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Original article

Phytochemical analysis and pH of ethanol extracts of selected Plants as Bactericide against Bacterial blight of Soybean (*Glycine max* L.) caused by *Pseudomonas syringae*

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ABSTRACT

Ethanol Extracts of *Allium sativum, Diospyrous mespiliformis* and Bactericide (Z-Force solution) were subjected to pH and phytochemical susceptibility testing against bacterial blight of soybeans caused by *Pseudomonas syringae*. Ethanol Extracts of *Allium sativum* showed the highest mean pH values at (10.5) followed by Ethanol Extracts of *Diospyrous mespiliformis* at (7.1) which are both alkaline, while Z-Force solution showed the least mean pH values at (5.1) which is acidic on the pH scale. Ethanol extracts of *Allium sativum* (Bulb) showed the presence (+) of Alkaloids, Flavonoids, Glycoside, Phenols, Terpenoids, Saponins, Steroids and Tannins while ethanol extracts of *Diospyrous mespiliformis* (Root, Leaf and Stem) showed the presence (+) of Alkaloids, Saponins, Steroids and Tannins. Among all the plant extracts used in this studies, highest percentage composition was recorded in *Allium sativum* (Bulb) (Alkaloids, 4.39%). We recommended that extracts with wider pH and permeability rate such as ethanol extracts of *Allium sativum* and *Diospyrous mespiliformis* should be adopted as control agents for bacterial blight of soybeans. bacterial pathogens.

Keywords: pH, phytochemicals*, Allium sativum, Diospyrous mespiliformis,* Bactericide solution

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INTRODUCTION

pH plays an extremely important role in influencing the growth of micro-organisms [2], the optimal conditions for growth of *Mucor racemosus*, are at pH 4.5. Reetha and Senthilkumar [9] studied the effect of temperature and pH on growth of *Trichoderma harzianum* and reported that pH differences among the isolates were significant. The radial growth of *Trichoderma harzianum* responded differently to various pH levels. These authors reported that the maximum mycelia weight of *Trichoderma harzianum* was observed at pH 7-7.5 and minimum growth was observed at pH 6 and low growth was observed at pH 5. Gulis [4] studied the in-vitro physiological studies on *Phytophthora syringae* that causes root rot of almond, and reported that the best pH level for the growth of the bacteria to be pH 6. Amadi *et al.* [1] carried out biocontrol and environmental studies and reported that low pH of fruit juices greatly limits the number and the type of bacteria that can survive or grow at this low pH but some bacteria with low pH than that of the fruit juice can grow at this condition. Singh *et al.,* [11] studied the optimal physical parameters for growth of *Trichoderma* species at varying pH, temperature and agitation and it revealed that most favorable pH ranges between 5.5 and 7.5.

Plants are composed of an array of complex mixtures of secondary metabolites, which exhibit therapeutic effect; - these are called phytochemicals and include alkaloids, glycosides, terpenoids and phenols [2]. Phytochemicals in medicinal plants can be used directly for therapeutic purposes or as a precursor for the synthesis of pharmaceuticals. Some plants growing in the wild are strictly used for therapeutic applications [9]. Alkaloids exhibit important pharmacological uses as analgesics, antibacterial, anti-malarial, anticancer, anti-hypertensive. Tannins are antiseptic in nature and hasten healing of wounds. Flavonoids have been shown to exhibit pharmacological effects as anti-allergic, anti-inflammatory, antioxidant, anti-cancer, antibacterial, antifungal and antiviral, agents [11]. Saponins defend plants against microbial attack, hence serve as antimicrobial and antifungal agents to humans. Terpenoids are extensively aromatic and are used in food and pharmaceutical industries for flavor, and odour improver [11]. Phenols are a precursor to a large collection of drugs, most notably aspirin, and also many herbicides and pharmaceutical drugs. Therefore, the aim of this research work is to determine the pH and Phytochemical composition of ethanol extracts of *Allium sativum, Diospyrous mespiliformis*, Z-Force (bactericide) and Antibiotics Solutions used for Sensitivity Test of Bacterial Blight of Soybean (*Glycine max* L.) caused by *Pseudomonas syringae* in Adamawa State.

