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Biodiversity, Ecology and Toxic Principles in Plants – Case Study: Fungal Biocontrol

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1. Introduction

Plants are made up of simple to complex natural plant derived compounds often referred to as toxic principles in plant. These natural compounds comprise acids, alkaloids, gossypol, glycosides, minerals, proteins, amino-acids, resins, resinoids, unclassified toxins and plants causing mechanical injury (http:// quizlet.com/3348648/toxic-principles-in-plants-flash-cards/ 2010). They are valuable components of plants, animals as well as man where they contribute to metabolism, resistance against pests and diseases (Shafique *et al*., 2011). It has been argued that some purified forms are beneficial in the pharmaceutical world (http://www.blueplanetbiomes.org/amason.htm2009). Biodiversity is defined as the variety of species, their genetic make-up, and natural communities in which they occur. It comprises all native organisms (plants and animals) and the processes that sustain life on earth. It is the variation of life in an area. It can be genetic or ecological. Genetic diversity deals with various genes needed by organisms to survive. On the other hand, ecological diversity is the diversity of all living things within an ecological zone such as rainforest, savannah, desert, arid and semi-arid ecologies (http://www.blueplanetbiomes.org/ amason.htm2009).

Biodiversity is a vital component of an ecosystem. It is found wherever there is life where it maintains oxygen balance in the air, replenishes the soil, purifies water, protects against erosion, flood, acts as wind shield, regulates storm and climate damage. Rapid loss of biodiversity is on the increase, due to demands from growing global population, technology advancement and societal consumption. In response to the above challenge, there have been concerted efforts to explore the environment and to utilise available resources for sustainable development. One of such effort is directed towards biological control (biocontrol) of fungal pathogen. The approach generally utilises living organisms (plants and/or animals) or natural products derived from these organisms called biofungicides. This approach relies on basic principles of plant pathology and disease triangle to achieve fungal control (Bailey, 2004). It employs the principle of Koch postulate, assessment of growth characteristics, site of infections, host range for crop tolerance and efficacy on other pests.In the past, some group of thought believe this approach may not be suitable other than greenhouses and forestry. However, the existence of broad spectrum biofungicides coupled with consumer`s attitude towards food safety, synthetic pesticide usage, demand for environmentally friendly biofungicides and preference for organic or certified fungicide-free-produce have made biocontrol appealing (Peluola, *et al*. 2010, Bailey, 2010). Biofungicides of plant origin is a promising management strategy for a safer and healthier environment (Obi, 1991, Sangoyomi, 2004, Peluola, 2005, Peluola *et al*., 2007; 2010, Akinbode and Ikotun, 2008, Shafique *et al*., 2011). Their occurrence within genetically similar species may not differ; however, recent researches suggest variations in their potency against fungi pathogens, their morphology and state of their by-products. Further research (Shafique *et al.*2011) also established the occurrence of these toxic materials in different forms on plants. However, there are fewer researches aimed at examining the possible diversity of toxic principles in plant due to ecological or geographical locations where such plants are found. In order to maximally benefit from arrays of potentials available from plant products, the needs to evaluate promising plant products in relations to ecological zones where they occur is imperative. Proximity to natural ecosystem where such potential biopesticides are found is paramount to sustainable production of active compounds and subsequent utilization for sustainable development. This chapter focuses on the authors' experiences on extraction, bioassay and effectiveness of potential biofungicides across agro-ecological zones in the control of fungal pathogens of arable crop. Judicious application of the complex interaction between ecology, biodiversity and toxic principles in plants is a feasible tool for sustainable management of fungi pathogens.

2. Diversity of biofungicides of plant origin

Biofungicides exhibit potential and physical variations (Peluola 2005). Its potential lie in variations in factors necessary to determine its ability to manage or control the target organism. Such factors include variation in fungitoxicity (fungistatic or fungicidal), minimum inhibition concentration, effectiveness or sensitivity to target organism and stability of active component on storage. Mechanisms of action are both physical and biochemical (Bailey, 2010). Fungi pathogens respond in three different ways to treatment with plant metabolites. They are either inhibitory, stimulatory or no effect. Inhibitory effect occurs when the presence of plant metabolites lead to a reduction in growth or complete death of fungal pathogens. Stimulatory lead to an increment in the growth of fungal pathogens above the untreated and no effect is a similar response in growth as in untreated pathogen. In a search for potential biofungicides, twelve tropical plants were evaluated for their fungitoxic effect on some fungal pathogens. The result showed that active components extracted in water were either inhibitory or had no effect. In contrast, ethanol and hexane extracts were stimulatory on the pathogen *Colletotrichum destructivum* O Gara treated with *Parkia biglobosa* (L.) leaf extract and *Morinda lucida* at (-20.0 and -23.9%) ethanolic extract and (-22.0 and -66.4%) hexane extract respectively (Peluola, 2005). On the other hand, physical variation has to do with variation in visual observation, morphology and state of biofungicides.

