DIAGNOSIS AND MANAGEMENT OF 'DANZAZZALAU' DISEASE OF ONION (*ALLIUM CEPA* L.) IN KEBBI STATE, NORTHWEST NIGERIA

BY

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M.Sc./AGRIC./31279/2012-2013

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (M.Sc.) DEGREE IN CROP PROTECTION

DEPARTMENT OF CROP PROTECTION,
FACULTY OF AGRICULTURE,
AHMADU BELLO UNIVERSITY,
ZARIA NIGERIA

APRIL, 2018

DECLARATION

I declare that the work in this dissertation entitled "Diagnosis and Management of 'Danzazzalau' Disease of Onion (*Allium cepa* L.) in Kebbi State, Northwest Nigeria" has been carried out by me in the Department of Crop Protection. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

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CERTIFICATION

This dissertation entitled "DIAGNOSIS AND MANAGEMENT OF 'DANZAZZALAU' DISEASE OF ONION (*ALLIUM CEPA* L.) IN KEBBI STATE, NORTHWEST NIGERIA" by Abdulbasit SHIYANBOLA meets the regulations governing the award of the degree of Master of Science (M.Sc.) in Crop Protection of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to Allah (SWT) who made it possible for me to complete it, and my family who stood firmly by me.

ACKNOWLEDGEMENT

All glory, honour and adoration are to Allah (SWT) for all His privileges and mercies upon me in seeing me completing this programme.

My most profound and sincere gratitude goes to Prof. O. Alabi (Chairman Supervisory Committee) for her constructive criticism that shaped this work into what it is. Prof. O. O. Banwo and Dr. S. E. L. Alao, other members of my supervisory team are appreciated for their advice, suggestions, guidance and encouragement. My sincere thanks and appreciation goes to the Head, Department of Crop Protection, Prof. O. O. Banwo for providing facilities when requested and conducive environment throughout my study period. Drs. Uyovbisere, E. and Anjorin, S. T. who contributed to aspects of laboratory identifications and experiments, accept my deepest appreciation. Management of the Institute for Agricultural Research (IAR) is appreciated for financial support to Kebbi State to collect samples. The Centre for Agriculture and Bioscience International (CABI), Egham, Surrey, United Kingdom is appreciated for identifying the fungi isolates. I wish to thank staff of Kebbi State Agricultural and Rural Development Authority (KARDA) for their assistance during sample collection.

To my late dad, Sheikh Abdulfatai A. Shiyanbola and mum Hajia Haolat A. Shiyanbola, I remain eternally grateful for the love and sacrifice that ensured I get the best education, may Allah (SWT) reward you both with paradise. To my numerous siblings, uncles, aunties, cousins, nephews, nieces and in-laws for their moral and financial support without which this work may not have been accomplished, I pray to Almighty Allah to continue to bless you all.

Messrs Abdulrahman, A., Due, J., Usman, S., Jibril, I., Bawa, I., Yusuf, A., Mrs. Nuhu, F. and Salihu, A. A. are appreciated for their technical inputs during the laboratory work. Mrs. Mamza, W. S., Kwanashie, A. J., Muhammad, B., Abdullahi, A., Yakubu, I., Peter, A., Lurwanu, Y., Abdulsalam, S., Olatunji, J. O., Wali, A. S, Mohammed, S. S. and late Fatima, Y. U are all appreciated for the academic and moral support given during my study. Members of the Department of Crop Protection, ABU, Zaria are appreciated for making my stay very conducive. I wish to thank the following friends for their moral support and prayers: Mal. Usman, I. (Daddere), Abdulraheem, A., Yusuf, A. A., Ibrahim, M., Sadiq, M. A., Jinadu, L. A., Ahmed, U. O., Mrs. Ogungbemi, E., Adeyemo, C., Mohammed, R. N., Abdulwahab, M. J. and Abdulsalam, N. This acknowledgement will be incomplete without saying a big thank you to my course mates namely Musa, N. F., Olatunde, G., Abdullahi, M. U., Isa, A. D., Badamasi, H., Abdulfatah, J. I., Kabir, A. and my senior colleagues, Jibrin, M. O., Ajene, I. J., Chindo, A. C. and Bulus, J. may the Almighty God bless you all.

ABSTRACT

Onion is an important vegetable crop in Nigeria grown in both wet and dry seasons though it fetches higher profit when cultivated in the dry season. The crop yield is limited by some production constraints, notable of which is the 'Danzazzalau' disease in Kebbi State. Fungi isolated from infected pseudostem and roots of onion were *Fusarium* sp. and *Thielavia* sp. Pathogenicity test was conducted using these fungi and *Fusarium* sp. proved to be pathogenic on 6-week old onion seedlings. *Fusarium* sp. was identified as a *Fusarium* species belonging to biological group clade V. by Centre for Agriculture and Bioscience International (CABI), Egham, Surrey, United Kingdom. Trials were carried out in the screenhouse and field during 2015 dry season at Samaru, Zaria to study the effects of water regimes, manure rates and mulch treatments for the management of 'Danzazzalau' disease of onion crop. The treatments consisted of three water regimes (3, 10 and 21-day), three manure rates (0 Kg/plot, 40 Kg/plot, and 80 Kg/plot) and three mulch types (polyethylene sheet, rice straw and no mulch) laid out in a Split Plot Design (SPD) with three replications. Water regime was the main plot while manure rates and mulch types were the sub-plots. Incidence and severity of the disease and its effect on onion growth and yield were determined. Lowest disease incidence (36.02 %) and severity (32.93 %) were obtained at the 21-day water regime. The 21-day water regime and rice straw mulch at 1.2 mt/ha produced highest bulb yield of 4.67 t/ha and 4.80 t/ha respectively which were significantly ($P \leq 0.05$) higher than the other treatments. The efficacy of aqueous and methanol crude extracts obtained from *Jatropha curcas*, *Moringa oleifera* and *Eucalyptus globulus* leaves at 5, 7.5 and 10 g/ml on growth and development of *Fusarium* clade V. was tested *in vitro*. Aqueous extract of Moringa at 10 g/ml (5 % w/v) when used to amend culture media led to decrease in number of conidia

produced by 91.53 %, however when Jatropha and Eucalyptus extracts at 10 g/ml (5 % w/v) were used conidial numbers were reduced by 42.3 % and 19.04 % respectively. Conidial dimensions were reduced (6.32 %, 6.05 % and 0.64 %) when 5 g/ml (2.5 % w/v) aqueous extract of Jatropha, Eucalyptus and Moringa were used to amend the culture media respectively. Methanolic extract of Eucalyptus at 10 g/ml (5 % w/v) when used to amend culture media conidial number was reduced by 88.3 % followed by 64.5 % and 20.10 % reduction when Moringa and Jatropha extracts were used respectively. Conidial dimensions were reduced by 5.31 %, 3.20 % and 1.28 % when 5 g/ml (2.5 % w/v) methanolic extract of Eucalyptus, Jatropha and Moringa were used to amend the culture media respectively. Methanolic extract of Eucalyptus evaluated *in vitro* at 10 g/ml (5 % w/v) was found to be effective in reducing the number of conidia produced and conidial dimensions of *Fusarium* clade V tested. A combination of 21-day water regime and rice straw mulch at 1.2 mt/ha were found to be effective in the management of 'Danzazzalau' disease of onion and may thus be a good component of an integrated disease management (IDM) programme for irrigated onion production.