MATERIALS AND METHODS

Study Area

The area has tropical climate marked by dry and rainy seasons, the rainy season commences around May and ends in the middle or late October while the dry season starts at October or November and lasts to April. The main annual rainfall ranges from 700 mm in the north-western part to 1600 mm in the central geopolitical (Girie and Yola south local government area) part of the state. Maximum temperature is about 40^{o}C around April

while minimum temperature could be as low as 18.3°C between December and early January. Relative humidity in the area is about 26% in the month of January while February has the lowest value of 16%, the month of July and august usually have the peak with relative humidity of about 80%. Yola lies between latitude 7° and 11° north of the equator and between longitude 11^0 and 14^0 east of the Greenwich meridian.

Determination of the pH of *Ethanol Extracts of Allium sativum* (Bulb), *Diospyrous mespiliformis* (Leaves, Stem Back and Roots), Z-Force Solution and Antibiotics Solutions used for Sensitivity Test

A buffer of pH 7.2 was prepared by dissolving one pellet of aluminum chloride in one hundred (100 mLs) of distilled water, this was used for the calibration of the pH meter. The buffer prepared as mentioned above was poured into a beaker and the pH electrode dipped into it. The pH of the meter was adjusted to 7.2 and allowed to stay for 10 minutes after which it was removed and rinsed thoroughly with distilled water from a wash bottle. The pH of each sample was taken by inserting the electrode the pH meter (Jenway-430 pH/conductivity meter) into the sample when the pH was noted at steady value, the electrode was rinsed thoroughly after reading and recording the pH value for each sample to avoid contamination, and this process was repeated for the three samples and at four (4) different concentration levels. This same process was repeated for all the eleven (11) antibiotics used in this research for in-vivo sensitivity test.

Sterilization of Materials

All the glass wears such as Petri dishes, beakers, test tubes, conical flasks and steering rods used for the experiments were sterilized in an oven at 160°C for 60 min. The inoculating needle, forceps and the cork borer were sterilized by flaming over a Bunsen burner flame and allowed to cool by dipping them into methanol. The prepared media were sterilized by autoclaving for 15 minutes at 10 lbs pressure at 121°C and allowed to cool.

Phytochemical Analysis of the Plant Extracts

The ethanolic extracts of the three plants that were used *Allium sativum* and *Diospyros mespiliformis*) were subjected to phytochemical analysis in Biochemistry Laboratory of Modibbo Adama University, Yola in which the chemical composition of these plant extracts were determined, this was done so as to know the active components present in the extracts.

Qualitative Phytochemical Analysis

The qualitative phytochemical screening of samples was carried out so as to determine the presence of alkaloids, flavonoids, steroid, phenols, tannins, saponin, glycosides and terpenoids.

a. Test for alkaloids

To 2 mLs of plant extract, 2 mLs of concentrated hydrochloric acid was added. Then 3 drops of Mayer's reagent was also added. Presence of green colour or white precipitate indicates the presence of alkaloids.

b. Test for flavonoid

i. To 2 mLs of plant extract 1 mLs of aqueous Sodium hydroxide solution

was added and observed for the formation of yellow-orange coloration.

ii. 2 mLs of plant extract was treated with 4 drops of concentrated sulphuric acid and observed for the formation of orange colour.

c. Test for glycosides

To 2 mLs of plant extract, 1 mLs of glacial acetic acid and 5% ferric chloride was added, to these 3 drops of concentrated sulphuric acid was also added. Presence of greenish blue colour indicates the presence of glycosides.

d. Test for phenols

To 1 mLs of the extract, 2 mLs of distilled water followed by 5 drops of 10% ferric chloride was added. The formation of blue or green colour indicates presence of phenols.

e. Test for tannins

To 1 mLs of the plant extracts, 2 mLs of 5 % ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

f. Test for terpenoids

5 mLs of extract of plant was mixed with 2 mLs of CH_3Clin a test tube and then 3 mLs of concentrated H_2SO_4 was carefully added to the mixture to form a layer. An interface with a reddish brown colouration indicates that terpenoids constituent are present.

g. Test for saponins

To 1 mLs of plant extracts, 5-10 mLs of distilled water was added and shaken in a graduated cylinder for 15 minutes; formation of 1 cm layer of foam indicates the presence of saponins.

h. Test for steroid

To 1 mLs of plant extract, equal volume of chloroform and 3 drops of concentrated sulphuric acid was added. Formation of brown ring indicates the presence of steroid.