Biodiversity of biofungicides can be viewed in two dimensions, the intra–specific and inter-specific diversity.

2.1 Intra-specific diversity

Intra-specific diversity deals with the intrinsic factors that lead to variation in diversity. The following case studies are used to demonstrate intra-specific diversity among some medicinal plants and to understand possible link between ecology, biodiversity and toxic principles in plant.

2.1.1 Case study 1: Diversity of neem seed oil (*Azadirachta indica* (A.) Juss) across agro-ecological zones

In a survey conducted across five savannah agro-ecological zones in Nigeria, West- Africa (Table 1) to determine the physical variations of neem seed oil across the savanna agro-ecological zones (AEZs) of Nigeria. Samples were collected from derived savanna (DS) southern guinea savanna (SGS), Northern guinea savanna (NGS), and Arid/semi- arid savanna (ASS) zones of Nigeria occupying about 80% of Nigeria land. Over 70% of West African land is situated in the savanna zones. (Isichei and Awodoyin,1990; Awodoyin et al., 1997). Oil yielding capacity of neem seed, colour of oil and neem cake (residue left over after the extraction of oil from neem seed) and physical state of oil were examined. Neem seed oil from savanna AEZs of Nigeria varied in all the parameters observed i.e. (oil yielding capacity, colour and state at 4°C). The highest volume of oil produced per kg of seed is 276ml for seeds from Hadejia (ASS) and lowest volume of oil of 142ml was obtained from Bida (SGS). The trend volume of oil extracted from 1kg of seed was as follows: Hadejia (ASS) 276.00ml > Zaria 270.00ml (NGS) > Ogbomosho (DS) 208.00ml > Mokwa (SGS) 183.00ml > Ilorin (DS) 170.00ml > Kaduna (NGS) 156.00ml and > 142.00ml in Bida (SGS) per kilogram of seed (Table 2).Variation observed in the oil may be due to change in climatic factors eg annual rainfall, temperature, soil and humidity as we moved up the Northern parts of Nigeria.

Location	Agro-ecological zones (AEZs)	State	Collection co-ordinates
Oghomosho	Derived Savanna (DS)1	Oyo	8°13′N,4°26′E
Ilorin	Derived Savanna (DS)2	Kwara	8°50′N,4°55′E
Mokwa	Southern Guinea Savanna (SGS)1	Niger	9°18′N,7°38′E
Bida	Southern Guinea Savanna (SGS)2	Niger	9°08′N,7°05′E
Abuja	Derived Savanna (DS)3	FCT	9°05′N,6°01′E
Kaduna	Northern Guinea Savanna (NGS)1	Kaduna	10°52′N,7°44′E
Zaria	Northern Guinea Savanna(NGS) 2	Kaduna	11°11′N,7°38′E
Kano	Sudan savanna	Kano	11°58′N,8°30′E
Gumel	Sudan savanna	Jigawa	12°631′N,9°38′E
Hadejia	Sahel savanna	Jigawa	12°45′N,10°05′

Where FCT= Federal Capital Territory

Table 1. Agro-ecological zone for the collection of neem seed and leaf samples in Nigeria, West-Africa.

In Nigeria, distinct variation exist across agro-ecological zones especially in respect to annual rainfall (1200-1500mm for southern guinea savanna (SGS) and 300-500mm for Arid and semi-arid savanna (ASS) zones) and length of growing season (7-9 months, for SGS and 3-4months for ASS). This result suggests the importance of drier savanna in the production of neem seed oil. Color of neem seed oil and neem cake also varied by agro-ecology. Neem trees in Nigeria contain abundant oil in the range of 140 ml/kg-276 ml/kg (Table 2) relative to 150 ml/kg-200 ml/kg obtainable in the USA and Asian countries (HDRA, 1999).

2.1.2 Fungitoxicity and control of seed and soil-borne fungal pathogens of cowpea (*Vigna unguiculata* (L). Walp) with neem seed oil

Fungitoxicity test on neem seed oil extracted across the savanna agro- ecological zones *(AEZs)* of Nigeria were further tested on mycelial growth of *Colletotrichum capsici (Syd.) Butler and Bisby)* and *Macrophomina phaseolina* Tassi Goid of cowpea in Nigeria. Biplot was employed to access variation and interactions across the AEZs. Interaction and variations among entries and environments and between entries and environments were accessed.