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CHAPTER ONE

1.0 INTRODUCTION

Onion (*Allium cepa* L.) belongs to the family Alliaceae, it is a monocotyledonous, cross-pollinated crop cultivated mainly as a cool season vegetable (Jilani and Ghafoor, 2003). The crop is grown in most regions of the world, with China, India and United States of America being the largest producers (FAOSTAT, 2014). In Nigeria, 1.32 x 10^6 mt are produced on 42,000 hectares of land which makes it ranks fourth after Egypt, Algeria and Morocco in Africa (FAOSTAT, 2014). Profitable production of onions is favoured by the inclement conditions obtained around the northwestern region of Nigeria. Reports of the National Agricultural Extension and Rural Liaison Services and Federal Department of Agricultural Extension (FDAE) in 2015 showed that the rich alluvial deposits along the River Niger provide suitable soil for its cultivation. The 119.10 x 10^3 and 109.90 x 10^3 mt estimates at Sokoto and Kebbi States respectively in 2014 are highest throughout the country.

World Food Programme reported global usage of onions as food and condiment. Its unique flavour and odour makes it indispensible in preparation of many traditional dishes. The numerous ways in which it is prepared from boiling, frying, stewing, baking, pickling, to eating raw make it a versatile food source (WFP, 2014).

Production of onion as a crop is however, constrained by numerous abiotic and biotic factors. In Nigeria, abiotic factors include nutrient deficient soil, shortage of supplementary irrigation facilities, inadequate inputs and poorly defined post-harvest storage and value addition strategies (Ibrahim, 2014). Pests and diseases have been reported to reduce yield and quality of produce (Currah and Felicity, 1990). Major insect pests include onion thrips, cutworms, leaf miners, onion maggot (*Hylemya antique* Meig.) and several species of mites decimate the crop. Economic diseases of

the crop induced by bacteria, nematodes, viruses, parasitic flowering plants and fungi in the northwestern Nigeria agro-ecology have been listed by Emechebe *et al*. (1980). Some of these diseases include bacterial soft rot, Iris Yellow Spot Virus disease transmitted by thrips (*Thrips tabaci* Lind); root knot nematode disease caused by *Meloidogyne* spp. *Colletotrichum gleoesporioides* causes the onion twister disease which is very prevalent in northwest Nigeria, *Alternaria porri* causes purple blotch; *Peronospora destructor* causes downy mildew disease and neck rot disease caused by *Botrytis allii* are other fungal diseases of this crop. These pathogens cause yield reductions of 50-70 % which discourage production by the farmers.

A relatively new disease of onion was reported by the Kebbi State Agricultural and Rural Development Authority (KARDA) during the 2009 Annual Cropping Scheme Meeting of the Institute for Agricultural Research, Ahmadu Bello University, Zaria. Symptoms ascribed to the disease were rapid protrusion and clock-wise curl of main leaf, die-back and eventual wilting of the whole plant. The plant can be infected at all stages of growth leading to 100 % loss of the crop as reported by the farmers and it discouraged them from continued production. Adopted strategies to manage the disease by farmers have not been successful. The local name 'Danzazzalau' given to the condition reflects the sudden protrusion and curling of main leaf that the farmers observed (IAR Cropping Scheme Report, 2009).

1.1 Justification of the Study

Onion is a highly valued vegetable crop possessing culinary and medicinal values. Major constraints to sustainable production of the crop include high incidence of pests and diseases, nutrient deficient soils; inadequate irrigation facilities, expensive inputs and marketing that gives marginal returns to the farmers. Kebbi State produces 135,000 mt of onion with an average yield of 20-35 mt/ha annually, thus making

Kebbi State the second largest onion farming community in Nigeria after Sokoto State (Usman, 2013). Onion production in Nigeria has been declining due to pests infestation (NAERLS and FDAE, 2015) and Kebbi State particularly has had onion yield stagnated at 109 x 10^3 mt from 2012 to 2014 due to the incidence of a devastating disease locally called 'Danzazzalau'. Total loss especially at seedling stage has been claimed by farmers and all measures to alleviate the menace have proved abortive. When infected stand was uprooted, basal stem plate was rotted and central crown sloughed off. The disease was said to be severe where crop suffers environmental stress and all stages of onion are affected. The need to determine main cause and condition(s) that favour the disease is very important. In view of this, a study to identify the causal organism(s) inducing the disease and proffer management strategies against the disease was carried out.

1.2 Objectives of the Study

The objectives of this research are to:

i. isolate and identify fungi associated with the 'Danzazzalau' disease;

ii. assess the incidence and severity and evaluate some production strategies for the management of the disease;

iii. evaluate *in vitro* the efficacy of aqueous and methanolic crude extracts of some plant materials on the pathogen development.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin and Distribution of Onion

The centre of origin for onion is in Iran and Pakistan and, it was an established culinary ingredient of Egypt and India around 1500 B.C. Since ancient time, it has been of great economic importance for its medicinal and dietetic values (Brewster, 1994). It was a popular food in ancient Egypt, where it was depicted on tombs as early as 3200 BC and has been found in mummies. It is cultivated in most parts of the world, including India, Malaysia, Indonesia, Burma, Philippines, China, Africa, tropical South and Central America and the Caribbean (World Food Programme, 2014).

2.2 Botany of Onion

Onion, *Allium cepa* (L.) is an annual crop which belongs to the family Alliaceae. It is characterized by a pungent alliaceous compound ally-propyl-disulphide (Galeone and Pelucchi, 2007). The bulb consists of thickened bases of leaves attached to a small conical stem, which varies from flat to round in shape. Leaves are long, round, hollow and often greenish in colour, and flowers are small in terminal umbel (Obeng-Ofori *et al*., 2007). The leaves consist of a sheath at the bottom, which attaches around the stem at a node, and the blade which comes off the top of the sheath. Each new leaf forms in the center of the stem, which is inside the sheath of the previously formed leaf, making for a series of round sheaths concentrically arranged. All of the leaf sheaths taken collectively form a stem-like neck, called a false stem or pseudostem, coming off the top of the true stem (Lannoy, 2001).

2.3 Uses and Nutritional Value of Onion

Onion bulb which can be boiled and used in soups and stews, fried or eaten raw in salads is a highly valued vegetable crop possessing culinary and medicinal values. It is useful for the prevention of cardiovascular diseases, diminishes the risk of blood clots and protects against stomach and other cancers (Winston, 2011). It improves lung functions, especially in asthmatic patients. The more pungent varieties of the crop possess the greatest concentration of health-promoting phytochemicals (Galeone and Pelucchi, 2007).

Regular consumption of raw onion has, like garlic, been shown to lower high cholesterol levels and high blood pressure which help prevent heart disease by lowering high homocysteine levels, a significant risk factor for heart attack and stroke (Galeone and Pelucchi, 2007). In the Middle East, both raw and cooked onions are eaten at practically every meal. Arabian herbal medicine recognizes the crop to aid in treating deafness, ear infection, malarial fever, skin problems, stomach cramps, and to improve vision (Ghazanfar, 2007). It is also used to calm stomach upset. Onion along with other herbs is used to rid the digestive system of parasites and stimulates appetite (Ghazanfar, 2007). It also prevents oral infection and tooth decay, the tooth ache is often allayed by placing a small piece of onion on the bad tooth or gum (Bakhru, 2007).