Quantitative analysis of the chemical constituents

This analysis was carried to determine the amount or percentage concentration of the phytochemical constituents present in the plant extracts used in this research.

a. Alkaloid determination

Four (4) g of the sample were weighted into a two hundred and fifty (250) mLs beaker and one hundred and sixty (160) mLs of twenty percent (20%) acetic acid in ethanol was added and covered to stand for four (4) hours. This was filtered and the extract was concentrated using a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was then added drop wise to the extract until the preparation was complete. The whole solution was allowed to settle down and the precipitate was collected by filtration and was weighed.

b. Flavonoid determination

Four (4) g of the plant sample were extracted repeatedly with forty (40) mLs of eighty percent (80%) aqueous methanol at room temperature. The whole solution was filtered. The filtrate was later transferred into a crucible and

evaporated to dryness over a water bath and was weighed.

c. Determination of glycoside

Glycoside quantitative determination was conducted by weighing each of the pulverized powdered extract into a 250 cm^3 round bottom flask and about 200 cm^3 of distilled water was added to one gram of each dry powder sample and allowed to stand for 2 hours for autolysis to occur. Full distillation was carried out in a 250 cm^3 conical flask containing 20cm^3 of 2.5% NaOH (sodium hydroxide) in the sample after adding an antifoaming agent (tannic acid). Glycoside (100 cm^3), 8cm^3 of 6M NH_4OH (ammonium hydroxide), and 2 $cm^3$of 5% KI (potassium iodide) were added to the distillate(s), mixed, and titrated with 0.02M $AgNO_3$(silver nitrate) using a microburette against a black background, continuous turbidity indicates the end point. The quantity of glycoside in the sample was calculated using the formula below;

d. Total phenolic content (TPC)

Total phenolic content of the extract was determined by briefly, adding 0.1mLss of extract (200, 600 and 1000 µg/mLs), 1.9 mLs distilled water and 1 mLs of Folin-Ciocalteau's reagent were seeded in a tube, and then 1mLss of sodium carbonate was added. The reaction mixture was incubated at 25°C for 2hours and the absorbance of the mixture was read at 765nm. The sample was tested in triplicate and a calibration curve with six data points for catechol was obtained. The results were compared with catechol calibration curve and the total phenolic content of sample was expressed as mg of catechol equivalents per gram of extract.

e. Tannin determination

Four (4) g of each of the dried powdered samples was weighed into forty 40 mLs plastic bottle. Four (4) mLs of distilled water was shaken for one hour and was filtered. Then four (4) mLs of the filtrate was pipette out into a tube and mixed with three (3) mLs of 0.1m Fecl3 in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured in a spectrophotometer at 120nm wavelengths within 10minutes. A blank sample was prepared and the color was developed and read at the same wavelength. A standard was prepared using tannin acid to get 100ppm and measured.

f. Terpenoids Determination

The dried plant extract 100mg (wi) was taken and soaked in 9mLs of ethanol for 24hour. The extract after filtration was extracted with 10mLs of petroleum ether using separating funnel. The ether extract was separated in pre-weighed glass vials and waited for its complete drying (wf). Ether was evaporated and the yield (%) of total terpenoids contents was measured by the formula (wi-wf/wi×100).