Entries close to the origin possesses an average performance across all the environments, and entries close to each other have similar performance. Small angle between two environments vectors indicates strong positive association (similarities) between two environments. Ninety degree angles indicate no association and angle greater than 90^0 represent dissimilar (Hess *et al*.2002).

On *M. phaseolina*, neem oil extracted from Ilorin, Ogbomosho (derived savanna) and Mokwa (southern guinea savanna) performed similarly while strong positive association existed between Ogbomosho and Hadejia (Sahel savanna) samples. On *C. capsici*, samples from Bida (southern guinea savanna) and Ilorin (Derived savanna) were similar (fig 1) (Peluola, 2005; Peluola, *et al*., 2010).

Neem seed oil from savanna (AEZs) of Nigeria differed significantly in respect of their *in-vitro* effects on two cowpea fungal pathogens, *Macrophomina phaseolina* (Macrophomina blight) and *Collectotrichum capsici* (brown blotch). Thus, their effects on mycelial growth of the two fungi on APDA as well as their minimum inhibition concentrations and overall rating of efficacy were significantly different from one another. These differences are not attributable to variation in latitudinal location of the seed collection sites since oil extract from seeds collected from Hadejia in Sahel savanna and Oghomosho (in derived savanna) reduced the most the *in-vitro* radial mycelial growth of both fungi.

Location / AEZs	**Volume of oil in ml/Kg neem seeds**	**Color of oil**	**State of oil at 4^0C**
Ogbomoso (Derived savanna)	208.0	Brown	Solid
Ilorin (Derived savanna)	170.0	Dark golden brown	Solid
Mokwa (Southern guinea savanna)	183.0	Golden brown	Solid
Bida (Southern guinea savanna)	142.0	Brown	Liquid
Kaduna (Northern guinea savanna)	156.0	Golden brown	Liquid
Zaria (Northern guinea savanna)	270.0	Dark golden brown	Liquid
Hadejia (Sahel)	276.0	Dark golden brown	Liquid
Standard Error	20		
C.V. (%)	3.6		

Table 2. Physical variations observed in neem oil extracts from different Agro-ecological zones in Nigeria.
Color characteristics of AEZs oil were based on mycological color identification guide of Rayner (1970).

Neem trees occur abundantly in northern Nigeria. As shade and aesthetic trees, they line many streets in the towns; they are frequently used in shelter belts and as shade trees in farms. Neem trees produce large quantities of seeds that drop and decay, apparently with little or no value to farmers. However, neem seed oil is very easy to produce (HDRA,1999) and it can be produced locally like groundnut oil and used as an effective natural pesticide against pests and pathogens as reported by earlier researchers e.g (Ketkar and Ketkar,1993;

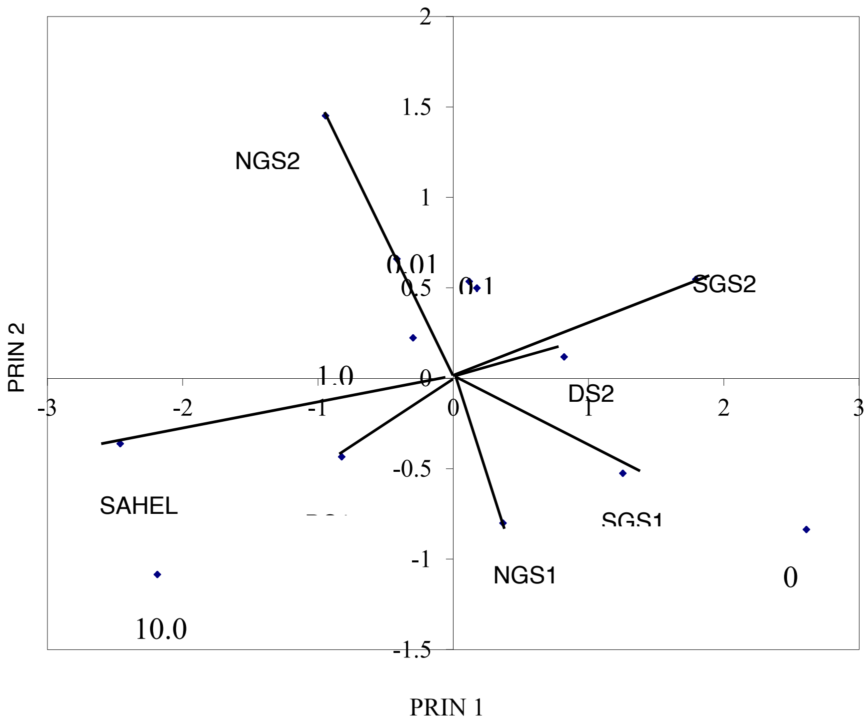
Ivbijaro,1990;Ivbijaro,1993;Obi,1991;Lale and Abdulrahman,1998). In the present study, there was no clear trend in relationship between the toxicity *in vitro* of neem seed oil to the two fungi pathogen and latitudinal location of the seed collection sites. For example, the highest toxicity to *M. phaseolina* was produced by oil extracted from seed collected from Hadejia in Sahel savanna which was followed by oil extracted from seeds collected from Ogbomosho (derived savanna),which both are at two ends of the country from wet to arid environments. Similarly the greatest reduction in mycelial growth *in vitro* of *C. capsici* occurred in APDA containing oil from neem seeds collected at Hadejia which was followed by oil from seeds collected at Ogbomosho. In this case, however, the next most effective oil extracts were from seeds collected from Kaduna and Zaria, in that order, both located in the northern Guinea savanna. Also, oils from seeds collected at two locations in the southern guinea savanna (Mokwa and Bida) and one location in the derived savanna (Ilorin) have no effect on *M. phaseolina*. However, they were effective or moderately effective on *C. capsici*. It appears that the fungal toxicity of oil extracted from neem seeds is location specific and is probably associated with ecotype or variety or strain of neem grown in these environments as suggested by Dales (1996) and Pamplona and Roger (1999).
The report of biplot analysis generally corroborated locational specificity of neem oil at varied concentration tested. This is probably the first report of a study in which fungal toxicity of neem seed oil was related to the geographical location of the trees that produced the seeds. The results suggest that it may be necessary to evaluate the relative efficacy of neem seed oil from different locations in any effort to use the oil as pesticide against specific pathogens (Peluola *et a*l. 2010).