The bulb is a significant source of vitamin C and potassium, low in sodium content and contains 88 % water. One hundred gram (100 g) edible portion contains 60 calories of energy; proteins 1.5 g; fat 0.6 g; total sugar 7.2 g; other carbohydrates 0.3 g; thiamin 0.04 mg; riboflavin 0.02 mg; niacin 0.1 mg; vitamin C 7 mg; Fe 0.5 mg; Mg 16.5 mg; K 150 mg and Na 7 mg (Ado, 2001; Obeng-Ofori *et al.*, 2007).

2.4 Production and Yield of Onion

On a worldwide basis, onion ranks the fifth most important fresh market vegetable (FAOSTAT, 2014). As of 2014, worldwide production was estimated at 84.76×10^6 metric tonnes (mt), Africa produced 8.613×10^6 mt, and Nigeria produced 1.32×10^6 mt (Appendix A), Nigeria is the twenty-fourth top onion producing country of the world and fourth in Africa (FAOSTAT, 2014). Ayodele (1996) reported that in Nigeria, commercial production is predominantly in the northern region where an average bulbs yield of 25 t/ha is obtained from which a land area of between 0.1 and 0.2 million hectares is cultivated annually. In Nigeria, the crop is second only to tomatoes in importance among the vegetables and is mainly grown for its bulb (Hussaini *et al.,* 2000). Kebbi State which is located within the region has annual average production of 135,000 metric tonnes giving a yield of 20-35 t/ha (Usman, 2013). Table 1 shows the major producing states in North-Western Nigeria that include Sokoto, Kebbi, Kano, Zamfara, Katsina, Jigawa and Kaduna (NAERLS and FDAE, 2014).

Table 1: Production estimates of onion in Northwest zone of Nigeria, 2011 to 2015

State	Production (×1000mT)				
	2011	2012	2013	2014	2015
Sokoto	47.5	116.10	119.10	116.17	116.20
Kebbi	44.3	109.70	109.90	109.96	106.02
Zamfara	38.9	95.40	98.40	95.45	95.50
Kano	47.1	94.90	95.60	95.70	95.74
Katsina	31.4	36.90	38.20	59.45	59.87
Jigawa	42.5	56.80	56.80	37.30	37.41
Kaduna	16.3	18.50	18.50	48.80	51.36

Source: NAERLS and FDAE (2015).

2.5 Environmental Requirements for Cultivation of Onion

The Asian Vegetable Research and Development Centre (AVRDC, 1990) reported that onion grows on most types of soil though; well drain ed medium textured soil with pH 6.0-7.0 is preferred. Planting on flat beds enhances good yield. Optimum temperature requirements for the crop are between 15-25 ^{o}C. Growing onion in warmer weather (above 35 ^{o}C) leads to bulb retardation (AVRDC, 1990). Low atmospheric humidity and clear bright days are necessary to ensure insect activity for pollination (Currah and Felicity, 1990). Vitalization which is the initiation of flowering after extended exposure to low temperature is required for flowering and the lower the temperature (15-19 ^{o}C), the faster the vitalization process (AVRDC, 1990). The crop requires a good fertilizer application; the amount of nutrients required for onion production varies from location to location depending on the inherent soil fertility. Fertilizer rate of 65 kg N, 40 kg P and 45 kg/ha is recommended in Nigeria (NAERLS and FDAE, 2015).

2.6 Pests and Diseases of Onion

The crop is attacked by numerous pests and diseases especially in tropical regions where environmental conditions favour their development.

2.6.1 Pests of onion

The common insect pests are thrips (*Thrips tabaci* Lind.), onion maggot (*Hylemya antique* Meig.), seed corn maggot (*Hylemya cilicruca* Rondani.), salt marsh caterpillar (*Estigmene acred* Drum.), wireworms of the genus *Limonius* and cutworms of the family Noctuidae, leaf miner (*Liriomiza langei* Frick.) and several species of mites (Ibrahim and Adamu, 2010; Mishra *et al*., 2014).

2.6.2 Diseases of Onion

In Nigeria, a number of diseases have been identified that affect the crop at different stages of growth and development (Emechebe *et al*., 1980). These include diseases induced by bacteria, viruses, nematodes, parasitic flowering plants and fungi.

2.6.2.1 *Diseases induced by fungi*

Onion twister disease

Colletotrichum gloeosporioides (teleomorph=*Glomerella cingulata*) incites twister disease, also known as anthracnose or seven curls. The fungus may be seed borne or soil borne and can survive on debris and alternate weed hosts (Nischwitz *et al*., 2008). Rain, wind, insects and irrigation water can spread the disease. Infection is favoured by high humidity and temperatures between 23-30 ^{o}C (Conn, 2012). Symptoms induced on affected onion plant include curling, twisting and chlorosis of leaves, roots may be stunted, and there may be an abnormal elongation of the necks. Bulbs of infected plants may appear slender and can decay rapidly when stored. In greenhouse transplant production, roots of young seedlings may exhibit a dark rot with spore masses, especially when root tips protrude from the growing medium. Disease may be patchy or wide-spread (Ebenebe, 1980; Schwartz and Mohan, 1995; Nischwitz *et al*., 2008).

Downy mildew of onion

The downy mildew of onion is induced by the fungus *Peronospora destructor* (Berk.) Casp. The disease symptoms at seedling stage is a furry, violet covering on the outside of the leaf or stem which is conspicuous when foliage is wet with dew, one or two days later the diseased leaf portions become pale green, then yellow, and finally collapse (Schwartz and Mohan, 1995; Hoepting, 2016b). Typically the symptom observed is the brownish-purple velvet-like sporulation of the pathogen on healthy green leaves. As the disease progresses, lesions which are slightly paler than the

normal leaf colour, enlarge and may girdle the leaf (Mishra *et al*., 2014). These lesions progress to a pale yellow followed by brown necrosis resulting in leaf tissues collapse. Infected seed stalks tend to remain pale yellow and the foliage is often invaded by other fungi, typically *Stemphylium* or *Alternaria* species (Aveling *et al.,* 1993; Hoepting, 2016a). The disease is usually observed initially in small patches progressing rapidly throughout the field. Bulbs can be infected and may either rot in storage, or if planted, give rise to pale green foliage seedlings. The fungus survives in volunteer onion plants, onion sets, plant debris or in the soil. The fungal spores are disseminated onto plants by winds and rain splash during cool wet weather, which is essential for disease development. Rain, dew or high humidity (≥95 %) is required for fungal spore germination and infection. The fungus grows internally and continues to produce spores as long as the weather remains cool and wet (Schwartz and Mohan, 2008; Conn, 2012; Hoepting, 2016b).