g. Saponin Determination

Four (4) g of each plant specimen were dispersed into forty (40) mLs of twenty percent (20%) ethanol. The suspension was heated over a water bath for 4hours with continuous stirring at about 55^0C. The mixture was filtered and the residue re-extracted with another forty (40) mLs of twenty percent (20%) ethanol. The combined extracts were reduced to two (2) mLs over a water bath at 90^0C. The concentrate was transferred into a two hundred and fifty (250) mLs separator funnel and four (4) mLs of di-ethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated; twelve (12) mLs of n-butanol was added. The combined n-butanol extracts were washed twice with two (2) mLs of five percent (5%) aqueous sodium chloride. The remaining solution was heated in a water bath; after evaporation, the samples were dried and weighed.

h. Estimation of Steroid content

An aliquot of 2mLs was taken from the powdered extract prepared in 50 mLs of distilled water and was shaken for 1hour. Sulphuric acid (4N, 2 mLs) and iron (III) chloride (0.5% w/v, 2mLss), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5mLs). The mixture was heated in a water-bath maintained at 70±2°C for 30minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780nm against the reagent blank and recorded.

i. Experimental Design

The experiment was a completely randomized design (CRD) and data collected were in triplicates and analysed using statistical analysis system (SAS) version 23.

Data Analysis

All the data obtained were analyzed using analysis of variance (ANOVA) to test for significance using statistical tool for applied sciences (SAS) version 23 and the means that were significant were separated using the least significant difference (LSD) at 5% probability level [10].

RESULTS

pH-Value of Ethanol Extracts of *Diospyros mespiliformis* (leaves, Stem-back and Roots), *Allium sativum* (Garlic), Bactericide (Z-Force solution) and Antibiotics

At P=0.05, the result in Table 1; shows significance difference in pH values among the Five different Concentration Levels of ethanol extracts of *Allium sativum, Diospyros mespiliformis* (leaves, Stem-back and Roots) and Bactericide (Z-Force solution) used in the experiment to control bacterial blight of soybeans caused by *Pseudomonas syringae* in-vitro and in-vivo. There was significance difference in pH values at ethanol extracts of *Diospyros mespiliformis* (leaves*)* among 5 mg/mLs, 10 mg/mLs, 15 mg/mLs and 20 mg/mLs has the highest pH (6.9) while 5 mg/mLs showed least pH (4.5). There was significant difference in pH values at ethanol extracts of *Diospyros*

mespiliformis (stem-back*)* among 5 mg/mLs, 10 mg/mLs, 15 mg/mLs and 20 mg/mLs has the highest pH (7.4). However, there is no significant variation between 10 mg/mLs and 15 mg/mLs. There was significance difference in pH values of ethanol extracts of *Diospyros mespiliformis* (Roots*)* between 5 mg/mLs and15 mg/mLs, 20 mg/mLs, 10 mg/mLs and 15 mg/mLs, 20 mg/mLs has the highest pH (9.4). However, there is no significant variation between 10 mg/mLs and 15 mg/mLs, 15 mg/mLs and 20 mg/mLs. There was significance difference in pH values for ethanol extracts of *Allium sativum* (Bulb*)* between 5 mg/mLs and 15 mg/mLs, 20 mg/mLs, 10 mg/mLs and 15 mg/mLs, 20 mg/mLs, between 10 mg/mLs and 15 mg/mLs, 15 mg/mLs and 20 mg/mLs has the highest pH (12.1). There was significance difference in pH values at Z-Force solution *(Bactericide)* between 5 mg/mLs and15 mg/mLs, 20 mg/mLs, 10 mg/mLs and 15 mg/mLs 20 mg/mLs, 15 mg/mLs and 20 mg/mLs has the highest pH (9.4). However, there was no significant variation between 10 mg/mLs and 15 mg/mLs.

Table 1: pH-Value of the Four different Concentration Levels on Ethanol Extracts of *Diospyros mespiliformis* (leaves, Stem-back and Roots), *Allium sativum* (Garlic) and Bactericide (Z-Force solution)

Concentration levels (mg/mLs)	*D. mespilifomis* Ethanol leaves Extract	*D. mespilifomis* Ethanol Stem-back Extract	*D. mespilifomis* Ethanol Roots Extract	*A. sativum* Ethanol Extract	Bactericide (Z-Force)
5 mg/mLs	4.5	5.2	7.0	8.8	4.4
10 mg/mLs	5.8	6.0	7.5	9.8	5.0
15 mg/mLs	6.2	6.4	9.0	11.3	5.1
20 mg/mLs	6.9	7.4	9.4	12.1	6.0
LSD (0.05)	0.6	0.5	0.6	0.8	0.4