2.1.3 Benefical effect of neem oil diversity

Other benefits from the oil such as provision of raw material for industries, neem cake production (good source of urea), soap making, source of income for the poor especially women and change in economic status of rural dwellers can be maximally utilized. Neem oil from derived (DS) and southern guinea savanna zones of Nigeria are solid at 4^oC while northern guinea savanna (NGS), arid and semi- arid savanna (ASS) are liquid. Similarly, as we move from the southern zones to the northern Nigeria, color of oil produced per AEZs varied from greyish Sepia (DS) to Sepia and Cinnamon (SGS), Umber to Fuscous black (NGS) and dark slate blue (ASS). According to Dales, (1996); Pamplona and Roger, (1999), differences in trees may be due to genetic components or environments where they are found. The inter-zonal variations may be due to closeness in physical and .environmental factors within the AEZs such as rainfall, relative humidity, temperature and soil type or genetic differences among neem trees. However, this study brings out the inter- zonal relationship and differences in neem seed oil across the savanna AEZs of Nigeria. It also identify agro- ecological zones of higher oil yielding capacity from neem seed. This information will be relevant to agro-allied company and industries of areas where maximum raw materials for their production are available. Decision on suitable site for the establishment of biopesticide industry in order to promote low cost but sustainable large scale production can be achieved.

2.2 Case study 2: Diversity of neem leaf extracts as potential biofungicdes for the control of anthracnose disease of cowpea (*Vigna unguiculata* (.L). Walp)

In a similar survey carried out to determine the variation in the fungitoxicity and effectiveness level of aqueous and ethanol neem leaf extract from savanna agro- ecological zones (AEZs) viz: derived savanna (DS 1, and 2), Southern guinea savanna (SGS 1, 2 and 3), Northern guinea savanna (NGS 1 and 2), Sudan savanna (SDS 1and 2) and Sahel savanna (SHS) zones of Nigeria. Graded extracts at concentration of 0, 5, 10, 25 and 50% were tested on *Colletotrichum destructivum* for fungitoxicity and effectiveness levels as highly, effective, moderately, slightly and not effective across AEZs. Data were subjected to ANOVA. Ethanol extract significantly (P≤ 0.05) reduced growth than aqueous extract across both concentration and locations. Effectiveness level of extracts were slightly to highly effective and significant (P≤ 0.05) across concentrations and locations. Growth inhibition of ethanol leaf extract ranged from 39.0% (SGS 1) to 64% (SHS 1) relative to 8.13% (NGS1) and 64% (SGS 2 and SHS) aqueous.The percentage reduction in radial growth of *C. destructivum* produced by ethanol extract of neem was consistently superior to that of aqueous extracts across all locations and concentrations except for Sahel savanna and southern guinea savanna 2. Although the minimum effective concentration of both ethanol and aqueous leaf extracts were the same, ethanol extract of the leaf from eight of the ten locations were rated as highly effective compared to only three locations for the aqueous extract. These observations suggest that ethanol is a better extractor of fungitoxic substances of neem leaves than water. This agreed with the report of Lale and Abdulrahaman (1998), who showed that ethanol extracted more potent insecticidal components of either neem seed or seed oil than aqueous or hexane extracts.