Purple blotch of onion

The purple blotch of onion is induced by the fungus *Alternaria porri* (Ellis) Clif. Infected onions exhibit small white sunken area with purple centre on leaves. As the lesions enlarge, the leaves dry out and collapse (Tyagi *et al*., 1990; Schwartz and Mohan, 2008). Older leaves tend to be more susceptible than younger leaves. Symptoms begin as water-soaked lesions that usually have a purple centre. Edges of lesions become brown to purple and the leaf turns yellow. With time, dark brown to black concentric rings form throughout the lesions. These are areas of sporulation of the fungus. As the disease progresses, lesion may girdle the leaf causing it to collapse and die. Similar symptoms occur on seed stalks and infected stalks can collapse resulting in shriveled seeds. When bulb infection occurs, it is normally through the neck. If the fungus invades the bulb, the infected area is initially bright yellow, but

eventually turns a characteristic red wine colour (Schwartz and Mohan, 2008; Conn, 2012; Mishra *et al*., 2014). The fungus over-winters as mycelium in leaf debris and cull piles. Spores are formed during humid nights when leaf wetness periods are greater than twelve hours. As the morning dew dries, spores become air-borne and are disseminated to susceptible onion tissues. One-four days are needed for symptoms incubation. Disease development is greatest during prolonged periods of leaf wetness (Tyagi *et al*., 1990; Conn, 2012). The pathogen may also be seed-borne and it is reported as the most important foliar disease of onion in northern Nigeria (Marley, 2013).

2.7 Effects of Cultural Practices on Onion Production

2.7.1 Effect of irrigation on onion production

Onion requires 300-400 ml of water per week, a total of 2500-3000 ml per crop for direct-seeded crops (Hamasaki *et al*., 1999). More water is required during hot and windy conditions, and less during cool, overcast weather. The fact that onion is a shallow-rooted crop, with most of its roots found in the top 20 cm of soil makes it sensitive to small changes in water supply. Critical periods for irrigation are stand establishment and bulb development all through to maturity (Stewart and Nielson, 1990).

Over irrigation especially during cool weather can also result in a greater incidence of pink root rot, which in turn predisposes the bulbs to Fusarium rots at the post harvest stage. Pythium root rot also occur year-round especially when there is excessive moisture in the field (Halim and Mehmet, 2001). Excessive amounts of irrigation will lead to fertilizer leaching particularly nitrogen. The type of irrigation used has an effect on population dynamics of insects, diseases, weeds that affect production programme. For example, overhead irrigation leads to greater incidence of sour skin

caused by *Pseudomonas cepacia* compared with furrow irrigation (Halim and Mehmet, 2001).

Irrigation management uses scheduling to decide when to irrigate and how much water to apply (Stewart and Nielson, 1990). The process of irrigation consists of introducing water into the part of the soil profile that serves as the root zone, for the subsequent use of a crop (Hillel, 1998). Brouwer *et al*. (1989) stated that the purpose of irrigation scheduling is to maximize irrigation efficiencies by applying the exact amount of water needed to replenish the soil moisture to a desired level. Irrigation scheduling methods differ by the irrigation criterion or by the method used to estimate or measure this criterion. Irrigation scheduling is one of the most important tools for developing best management practices for irrigated areas (Al-Jamal *et al*., 1999). Halim and Mehmet (2001) studied the response of onion to different irrigation scheduling and subjected the crop to four irrigation treatments according to available soil water depletion fractions of 0.30, 0.50, 0.70 and no irrigation; they found that the yield and yield components except solids soluble in bulbs were affected by irrigation and soil water depletion fractions. The highest yield was obtained from the plots in which irrigation water was applied at soil water fraction level of 0.30.

Mateen *et al*. (2005) studied the effect of irrigation regimes on growth and yield of onion at 5-,10-,15- and 20-day in addition to the control treatment in sandy loam soil; they found out that 5-day irrigation regime treatment was the better irrigation regime compared with the other treatments in plant growth and onion yield. Mermoud *et al.* (2005) reported that onions prefer longer regimes between irrigations but deeper depths of irrigation. The irrigation frequency was found to be important with irrigating twice a week instead of once a day, leading to increase of the root zone

water storage, better crop water availability through the whole root zone and higher yields.

2.7.2 Effect of manure on onion production

Manure incorporation benefits both soil and crop as it improves tilth of heavy soils, and increases the water holding capacity of light soils. The full benefit of manure is obtained only when the soil is well cultivated, drained and limed (Russell, 2004). Manures are derived from crop debris, municipal wastes, animal wastes, industrial effluents etc. Animal waste make substantial contribution of nitrogen, phosphorus, potassium and other nutrient needs of plants. Total supply however, depends on the nature and size of animal enterprises and methods used in decomposing, storing, and application of the manure (Habtemariam, 2003; Russell, 2004).

Animal manures, particularly cattle dung, were the main source of nutrients for the maintenance of soil fertility in settled agriculture until the advent of mineral fertilizers (Ofori and Sanatanna, 1990). Farmers in developing countries, due to limited financial resources, can barely afford to purchase sufficient mineral fertilizers, and therefore, use animal manures whenever available. Farmyard manures are responsible for nutrient availability, improve soil physical properties (aggregation), and hence improve water retention capacity, infiltration rate and biological activity of soil (Kwakye, 1980). Application of farmyard manure (FYM) is reported to improve soil physical and chemical conditions and to conserve soil moisture (Sattar and Guar, 1989). The advantage of farmyard manure application, however, greatly depends, among others, on proper application methods, which increase the value, reduce costs, and enhance effectiveness (Teklu *et al*., 2004). Fresh farmyard manure should not be applied to horticultural crops as it contains organically bound elements, which are not available to plants until after decomposition. Fresh manures may also have a negative

impact on health of vegetable crops due to competition that arises between the decomposing microorganism and the crop for nitrogen. Therefore, farmyard manure should first be stored for 3-4 weeks in pits and then transported to field during soil preparation for planting. Farmyard manure has comparatively lower C: N ratio (10:1) and hence is more useful in enriching the soils than those with high C: N ratio (18:1) (Malik, 2000). Besides NPK, manures contain all other essential elements. However the nutrient contents of manures may vary depending upon the feed, bedding and the individual animal (Ofori and Sanatanna, 1990).

2.7.3 Effect of mulch on onion production

One of the traditional techniques that have been used since 1950's is the utilization of mulch in vegetable production. When mulch is spread over the soil surface, a favourable soil-water-plant relation is created. The microclimate surrounding the plant and soil is greatly influenced by mulch. For commercial production of vegetable crops in many regions of the world, the use of mulch has become an important cultural practice to maximize water use efficiency by the plant and to improve its growth (Awodoyin *et al*., 2007).

Organic or inorganic soil mulches influence the crop in many ways. Barrier can be provided by plastic mulches against weeds, moisture loss, nutrient loss, erosion, insect and disease injury while enhancing plant establishment and an earlier crop of higher quality (Dilipkumar *et al*., 1990). Organic mulching improves physical, chemical and biological properties of soil, as it releases nutrients to the soil and ultimately facilitates the growth and yield of crops (Dilipkumar *et al*., 1990). Organic mulching possesses an advantage for increased crop production through its positive effect on the soil environment which enhances crop growth and yield (Lal *et al*., 1980). Judicious use of crop residues help to maintain adequate infiltration rates, prevent surface

crusting, improve aggregation of soil and modify the transport and retention of water, heat and air in the soil (Agyenim-Boateng and Dennis, 2001). It was also reported that physical, chemical and biological properties of soils are affected by crop residues. According to the authors, when crop residues are applied as surface mulch they influence the soil positively by conserving moisture, improving soil structure thereby enhancing aeration, infiltration and water-holding ability. Mulching also reduces high soil temperature, controls soil erosion by reducing runoff and heavy impact of rain drops and preventing leaching of nutrients, promoting the activities of soil microorganisms as well as reducing weed competition (Agyenim-Boateng and Dennis, 2001).