Key
LSD: Least Significant Difference

At P=0.05, the result in table 2; shows significance difference in pH values among the Ethanol Extracts of *Allium sativum, Diospyros mespiliformis* and Bactericide (Z-Force solution) used in the experiment to control bacterial blight of soybeans caused by *Pseudomonas syringae* in-vitro and in-vivo. Ethanol Extracts of *Allium sativum* showed the highest meanpH values at (10.5) followed by Ethanol Extracts of *Diospyros mespiliformis* at (7.1) which were both alkaline, while Z-Force

solution showed the least mean pH values at (5.1) which is acidic on the pH scale.

At mean pH value of 10.5 the Ethanol Extracts of *Allium sativum* showed the best control of *Pseudomonas syringae* at 1.2 mm *in-vitro* and 9.1mm *in-vivo*. At mean pH value of 7.1 the Ethanol Extracts of *Diospyros mespiliformis* showed control of *Pseudomonas syringae* at 7.4 mm *in-vitro* and 18.3 mm *in-vivo* At mean pH value of 5.1 the Z-Force solution showed the least control of *Pseudomonas syringae* at 30.2 mm *in-vitro* and 52.7 mm *in-vivo*. This result implies that there was a huge significance difference among the pH values of the extract in controlling the bacterial blight of soybeans at P=0.05.

Table 2: Effect of the pH-Value of the Ethanol Extracts of*Diospyros mespiliformis*, *Allium sativum* (Garlic) and Bactericide (Z-Force solution) on the control of Bacterial Blight of Soybean

Extracts	pH-Value	pH Ratings
D. mespiliformis (Ethanol Extracts)	7.1	Alkaline
Allium sativum (Ethanol Extracts)	10.5	Alkaline
Bactericide (Z-Force)	5.1	Acidic

LSD: Least Significant Difference (= 1.8)

At P=0.05, the result in table 3; shows significance variation in pH values among the Antibiotics used in the experiment to control bacterial blight of soybeans caused by *Pseudomonas syringae* in-vivo. There is significant variation in the pH values of the antibiotics. However, there is no significant variation between Ampiclox and Erythromycin, Ampiclox and Pefloxacin, Ampiclox and Sparfloxacine, Amoxicillin and streptomycin, Pefloxacin and Sparfloxacine, Ciprofloxacin and Septrin. The result of the analysis shows that the entire antibiotics used in the study were alkaline with the exception of streptomycin and ofloxacin which are both acidic base on the pH ratings. Augmentin showed the highest pH-value of 9.1 followed by Chloramphenicol with pH-value 8.7 which were both alkaline, while ofloxacin showed the least mean pH values at (6.6) which is acidic on the pH scale.

Table 3: Effect of the pH-Value of Antibiotics on the Sensitivity of Bacterial Blight of Soybean caused by *Pseudomonas syringae* (in-vivo)

Antibiotics	pH-Value	pH Ratings
Augmentin	9.1	Alkaline
Ampiclox	7.5	Alkaline
Amoxicillin	7.1	Alkaline
Chloramphenicol	8.7	Alkaline
Ciprofloxacin	8.2	Alkaline
Erythromycin	7.7	Alkaline
Ofloxacin	6.6	Acidic
Pefloxacin	7.4	Alkaline
Septrin	8.1	Alkaline
Sparfloxacine	7.5	Alkaline
Streptomycin	6.9	Acidic

LSD: Least Significant Difference (= 0.3)

Quantitative and Qualitative Phytochemical Analysis of the Ethanol Plant Extracts used