Where PRIN= Principal component; DS= Derived savanna 1&2; NG= Northern guinea savanna 1&2; SGS=Southern guinea savanna1&2;

Fig. 1. Biplot showing the relationship between neem seed oil extracted from different location in the savanna AEZs of Nigeria at different concentration.

Locations/ concⁿ (%)	0.01	0.1	1.0	10.0	MIC(%)	Level of Effectiveness
Ogbomosho	0	0	3.7	25.9	1.0	(ME)
Ilorin	0	0	0	0	NA	(NE)
Mokwa	0	0	0	0	NA	(NE)
Bida	0	0	0	0	NA	(NE)
Kaduna	0	0	0	0	NA	(NE)
Zaria	0	0	0	24.1	10.0	(ME)
Hadejia	0	0	19.6	33.3	1.0	(ME)
Overall mean	0	0	23.3	83.3		
Standard Error	0	0	2.76	5.71		
CV	0		31.36	18.14		

Values are means of three replicate plates per treatment per pathogen. Where MIC = minimum inhibition concentration, $conc^n$ (%)= Percentage concentration of oil extract, NE=Not effective; ME=Moderately effective;

Table 3. Percentage inhibition of radial growth of the *M. phaseolina* treated with oil extracts from different agro ecological zones.

Locations/concⁿ (%)	0.01	0.1	1.0	10.0	MIC(%)	Level of Effectiveness
Ogbomosho	39	36.2	36.7	66.1	0.01	(HE)
Ilorin	15.2	21.6	37.3	43.5	0.01	(EE)
Mokwa	23.7	23.7	28.2	37.3	0.01	(ME)
Bida	16.7	23.2	37.9	44.1	0.01	(EE)
Kaduna	20.5	35.6	37.9	65.3	0.01	(HE)
Zaria	ND	45.8	55.4	54.8	ND	(EE)
Hadejia	23.8	35	50.3	74.6	0.01	(HE)
Overall mean	138.9	221.1	283.7	385.7		
Standard Error	16.80	25.52	32.65	44.45		
CV	32.00	30.54	30.45	30.49		

Values are means of three replicate plates per treatment per pathogen. Where MIC = Minimum inhibition concentration., $conc^n$ (%)= Percentage concentration of oil extract NE=Not effective; ME= Moderately effective; EE= Effective; HE= Highly effective;

Table 4. Percentage inhibition of radial growth of the *C.capsici* treated with oil extracts from different agro ecological zones.

Regardless of the extracting medium, there were significant differences in the toxicity of leaf extracts to *C. destructivum*. However, neem leaf extract toxicity to the pathogen did not show the agro-ecological zones of the sites from which the leaves were collected. Thus although, across concentrations leaves from southern guinea savanna 2 had the highest toxicity, similar extracts from southern guinea savanna 1 had the least but two toxicity. In respect of the aqueous extract, the most efficacious extract across concentration was obtained from leaves collected from Hadejia (Sahel savanna) and in southern guinea savanna which are two contrasting agro-ecological zones especially in respect to annual rainfall (1200-1500mm for southern guinea savanna and 300-500mm for Sahel savanna) and length of growing season (7-9 months, for southern guinea savanna and 3-4months for Sahel savanna). Since the neem *in vitro* result of the present study suggests that leaf extract toxicity to *C. destructivum* is probably location specific, leaves for extraction should be collected from sites where leaves gave the highest toxicity, depending on the extraction medium.

2.3 Case study 111: Comparative efficacy studies across agro-ecological zones on neem seed oil, aqueous and ethanol leaf extracts for control of brown blotch pathogen

Efficacy studies among neem seed oil, aqueous and ethanol leaf extract were carried out with *Colletotrichum capcisi* causal organism of brown blotch of cowpea. Graded extracts of aqueous, ethanol and oil extracts were tested from the samples at varied concentrations. The effects of extracts were observed on mycelial radial growth and conidial germination of *Colletotrichum capsici*, causal organism of brown blotch disease of cowpea in Nigeria. Neem products inhibited the growth of *Colletotrichum capsici* above 50% across all agro-ecology in savanna zone of Nigeria in this study. Efficacy of the extract was significantly different at P< 0.001. There are indications of higher toxicity to the pathogen as minimum inhibitory concentrations of mycelial growth were observed at lowest concentration tested. This observation is in agreement with Owolade *et al*. (2003) who observed that neem leaf extract, reduce the incidence of *Fusarium moniliforme* Sheld on maize seeds. Peluola *et al*. (2007) also observed that neem leaf extract effectively inhibited the growth of *Colletotrichum destructivum in- vitro*.