Hochmuth *et al* (2001) reported that the main objectives of mulching are weed control, conservation of soil moisture, suppression of soil borne diseases and modification of temperature. Mulching effectively manipulates crop growing environment leading to increased yield and improved product quality by suppressing weed growth, ameliorating soil temperature, conserving soil moisture, reducing soil erosion, improving soil structure and enhancing organic matter content (Hochmuth *et al*., 2001; Awodoyin and Ogunyemi, 2005).

2.8 Taxonomy and Characteristics of *Fusarium* spp.

2.8.1 Nomenclature of *Fusarium* spp.

Fusarium spp. belongs to the class Hyphomycetes, family Hypocraceae of the order Hypocreales and the genus Fusarium with over twenty species (De Hoog *et al*., 2000). The most common species are *Fusarium solani, F. oxysporum, F. chlamydosporum, F. moniliforme, F. vasinfectum, F. pallidoroseum* and others (Nelson *et al*., 1981).

2.8.2 Morphological and Cultural Characteristics of *Fusarium* spp.

The mycelia of *Fusarium* spp. are either septate or non-septate (coenocytic) and consist of conidiophores which are variable. The conidiophores could be slender, short and simple or short branched irregularly or bearing a whorl of phialides, single or grouped into sporodochia. Phialides are cylindrical with a small collarete, solitary or produced as a component of branching systems (Booth, 1971). Conidia are phialospores, hyaline and variable principally of two kinds, that is macro and micro. The macro conidia are several celled slightly curved or bent at the pointed ends typically canoe shaped and has width and length of 2.5-4.0 and 11-70 µm respectively (Sutton *et al*.,1998; Barnnet and Hunter, 2006). The microconidia 2.4× 4-8 µm are formed on long or short simple chain, one celled, ovoid or oblong and hyaline (Sutton *et al*., 1998; Barnnet and Hunter, 2006). Chlamydospores when present are sparse in chains, thick walled, hyaline intercalary or terminal (Booth, 1971; Sutton *et al*., 1998). When grown on potato dextrose agar (PDA), colonies of *Fusarium* spp. are usually fast growing covering 9 cm Petri dish within eight days at 25-28 ℃ and producing pale or brightly coloured cottony aerial mycelium (Booth, 1977). The thallus varies from whitish to yellow, brownish, pink, reddish or lilac shades (Booth, 1977; Burgess and Liddell, 1983). When grown on Sabourad dextrose agar at 25 ℃, it produces wooly to cottony, flat spreading colonies (Sutton *et al*., 1998).

2.9 Management of Plant Diseases with Botanicals

Plant extracts have found useful place as bio-pesticides in the control of diseases of crop plants. Botanical pesticides are alternatives to synthetic chemical pesticides for pest management derived from plant roots, barks, seeds, buds, leaves and fruits (Al-samarrai *et al*., 2012). They have several advantages over the synthetic pesticides due to the fact that they are cheap, easy to prepare, non-poisonous to human due to short life span and in most cases readily available and have more than one active ingredient

which work synergistically making it difficult for pests to develop resistance (Tello *et al*., 2013). Consequently, they offer a greater role in the production and postharvest protection of food in most developing countries. Takahashi *et al*. (2004) listed a number of plants with pesticidal properties to include neem (*Azadirachta indica*), basil/scent leaf (*Ocimum gratissimum*), castor (*Ricinus comunis*), physic nut (*Jatropha curcas*), drumstick (*Moringa oleifera*), eucalyptus (*Eucalyptus globulus*) etc. Investigations on mechanisms of disease suppression by plant products have indicated that the active principles present in them act on the pathogen directly causing severe damage by promoting cellular disorganization and degradation that makes germination unviable (Amadioha, 2000). The components of plant products, such as phenolic compounds, terpenic alcohols and aldehydes, alter membrane permeability, denature and precipitate cell proteins, inactivate enzymes and cause leakage of amino acids from microbial cells of pathogenic fungi, thus being antimicrobial (Hamilton-Kemp *et al*., 2000; Sangeetha *et al*., 2010).

The potential use of plant extracts to control plant pathogens has been reported in different laboratory, green house and field studies hence, botanicals offer a more economical and efficient alternative for disease control (Hernandez *et al.*, 2010). In Nigeria, plant extracts have been used to control fungal diseases of plant such as anthracnose of sorghum (Akpa *et al*., 1991), brown blotch of cowpea (Owolade and Osikanlu, 1999), black sigatoka of banana (Okigbo and Emoghene, 2004), yam tuber rot (Okigbo and Nnemeka, 2005), pea root rot (Abdulaziz and Younes, 2010) and pawpaw fruit rot (Ebele, 2011).

2.9.1 *Jatropha curcas* L.

Jatropha curcas L. belongs to the family Euphorbiaceae. The plant is also known as physic nut or purging nut in English (Openshaw, 2000; Wurdack, 2008). In Nigeria,

Jatropha is cultivated all over the country from North to South where it is either used as fencing materials in villages or demarcations between farms (Wurdack, 2008). The aqueous extracts of *Jatropha* leaves were effective in controlling *Sclerotium* sp., *Rhizoctonia sp* and *Piper nigrum* as reported by Carcia and Lawas (1990). Extracts from all parts of *Jatropha* plant have shown plant protection and insecticidal effect against various pests (Grainge and Ahmed 1988; Habou *et al*., 2011). The toxicity of *Jatropha curcas* oil and seed cake against other insect pests have also been reported by many authors such as Musa *et al. (*2011), Satyawati *et al*. *(*2011), Habou *et al*. (2011), Ratnadass and Wink (2012) with promising results (high mortality).

2.9.2 *Moringa oleifera* Lam.

Moringa oleifera belonging to the family Moringaceae is an effective remedy for malnutrition and its fungicidal activity on some fungi species has been reported. The Moringa tree is cultivated and used as a vegetable (leaves, green pods, flowers, roasted seeds), for spice (mainly roots), for cooking and cosmetic oil (seeds) and as a medicinal plant (all plant organs) (Lucienne *et al*., 2006). Roots, flowers, bark, stem, leaves and seeds of Moringa possess antimicrobial properties (Anjorin *et al*., 2010; Dwivedi and Enespa, 2012).The roles of aqueous extracts of various parts of Moringa in enhancing plant growth and productivity have been explored, making it even a more valuable plant species (Fahey, 2005). The fungicidal effect of Moringa extracts on soil-borne fungi such as *Rhizoctonia, Pythium* and *Fusarium* was reported by Moyo *et al*. (2012). Dwivedi and Enespa (2012) indicated that extracts of *Moringa oleifera* (leaf, bark and seed) at 75 % (v/v) concentration showed significant inhibition in the mycelia growth of *Fusarium solani* and *Fusarium* clade V. f. sp. *lycopersici*.

2.9.3 *Eucalyptus globulus* Labill.

Eucalyptus globulus was discovered in islands of Tasmania in 1792 by French explorers. The genus *Eucalyptus* contains about 600 species, of all the species, *Eucalyptus globulus* is the most widely cultivated in subtropical and Mediterranean regions (Takahashi *et al*., 2004).