The result in Table 4: Shows the Qualitative Phytochemical Composition of Ethanol Extracts of *Diospyros mespiliformis* and *Allium sativum* which showed the presence or absence of the secondary metabolites; Alkaloids, Flavonoids, Glycoside, Phenols, Terpenoids, Saponins, Steroids and Tannins. Ethanol Extracts of *Allium sativum (Bulb)* showed the presence (+) of all the phytochemicals, while Ethanol Extracts of *Diospyrosmespilifomis* (Root, Leaf and Stem) showed the presence (+) of Alkaloids, Saponins, Steroids and Tannins. However, Ethanol Extracts of *Diospyros mespiliformis* (Root, Leaf and Stem) showed the absence (-) of Flavonoids, Glycoside, Phenols and Terpenoids with the exception of Ethanol Extracts of *Diospyros mespiliformis* (Leaf) which showed the presence (+) of Flavonoids and Phenols.

Table 4: Qualitative Phytochemical Composition of Ethanol Root, Leaf, Stem Extracts of *Diospyros mespiliformis* and *Allium sativum*

Phytochemicals	Phytochemical Composition (+/-)			
	Diospyros mespiliformis			*Allium sativum*
	(Roots)	(Leaves)	(Stem)	(Bulb)
Alkaloids	+	+	+	+
Flavonoids	-	+	-	+
Glycoside	-	-	-	+
Phenols	-	+	-	+
Terpenoids	-	-	-	+
Saponins	+	+	+	+
Steroids	+	+	+	+
Tannins	+	+	+	+

Key

_: Absent
+: Present

The result in Table5: Shows the Quantitative Phytochemical percentage (%) Composition of the secondary metabolites which are present in the Ethanol Extracts of *Diospyros mespiliformis* and *Allium sativum*; ethanol extracts of *Allium sativum* (*Bulb)* showed the presence (+) of all the phytochemicals with Alkaloids showed the highest percentage Composition of (4.39%) while Glycoside showed the least percentage concentration of (0.03%). In the ethanol extracts of *Diospyros mespiliformis* (Leaf), although Glycoside and Terpenoids were absent, Alkaloids showed the highest percentage Composition of (2.04%) while Steroids showed the least percentage concentration of (0.06%). In the Ethanol Extracts of *Diospyros mespiliformis* (Stem), Flavonoids, Glycoside, Phenols and Terpenoids were absent, however Tannins was present and showed the highest percentage Composition of (1.81%) while Steroids showed the least percentage concentration of (0.20%). In the Ethanol Extracts of *Diospyros mespiliformis* (Roots), Flavonoids, Glycoside, Phenols and Terpenoids were absent, however Tannins was present and showed the highest percentage Composition of (2.95%) while Steroids showed the least percentage concentration of (0.11%). Among all the parts of the plants used in this studies

(Ethanol Extracts of *Diospyros mespiliformis* (Root, Leaf and Stem), and *Allium sativum(Bulb)),* highest percentage Composition was recorded in *Allium sativum* (Bulb) (Alkaloids, 4.39%) while the least percentage concentration was also recorded in *Allium sativum* (Bulb) of (Glycoside, 0.03%).

Table 5: Percentage Quantitative Phytochemical Composition of Ethanol Root, Leaf, Stem Extracts of*Diospyrosmespilifomis*and*Allium sativum*

Phytochemicals	Phytochemical Composition (%)			
	Diospyros mespiliformis			*Allium sativum*
	(Roots)	(Leaves)	(Stem)	(Bulb)
Alkaloids	0.54	2.04	0.89	4.39
Flavonoids	-	0.77	-	1.03
Glycoside	-	-	-	0.03
Phenols	-	1.49	-	0.54
Terpenoids	-	-	-	0.19
Saponins	0.38	1.69	1.03	1.74
Steroids	0.11	0.06	0.20	0.27
Tannins	2.95	1.01	1.81	3.65

