant activities in bark and roots of neem (*Azadirachta indica*) and Mahaneem (*Melia azedarach*). J. Am. Pharm. Sc., 4: 28-34.
30. Brahmachari, G. (2004). Neem-an omnipotent plant: A retrospect ChemBioChem. 5: 408-421.
31. Gajalakshmi, S. and S.A.Abbasi (2004). Neem leaves as a source of fertilizer cum- pesticide vermicompost. Bioresour., Technol., 92-291-296.