The essential oil from Eucalyptus species possesses wide range of biological activity including anti-bacterial, fungicidal, insecticidal and herbicidal. The volatile oil from lemon scented eucalyptus and its major constituent, the monoterpene citronella possess a wide spectrum of fungicidal activity and inhibit the growth of fungal pathogens (Batish *et al*., 2008). The oil also contains citronellol, limonene, p-cymeneand isopulegol, plant tissues of *E. globulus* contain arrays of secondary metabolites viz., alkaloids, flavonoids, quinines, tannins, steroids, phenolics, saponins, resins, essential oils, various organic acids and other compounds that can be used to develop environmentally safe crop protection packages (Tripathi and Shukla, 2007).

2.10 Integrated Disease Management Strategies for Onion

The basic aim of any plant disease management strategy is to reduce the economic and aesthetic damage induced by plant diseases (Marley, 2013). Management strategies for diseases of onions consist of many approaches which are cost effective and eco-friendly, these include site selection, cultivar/landrace selection, cultural practices in the field such as use of organic manure, sanitation, plant spacing, intercropping, scheduling of water application, use of mulch cover, fungicide sprays in the field, and postharvest treatments (Stone *et al*., 2004). In addition to these cultural measures, monitoring environmental factors (temperature, moisture, soil pH, and soil nutrients), disease forecasting and establishing economic thresholds are

important to the management scheme (Agrios, 2005). Control of post-harvest diseases is achieved using integrated management on the field or after harvest treatments or preferably a combination of both. Management strategies must be efficient and cost-effective and safe to consumers, agricultural workers and the environment. The integration of a number of practices with the aim to reduce or eliminate negative side effects caused by chemicals used for managing major onion diseases is the most realistic option for managing the problem. Field burning of crop residues, soil tillage, fertilization, irrigation, weeding, and other agricultural practices used to control or reduce losses from plant disease can be beneficial to the environment and thereby maintain sound ecological balance (Maloy, 2005).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Determination of Causal Organism of 'Danzazzalau' Disease

3.1.1 Collection of onion seeds and seedlings samples

In October 2014, onion seeds and seedlings were collected from two farm communities each of Aliero and Maiyama Local Government Areas of Kebbi State, Nigeria (Fig. 1). The nursery beds at Rayin Guzuma and Jandutsi in Maiyama LGA had water supplied from artesian wells while those at Kawari and Gezoji in Aliero LGA had pump-water supplied. Each bed was approximately 3×2 m^2 raised with seed drilled across and mulched with thatched grass. The age of seedlings was about 4 weeks according to the farmers. Locations visited were facilitated by Extension staff of Kebbi State Agricultural and Rural Development Authority (KARDA). During the visit, a list (Table 2) was administered to farmers to obtain information on production practices adopted. At Gezoji, farmer-saved seeds "Barke" was collected, and brought to the Department of Crop Protection, ABU, Zaria, for further work.

Transplanted seedlings at the locations visited were observed and information on the following were noted, viz: area of field, water source, weeks-after-transplanting, type of crop mixtures, fertilizer and pesticide use, local name of the disease, brief description of noticed symptoms, time the symptoms were noticed on plants and how they have tried to manage the disease. Specifics of location were derived using the hand-held Global Position System (GPS) navigator (GARMIN®, USA). Figure 1 is the LGAs visited. At each field, between 25 to 30 plants (comprising both diseased and apparently healthy plants) with attached soil were randomly uprooted with hoe, wrapped with sack cloth, labeled and brought to the laboratory of Department

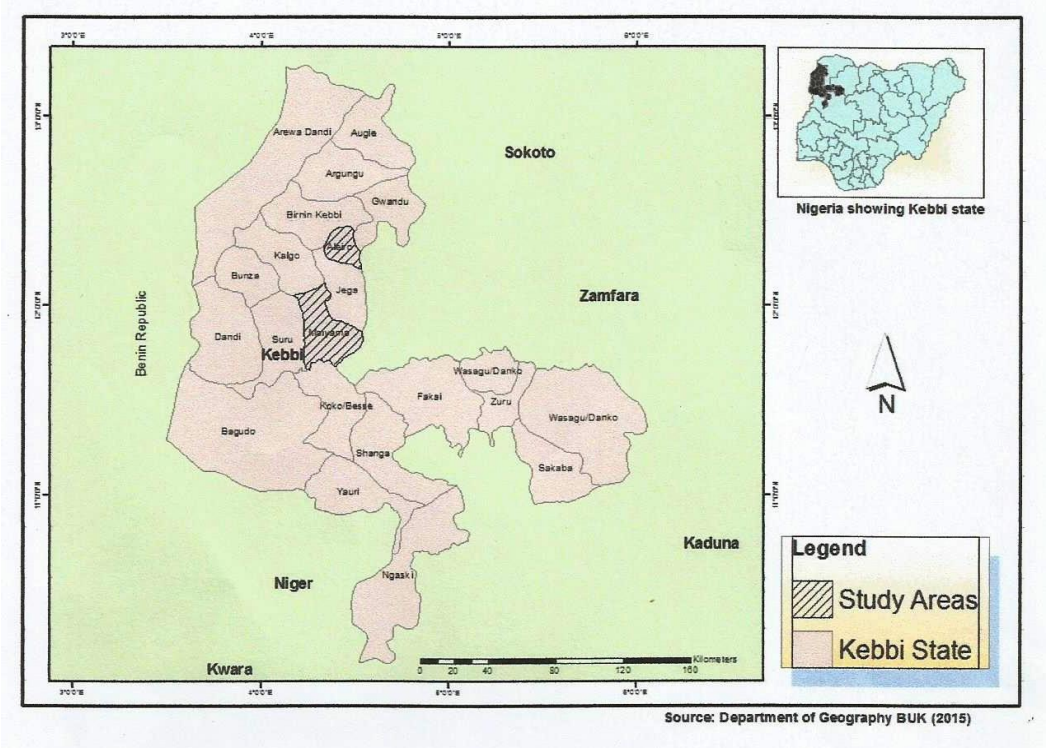


Fig 1: Local Government AreasVisitedin Kebbi State

of Crop Protection, Zaria, for further investigations. The plants were transferred to plastic pots and maintained in the screen house for monitoring.

3.1.2 Isolation and identification of fungi from samples

One each of healthy-looking and diseased plant from the farms visited were washed thoroughly with running water, roots 3 mm long were cut with a pair of scissors into Petri dish, surface sterilized in 1 % sodium hypochlorite (NaOCl) solution for 3 minutes, rinsed in three changes of sterile distilled water (SDW) and dried between folds of sterile filter paper. Eight 3 mm long pieces from the root tip, pseudostem and leaf tissues obtained using sterile blade were aseptically transferred to potato dextrose agar (PDAs) media amended with streptomycin sulfate. The PDAs consisted 15 g of agar agar and 20 g of dextrose poured into filtered potato broth, with 0.6 µg/L streptomycin sulfate added after autoclaving at 121 °C for 20 minutes and cooled to 45 °C. Twenty milliliter of media at 45 °C was dispensed into 9 mm diameter Petri dishes. Five plates containing surface sterilized plant parts each were arranged on laboratory bench at ambient conditions and observed for growth and development of fungal mycelia.