Key
_: Absent

DISCUSSION

pH values among the Five different Concentration Levels of Ethanol Extracts of *Allium sativum, Diospyros mespiliformis (*leaves, Stem-back and Roots*)* and Bactericide (Z-Force solution) used in the experiment to control bacterial blight of soybeans caused by *Pseudomonas syringae* in-vitro and in-vivo shows significance difference. Ethanol extracts of *Allium sativum* showed the highest mean pH values at 10.5 followed by Ethanol Extracts of *Diospyros mespiliformis* at 7.1 which are both alkaline, while Z-Force solution showed the least mean pH values at 5.1 which is acidic on the pH scale. The result of the analysis shows that the antibiotic used in the study is alkaline with the exception of streptomycin and ofloxacin which are both acidic base on the pH ratings. Augmentin showed the highest pH-value of 9.1 followed by Chloramphenicol with pH-value 8.7 which are both alkaline, while ofloxacin showed the least mean pH values at 6.6 which is acidic on the pH scale. This finding is in agreement with the report made by [12] that most isolates preferred pH levels of 4.96 – 5.15. Also, Amadi *et al.* [1] in his study concluded that certain alkaline medium with wide pH range can be used to inhibit the mycelia growth and sporulation of *A. parasiticus* in order to prevent it from damaging our crops. However, the finding in this study contradicts the findings made by [5] who reported that, the optimum growth at pH 7 and pH 9 and poor growth at pH 5.

Phytochemical screening of the test plants *Diospyros mespiliformis (*Root, Leaf, Stem*)* and *Allium sativum* (Bulb) used in this study was carried out to determine the exact phytochemical contents. Results of the phytochemical screening carried out showed that Ethanol Extracts of *Allium sativum* (Bulb) showed the presence (+) of all the phytochemicals, while Ethanol Extracts of *Diospyros mespiliformis* (Root, Leaf and Stem) showed the presence (+) of Alkaloids, Saponins, Steroids and Tannins. However, ethanol extracts of *Diospyros mespiliformis* (Root, Leaf and Stem) showed the absence (-) of Flavonoids, Glycoside, Phenols and Terpenoids with the exception of Ethanol Extracts of *Diospyros mespiliformis* (Leaf) which showed the presence (+) of Flavonoids and Phenols. Among all the parts of the plants used in this studies (Ethanol Extracts of *Diospyros mespiliformis* (Root, Leaf and Stem), and *Allium sativum (Bulb)),* highest percentage Composition was recorded in *Allium sativum* (Bulb) (Alkaloids, 4.39%) while the least percentage concentration was also recorded in *Allium sativum* (Bulb) of (Glycoside, 0.03%). Eleazu *et al.* [3] reported that phytochemicals occur naturally in plants and form part of plants defense mechanisms against diseases. Krishnaiah and Sarbatly [6] and Eleazu *et al.*[3] reported that phytochemicals are classified into primary and secondary, based on their activity in plant metabolism. The primary ones comprise of sugars, amino acids, proteins and chlorophyll, while secondary ones include the phenolic compounds such as tannins, flavonoids, alkaloids, saponins, anthraquinones, phlobatannins, proanthocyanidins, etc. Likewise, Prakash and Hosetti [8] and Zongo *et al.* [13] reported that phenolic compounds possess considerable antimicrobial properties, which was attributed to their redox properties. The finding made by Prakash and Hosetti [8] attributed antimicrobial properties of

plants to the presence of secondary metabolites.

CONCLUSION

pH values among the ethanol extracts of *Allium sativum, Diospyros mespiliformis* (leaves, Stem-back and Roots*)* and Bactericide (Z-Force solution) used in the experiment to control bacterial blight of soybeans caused by *Pseudomonas syringae* in-vitro and in-vivo. Ethanol Extracts of *Allium sativum* showed the highest mean pH values followed by Ethanol Extracts of *Diospyros mespiliformis* which are both alkaline, while Z-Force solution showed the least mean pH values which is acidic on the pH scale. All the antibiotic used in the study are alkaline with the exception of streptomycin and ofloxacin which are both acidic base on the pH ratings. Augmentin showed the highest pH-value followed by Chloramphenicol with pH-value which are both alkaline, while ofloxacin showed the least mean pH values which is acidic on the pH scale.