Location/conc^n (%)	5	10	25	50	MIC(%)	Level of effectiveness
Oghomosho	40.9	48.4	59.7	64	5	HE
Ilorin	30.6	38.7	40.9	53.8	5	EE
Mokwa	29.6	32.8	45.2	49.5	5	EE
Bida	51.6	53.2	59.1	71.5	5	HE
Abuja	48.4	47.3	59.1	80.1	5	HE
Kaduna	ND	ND	62.9	71.5	ND	HE
Zaria	32.3	38.2	76.3	96.2	5	HE
Kano	53.8	60.2	65.6	76.9	5	HE
Gumel	14.5	50.5	55.4	67.7	5	HE
Hadejia	51.6	53.2	59.1	71.5	5	HE
Overall mean	35.3	42.3	58.3	70.3		
SE	4.2	2.8	3.1	4.2		

Values are means of three replicate plates per treatment per pathogen. Where MIC = minimum inhibition concentration, conc^n (%)= Percentage concentration of leaf extract NE=Not effective; ME= Moderately effective; EE= Effective; HE=Highly effective;

Table 5. Percentage inhibition of radial growth of the *C.destructivum* treated with ethanol neem leaf extracts from different agro ecological zones.

Neem leaf extracts (aqueous or ethanol) compared favourably well with their seed oil counterparts at 10% extract concentrations (Table 6). Effectiveness levels appear to be location specific, especially at lower concentrations. For example, derived savanna1 was slightly effective (SE) 1-20% inhibition in aqueous leaf extract, effective (EE) 41-60% inhibition in ethanol and highly effective (HE) above 60% inhibition in seed oil extract at 10% extract concentration. However, effectiveness levels in derived savanna 2 was all effective (EE) 41-60% inhibition across aqueous and ethanol leaf extract and seed oil extract while Sahel savanna gave highly effective (HE) above 60% inhibition on the pathogen at 10% neem extract concentration (Table 6). At higher concentrations of 25 and 50% leaf extract, aqueous and ethanol leaf extracts gave higher inhibitions above 60% inhibition on the pathogen. However, ethanol neem leaf extract was not significantly different from aqueous neem extract in toxicity to the pathogen. According to Obi, (1991) and Lale and Abdulrahaman (1998), synthetic solvents are better extractor of bio-active component in plant materials. Effectiveness level at these concentrations (25 and 50%) were all highly effective (HE) above 61% inhibition with the exception of derived savanna 2 which was effective (EE) at 41-60% inhibition. Hence, aqueous extracts are economically viable and recommended for use at farm level due to its reduced cost. Minimum inhibition concentration (MIC) showed that neem leaf extract inhibited the growth of the pathogen at the lowest concentrations (5% neem leaf and 0.01% seed oil extract) tested. The drier savanna (The Sahel) consistently gave higher inhibition of growth on the pathogen when tested in neem seed oil, aqueous or ethanol neem leaf extract in this study (Table 6). This might be due to reduced volume and number of rainy days per year in the far north compared with the south annual rainfall (1200-1500mm for southern guinea savanna and 300-500mm for Sahel savanna) and subsequent length of growing season (7-9 months,

Locations /concentration (%)	AQ 10	LE	ET 10	LE	SOE 10	LE
Oghomosho	9.6	SE	42.9	EE	66.1	HE
Ilorin	49.2	EE	44.1	EE	43.5	EE
Mokwa	79.7	HE	40.7	EE	37.3	ME
Bida	78.0	HE	76.3	HE	44.1	EE
Abuja	24.3	ME	45.2	EE	ND	ND
Kaduna	27.7	ME	51.4	EE	65.3	HE
Zaria	35.0	ME	27.7	ME	54.8	EE
Kano	39.0	ME	55.4	EE	ND	ND
Gumel	45.2	EE	63.3	HE	ND	HE
Hadejia	79.7	HE	76.3	HE	74.6	HE
Overall mean	46.6		52.3		55.1	
Standard Error	7.8		5.0		5.3	

Values are means of three replicate plates per treatment. Where LE= Level of effectiveness; SOE= Seed oil extract from neem; ET= Ethanolic neem extract; AQ= aqueous neem extract; NE=Not effective (-0); SE= Slightly effective (1-20%); ME=Moderately effective (21-40%); EE= Effective (41-60%); HE=Highly effective (61-100%)