Five-to-seven day-old hyphal tips from distinct fungal colonies were transferred to fresh plates and cultural and microscopic characteristics of the fungi were recorded and identification was based on monographs of Watanabe (2002) and Barnett and Hunter (2006). The pure cultures were sent to Centre for Agriculture and Bioscience International (CABI), Egham, Surrey, United Kingdom for confirmation of identity.

3.1.3 Test for pathogenicity of isolated fungi

Fourteen day old mycelia mat of each fungus was scrapped with edge of flame-sterilized glass slide after pouring 10 ml SDW. To this was 200 ml SDW added and blended using an electric Turbo blender (Binatone®, Japan) at low speed for 3 minutes. Suspension was filtered through double layer muslin cloth, a droplet of

filtrate was placed on haemocytometer and number of conidia per milliliter counted. Total number of spores/ml was calculated using the formula below,

$$\text{Number of spores/ml} = \frac{n}{256} \times 4 \times 10^6$$

where: n = number of conidia counted in the chambers

256 = constant volume obtained from 16 x 16 squares of haemocytometer, 4 x 10^6 = constant

Inoculum suspension obtained was stored in the refrigerator pending use about 12-14 hours later.

Already dressed "Barke" onion seeds with 20 % thiamethoxan+ 20 % metalaxyl- M+2 % difenoconazole (Apron Star, Sygenta Crop Protection®) was sown in heat-sterilized 3:1 loam:sand soil contained in 1.5 m x 1.0 m sized tray. Cow dung at 0.5 kg/m^3 was incorporated into soil prior to drilling in the seeds. Grass was used as mulch, water applied daily and regular hand removal of weeds was carried out.

Roots of six week-old seedlings raised in the glasshouse were drenched into 10 ml 1×10^6 and 1×10^3 conidia/ml inoculum suspension for 3 minutes before transplanting into heat-sterilized soil contained in pots (15 cm diameter). For dip method of inoculation, the roots were dipped into 10 ml 1×10^6 and 1×10^3 conidia/ml inoculum suspension for 3 minutes before transplanting. After transplanting, seedlings were watered (50 ml per pot) then covered with polyethylene sheets overnight during the experiment. Three potted onion seedlings of the isolated fungi were arranged in the screen house and observed for development of typical 'Danzazzalau' symptoms until 28 days after inoculation. Leaves showing symptoms were collected, taken to laboratory for re-isolation of the organism which was later compared with the original culture to confirm Koch's postulates.

3.1.4 Effect of methods of inoculation on disease development

Inoculation of six week-old seedlings by root dip (T_1) and soil drench (T_2) was conducted in the glasshouse of the Department of Crop Protection, ABU, Zaria. Root dip involved dipping the seedling roots in 10 ml of 1×10^6 and 1×10^3 conidia/ml inoculum suspensions for 3 minutes before transplanting into heat-sterilized soil while for soil drench, 10 ml of inoculum suspension was poured at the base of each of the transplanted seedlings. Seedlings inoculated with 10 ml SDW served as control. Each treatment was repeated 6 times and pots were covered with polyethylene sheets overnight. Labeled pots were arranged in the glasshouse in a Completely Randomized Design (CRD) and observed daily for the appearance of developing symptoms, and disease scores taken at 7, 14, 21 and 28 DAI.

3.2 Evaluation of some Cultural Practices Effects on 'Danzazzalau' Disease and Yield of Onion

Field trial was conducted at the Institute for Agricultural Research (IAR), Zaria irrigation farm (11° 10' N 07° 38' E, 685 m above sea level) in January 2015. Land was ploughed, disc-harrowed, and experimental plots demarcated into 6 m^2 gross size basins. The basins were separated by a 0.5 m wide channel on the four sides. A split plot design with the main plot as three supplementary irrigation regime (I) of 3, 10, and 21 days; while three manure levels (C) (cow dung at 0, 40 and 80 kg/plot and two mulch materials (M) white polyethylene sheet, dried rice straw and no mulch made up the sub-plots. All treatments were replicated three times. Cow dung manure was obtained three weeks before application on the field from the National Animal Production Research Institute (NAPRI, Shika, Zaria). The polyethylene sheets thickness was 0.38 mm while rice straw was collected from Bomo village, Sabon-gari LGA, Zaria.

Each of the sunken beds had 63 six week-old seedlings transplanted and spaced 25 cm x 20 cm to give a plant population of 105,000/ha. The 81 beds had irrigation water pumped in for 2 h through a pumping machine (Honda®, Japan) from nearby IAR dam immediately after transplanting. The 3, 10 and 21- day water regime treatments had 15, 7, and 5 applications of irrigation respectively each lasting 1 hour. Three weeks before seedlings transplant, appropriate amounts (multiples of 82 g/m^2) of cow dung was broadcast into labeled beds and incorporated into the soil. After transplanting, labeled plots had 720 g rice straw per basin arranged along stands mulch while a cut, 4 to 5 cm diameter was made per seedling on the stretched out polyethylene sheet. Plots without cow dung, rice straw or polyethylene sheet treatment served as control. The fertilizer N: P: K 15:15:15 at the rate of 309 g/m^2 was applied as side dressing twice at 3 and 6 weeks after transplanting. Weeds were controlled through manual hoe weeding as needed.

Disease incidence and severity were assessed at 9 weeks after transplanting (WAT) and crop harvested at 16 WAT. Number of plants showing typical symptoms of 'Danzazzalau' was taken and used to calculate incidence as percent of total number of plants per treatment using the formula below:

$$DI(\%) = \left(\frac{x}{n} \times 100 \right)$$

where:

DI (%) = disease incidence

x = number of infected plants per treatment

n = total number of plants per treatment

Being an undescribed disease, symptoms observed in the tests for pathogenicity (Plate I) were used to determine the disease severity scale based on proportion of infected tissue.

A newly developed 1-5 disease severity scale designed for 'Danzazzalau' disease is described below:

1= no symptom expressed; healthy plants
2 = (1-25 %) of leaves curled;
3 = (26-50 %) of leaves curled;
4 = (51-75 %) of leaves curled;
5 = (76-100 %) of leaves curled and toppled over.

Disease severity (DS) was calculated using the formula below:

$$(\text{DS }\%) = \frac{\text{Sum of disease ratings}}{\text{Total number of plants} \times \text{maximum grade}} \times 100$$

Other components of yield assessed include: number of bulbs per plot (NBP); bulb diameter (BDM) and bulb yield (BYD). Diameter of nine bulbs per plot was determined using a vernier caliper while the bulbs in each plot were weighed and expressed in tonnes per hectare.

3.3 *In* vitro Evaluation of Aqueous and Methanol Crude Leaf Extracts of some Plants on *Fusarium* sp.

3.3.1 Plant materials collection and crude leaf extractions

3.3.1.1 *Aqueous crude leaf extraction*

Leaves of *Jatropha curcas* L., *Moringa oleifera* Lam. and *Eucalyptus globulus* Labill. were collected from the environs of the Institute for Agricultural Research (IAR) farm. The leaves were washed with tap water and air dried on laboratory bench for 48 hours. The leaves were crushed with a mortar and pestle, 5 g, 7.5 g and 10 g (2.5 %, 3.75 % and 5.0 % w/v) each of the leaves were soaked separately in 200 ml sterile

distilled water (SDW), agitated on shaker (Edmund Bühler®, Germany) for four hours and filtered through Whatman No. 1 filter paper and was concentrated in water bath set at 60 °C for 24 hours.