The phytochemical screening carried out showed that Ethanol Extracts of *Allium sativum* (Bulb) showed the presence (+) of all the phytochemicals, while Ethanol Extracts of *Diospyros mespilifomis* (Root, Leaf and Stem) showed the presence (+) of Alkaloids, Saponins, Steroids and Tannins. However, Ethanol Extracts of *Diospyros mespilifomis* (Root, Leaf and Stem) showed the absence (-) of Flavonoids, Glycoside, Phenols and Terpenoids with the exception of Ethanol Extracts of *Diospyros mespiliformis* (Leaf) which showed the presence (+) of Flavonoids and Phenols. Among all the parts of the plants used in this studies (ethanol extracts of *Diospyros mespiliformis* (Root, Leaf and Stem), and *Allium sativum (Bulb)),* highest percentage Composition was recorded in *Allium sativum* (Bulb) (Alkaloids) while the least percentage concentration was also recorded in *Allium sativum*(Bulb) of (Glycoside).

REFERENCES

1. Amadi, J.E., Nwaokike, P., Olahan, G.S. and Garuba, T. (2014). Isolation and identification of fungi involved in the post-harvest spoilage of guava (*Psidium guajava*) in Awka metropolis. *International Journal of Engineering and Applied Sciences* **4**, (10):21 – 43.

2. Arnold, W. M. and Weaver, R. H. (2008). Quick micro techniques for 5:109-118. The identification of cultures. *Journal of Laboratory Clinic and Medicine*.33:1334.

3. Eleazu, C. O., Eleazu, K. C., Awa, E. and Chukwuma, S. C. (2013) Comparative study of the phytochemical composition of the leaves of five Nigerian medicinal plants. *Journal of Biotechnology and Pharmaceutical Research*, **3**: 42-46.

4. Gulis, V. (2005). Are there any substrate preferences in aquatic hyphomycetes? *Mycology Research*, **105**, 1088 – 1093.

5. Javed, S., Shahid, A. A., Haider, M. S., Umeera, A., Ahmad, R. and Mushtaq, S. (2012). *Nutritional ,*

phytochemical potential and pharmacological evaluation of Nigella Sativa (Kalonji) and Trachyspermum Ammi (Ajwain). *6*(5), 768–775. https://doi.org/10.5897/JMPR11.1341

6. Krishnaiah, D. R. and Sarbatly, B. A. (2007). Phytochemical antioxidants for health and medicine: A move toward nature. *Biotechnology and Molecular Biology Review*, **1**: 97-104.

7. Molan, A. L., and Faraj, A. M. (2010). The effects of condensed tannins extracted from different plant species on egg hatching and larval development of Teladorsagia circumcincta (Nematoda: Trichostrongylidae). *Folia Parasitological*, **57:** 62-68.

8. Prakash, G. and Hosetti, B.B. (2010). Investigation of antimicrobial properties of *Dioscorea pentaphylla* from mid-Western Ghats, India.*Journal of Scientific World*, **8:** 91- 96.

9. Reetha, D. and Senthilkumar, P.K. (2014).Screening of antimicrobial properties of certain Indian medicinal plants. *Journal of Phytology*. **1**(3): 193-198.

10. Schaffer A.A., Stepansky A., Kovalski I., and Perl-Treves R. (2009), Variation in sugar levels and invertase activity in mature fruit representing a broad spectrum of *Cucumis melo* genotypes.*Genetic Research and Crop Evolution*.**46:** 53-62.

11. Singh, B.B., Chambliss, O.L. and Shanma, B. (2012). Recent advances in breeding. *Journal of international research for agricultural sciences (JIRAS)*.**5**,(3):Pp. 215-221.

12. Wasantha, K. L. (2014). Influence of Carbon, Nitrogen, Temperature and pH on the Growth and Sporulation of Some Indian Isolates of *Colletotrichum gloeosporioides* causing Anthracnose Disease of Papaya (Carrica papaya L). *Journal of Microbiology Research*. **8** (7): 685-688

13. Zongo, C., Savadogo, K., Somda, J.M., Koudou, J. and Traore, A.S. (2011). *In-vitro* evaluation of the antimicrobial and antioxidant properties of extracts from whole plant of *Alternanthera pungens* and leaves of *Combretum sericeum*. *International Journal of Phytomedicine*, **3**: 182 – 191.