Table 6. Comparative inhibition% of radial growth of *C .capsici* treated with aqueous, ethanol, or neem seed oil extracts at 10% neem extracts concentrations from different agro ecological zones.

for southern guinea savanna and 3-4months for Sahel savanna. Hence concentrated sap of neem trees in the north acted in synergy to increase toxicity of neem products above other agro-ecologies in the savanna of Nigeria. The result therefore suggests Sahel savanna as better location for producing neem based organic pesticides. It also suggests 25% concentration of aqueous neem extract across all agro-ecologies for use by the peasant farmers. It is well documented that neem products are effective against certain diseases of plant and animals across the globe (Neem Tree Farms, 2005, White, 2004, NRC, 1992). However, its activity against plant diseases across wider agro-ecology in its natural habitat will be very important for policy /decision makers, industrialists, large scale and peasant farmers in the area of maximizing profit from their farm produce. This result indicates that toxicity and control of fungi pathogens with biopesticides depend on location of extraction, extracting medium and the pathogen under control. However, the importance of diversity across a range of agro-ecology is evident in these studies.

3. Inter-specific biodiversity and fungal biocontrol

Biodiversity can also be explained as diversity among species of the same plant. Nature's provision is such that plant occurs in different forms within the plant family. Such is referred to as plant species diversity. Diversity within plant species can be observed in the physical (color, taste, size of development, and other agronomic characteristics) or on its active component. For example, garlic, (*Allium sativum*) is widely grown and consumed globally. It is believed to have antimicrobial and antifilaria potencies. Traditionally, *A. sativum* has been used in the treatment of worms and dysentery in children and adults. In the kitchen, garlic is used for seasoning. There are several varieties of garlic but the most popular ones are the common white and the common pink (Ngeze, 2001). In order to verify antifungal potentials of garlic; extracts from common pink and common white garlic were tested on mycelial radial growth, spores, and sclerotial production of *Macrophomina phaseolina* (Tassi) Goid, *Colletotrichum destructivum* O gara and *C. capsici* (Syd) Butler and Bisby pathogens of cowpea *in-vitro*.

At 4days after inoculation, the *in-vitro* result gave 100% inhibition on the mycelial, conidial and sclerotial germination of pathogens treated with both pink and white garlic extracts. However, at 5 days after inoculation, pink or white garlic extracted in ethanol generally gave reduced inhibition relative to their aqueous garlic extracts till seven days after inoculation. Media treated with white garlic extracts and inoculated with *M. phaseolina* gave highest inhibition percentages of 91.5 and 82.6% in aqueous and ethanol garlic extracts respectively relative to the control plate at highest concentration of 250ppm(Table 7).

Similarly, media treated with common pink garlic and inoculated with *M phaseolina* gave highest reduction of mycelial growth of 72.0 and 65.1% respectively on aqueous and ethanol pink garlic extract after 7days of inoculation. The least percentage inhibition of 27.7% was observed on *C. capsici* when treated with the common pink garlic while the white variety showed its least inhibition percentage of 34.45 % on *C. destructivum*. The result on *M. phaseolina* was not significantly different at $P \leq 0.05$ and compared favorably with conventional fungicide benomyl at 0.5gai/kg (recommended concentration rate).

The fact that white garlic extract consistently prove more effective at inhibiting the mycelial growth of the three cowpea pathogens than the pink garlic is an indication of variation in active constituent due to the existing garlic varieties.

Garlic extracts effectively inhibited the mycelial growth, conidial and sclerotial germination of the three pathogens *in vitro*. This observation agreed with the work of Sangoyomi (2004)

Pathogen	Pink Garlic[e]		White Garlic[e]		Benomyl
	Aqueous	Ethanol	Aqueous	Ethanol	
M. phaseolina	72.0a	65.1a	91.5a	82.6a	90.0a
C. destructivum	36.0b	34.2b	41.5b	33.39b	100.0a
C. capsici	22.0c	27.7c	54.1a	34.45b	100.0a

Values are means of three replicate plates per treatment. Where *M. phaseolina=Macrophomina phaseolina, C. destructivum = Colletotrichum destructivum & C. capsici = Colletotrichum capsici,* Garlic[e] = garlic extract

Table 7. Percentage inhibition of growth of three fungal isolate due to treatment with pink or white garlic extract and compared with benomyl.

who reported that aqueous extract of garlic effectively inhibited mycelia growth, conidia, pycnidia and sclerotial production of *Butryodiplodia theobromae, Aspergillus niger*, *Sclerotium rolfsii, Rhizoctonia solani,* and *Nattrassia mangifera*, fungal pathogens of yam in storage. Ismail *et al*., (2001) reported the control of Colletotrichum sp and *M. phaseolina* in jute using garlic extract at ratio 1:2. The result suggests common white garlic extracts a suitable biofungicide against cowpea fungal pathogens. It also explains the inter-specific biodiversity among the garlic spp. Further research may need to look into on-field trial of the spp before embarking on large scale production of the active constituents as biopesticide.

4. Eco-biodiversity and sustainable agriculture

In the classical examples on fungal biocontrol discussed above, it is evident that the role of eco-biodiversity in sustainable agriculture is significant in the search for sustenance of individual within and across her agro-ecology. Lately many products from neem (*Azadiractha indica* (A). Juss) had been patented for use in the developing and developed world (White 2004, Dales 1996 and NRC, 1992). In human medicine, its twigs have been used for the treatment of teeth disease and as a popular traditional remedy against malaria an endemic disease in the tropic. Every parts of the plant in the crude and refined forms had received much attention. The seed oil has been used as remedy against several pests and diseases of plant as insecticidal, fungicidal, nematocidal and bactericidal among others (HDRA 1999; Lale and Abdulrahaman, 1999; Onalo, 1999; Ketkar and Ketkar 1993; Saxena, 1993; Ivbijaro, 1990). The active ingredients found in neem seed oil are mostly the triglycerides i.e. oleic, stearic, and palmitic acids (Saxena, 1993) and are just like ordinary vegetable oil (National Research Council, 1992). Therefore it can serve as essential raw material in the pharmaceutical, agro-allied, soap and detergent industries among others. Products from neem are natural, biodegradable, environmentally friendly, less toxic and leave no residue in the environment. It is of tropical origin and popularly known for its risk-free effect to beneficial microorganisms. The safety of neem derivatives to beneficial microorganism, rapid biodegradability and soft mode of action makes it an appropriate biopesticide. Biotechnology and biological science research council (BBSRC) is an international organisation that deals with different aspects and innovations in biological research. The body recognises four levels of biodiversity as molecular, gene and genome, organism and habit and ecosystem diversity. Habitat and ecosystem diversity can be viewed in a similar context with ecological diversity which is the variation in species within and between different habitats and ecosystems. Phylogeny believes that all diversities are related through common ancestry. Nature is endowed with array of blessings necessary to meet man's sustainable needs within a location. Interference by man and the challenge of meeting the needs of increasing population led to huge depletion of natural resources. For example, it is reported that human activities increased extinction rate 1000 times its usual rate (www.pabiodiversity.org/what is biodiversity .html). Exploitation of our environment must be carefully done to prove our understanding of how management affects the ecology. The need to identify what is available, researches its usefulness and identifies strategies of managing and preserving them is important. For example, a particular specie is identified as prevalent in an ecological zone, there is much likelihood that the plant had survived competition from other plants species for it to remain dominant and to sustain dominance within its ecology. Ideally, local uses of such plant might have existed, further research meant to explore other benefits associated with plants capitalizing on their ability to survive competition and exploiting the value of functional biodiversity in sustainable agricultural context will be important.

BBSRC also identify as part of their main objectives the need to uunderstand the ecological significance of intra-specific variation and inter-relationships between inter- and intra-specific variation, investigating biodiversity processes in relation to different land-use patterns and different agricultural systems and also making efforts at conserving habitat and ecosystem diversity.

5. Conclusion

Agro-ecological methods, including organic farming practices, can help farmers protect natural resources and provide a sustainable alternative to costly industrial inputs.Agriculture can address environmental challenges, past approaches to combat hunger have tended to focus narrowly on a few types of crops, rely heavily on chemical fertilizers and ignore women farmers. Agricultural practices that emphasize increased production have contributed to the degradation of land, soil, and local ecosystems, and ultimately hurt the livelihoods of the farmers who depend on these natural resources.

'Moving Eco-agriculture into the Mainstream' was one of the 15points recommended by World Watch Institute's on Nourishing the planet team during this year earth day (April 18, 2011). In this era of global climate change and economic recession, the emphasis should be toward ecological agriculture as a means of generating both economic value and sustainable development (World Watch Institute, 2011). A global approach towards ecological agriculture utilizing the nature's provision within or across ecosystem is a move in the right direction. In a similar approach, recently, the United Nations Industrial Development Organisation (UNIDO) has embarked on a major initiative to promote appropriate low-cost, but sustainable technologies and techniques for large scale production of biopesticides from neem in West Africa (UNIDO, APRIL 2007, unpublished information). However, this is probably the first report of a study in which the fungitoxicity of botanicals were related to sites of collection across a wider range of agro-ecological zones.

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