Molten PDAs (85 ml) was dispensed into ten conical flasks (250 ml) each and 15 ml of each extract was added per flask, swirled clockwise and anticlockwise effecting mixture. Two grams of mancozeb 80 % WP (Z-Force, Jubaili Agrotec®) was dissolved in 200 ml SDW. Twenty millilitres of the PDAs amended with each extract and mancozeb was poured into five labeled Petri dishes.

3.3.1.2 *Methanolic crude leaf extraction*

The crushed plant leaves weighing 5 g, 7.5 g and 10 g (2.5 %, 3.75 % and 5.0 % w/v) were filled into thimble and extracted with 200 ml methanol (Sigma Aldrich®, England) in a soxhlet extractor for 8 hours. After complete solvent evaporation using a steam bath set at 60 °C for 24 h, the extracts were preserved in vials until when needed. Methanolic extracts were incorporated into PDAs as described in 3.3.1.1 above.



1= No symptom expression



2= 1-25 % of leaves curled



3 =26-50 % of leaves curled



4 =51-75 % of leaves curled



5 =76-100 % of leaves curled and toppled over

PlateI:Pictorial representation of the 1-5 disease severity scale used

Five millimeter discs of *Fusarium* sp. were taken from 9-day old culture with sterile corkborer No 4 and placed in middle of Petri dishes. Treatments consist of aqueous and methanolic extracts obtained from leaves of *Jatropha curcas*; *Moringa oleifera* and *Eucalyptus globulus*, PDAs without any extract but with fungus served as control, while PDAs amended with mancozeb served as standard check with each Petri dish taken as a repetition. Labeled Petri dishes were arranged in a Completely Randomized Design (CRD) on laboratory bench and mycelia diameter was measured at two-day interval for fourteen days, starting from 2 days after inoculation (DAI) along two perpendicular lines drawn at the bottom of Petri dishes. Percent mycelia growth inhibition (PI) was calculated using the formula described by Ashwani *et al*. (2012).

$$\text{Percent inhibition (PI \%)} = \frac{A-B}{A} \times 100$$

Where:

A= mycelia diameter in control plate

B= mycelia diameter in treated plate

At 14-day after inoculation, the mycelia from five Petri dishes per treatment were harvested into 500 ml beakers using scalpel, macerated in 200 ml SDW, using Turbo blender (Binatone®, Japan), and filtered through double layer muslin cloth. Conidia suspension obtained was homogenized and serially diluted. Micro-conidia were counted with the aid of haemocytometer at x 400 magnification. The values obtained were substituted into the formula cited in 3.1.3 above to calculate the number of conidia per millilitre (ml) for each repetition.

Length and width (µm) were obtained for fifty randomly selected conidia per repetition using an ocular micrometer at x 400 magnification, and mean was obtained by dividing total length or width by 50.

3.4 Data Analysis

Data collected were subjected to analysis of variance (ANOVA) using SAS software version 9 (2002). Significant means were separated using the Student-Newman-Keuls (SNK) test at 5 % level of significance.

CHAPTER FOUR

4.0 RESULTS

4.1 Fungi Isolated from Infected Onion Samples and their Identification

4.1.1. Details of onion farms visited

At the nursery of the farms visited for collection of samples, most of the farmers sourced their seeds from nearby local markets, exchange from friends and/or their own saved seed. The farmers at Gezoji used neonicotinoid 20 % + phenylamide –20 % + triazole 2 % (Dressforce, Jubaili Agrotec®) as seed dressing, while some others at Kawari, Rayin Guzuma and Jandutsi used 20 % thiamethoxan+ 20 % metalaxyl- M+2 % difenoconazole (Apron star, Sygenta Crop Protection®).

The size of the fields visited (Table 2) ranged between 0.25 and 0.50 ha, on which were transplanted 6 to 8 week old seedlings in sunken beds measuring about 3 m by 2 m. Sole cropping seems to be widely practiced at Gezoji and Rayin Guzuma while Kawari and Jandutsi had some fields cropped with chili pepper or tomato within the farm. With respect to time they observe infection of plants, most farmers claimed between 2 to 4 weeks after transplanting. A description of 'Danzazzalau' symptoms indicated that farmers at Kawari and Jandutsi observed protrusion of the main leaf while at Gezoji and Rayin Guzuma it was described as the whole plant rot. The farmers in Kawari and Gezoji tried managing the disease by withdrawing irrigation for 14-21 days and rouging while those at Rayin Guzuma did abandon infected fields and moved to new land. Only farmers at Jandutsi claimed they apply post-transplant pesticides 12 % carbendazim + 63 % mancozeb WP (Team®).

4.1.2 Pathogenicity of fungi associated with inoculated seedlings

Fungi isolated from diseased leaf, basal stem and root sections of onions include *Phoma* sp. *Rhizoctonia* sp. (Plate V); *Aspergillus* sp. (Plate VII); and *Fusarium* spp.

Table 2: Information for the visited onion farms in Kebbi State

LGA	Community	GPS Coordinates		Altitude (m)	Farm size (ha)	Seed source	Local seed name	Seed dressing pesticide used	Cropping pattern	Associated crop
Aliero	Kawari	12°19.536' N	004°29.232' E	276	0.35	FSS	Barke	Apron star	Mixed cropping	Pepper
	Gezoji	12°17.208' N	004°28.559' E	266	0.50	MM	Barke	Dress force	Sole cropping	n.a.
Maiyama	Rayin Guzuma	12°07.459' N	004°31.436' E	291	0.25	FSS	Yankasa	Apron star	Sole cropping	n.a.
	Jandutsi	12°10.023' N	004°28.657' E	257	0.40	LS	Yankasa	Apron star	Mixed cropping	Tomato

LGA = Local Government Area

FSS = Farmer-saved seed; LS = locally sourced; MM = Maiyama market

n.a. = Not applicable

Confirmed identities of fungi from CABI, UK were *Fusarium chlamydosporum*; and *Fusarium* clade V. (Plates II and III). The culture suspected to be *Phoma* sp. was confirmed to be a species of *Thielavia* (Plate VIII). Mycelia of *Fusarium* clade V. was cottony white with 4 to 5 concentric growth rings on solid media, well branched, with uniform growth. The fungus grew slowly to cover 9 cm diameter dish in 8 to 9 days. Conidiophores, phialides and micro-conidia were observed at x 100 magnification. Microconidia were smooth, hyaline, and ovoid to cylindrical. Characteristics of fungi associated with onion tissues are presented in Table 3.

Pathogenicity tests showed that *Fusarium* clade V. was pathogenic on onion seedlings when inoculated with the organisms at 10^6 conidia/ml concentration. Symptoms observed on leaves and roots were similar to those observed on the seedlings initially. Symptoms observed on inoculated plants were drying of leaves beginning from the tip, abnormal elongation and collapse of plants. Uprooted roots showed dark brown colouration. Other isolated fungi (*Aspergillus* sp (Plate VII); *Thielavia* sp.; *Fusarium chlamydosporum* and *Rhizoctonia* sp (Plates V and VI)) were not pathogenic as they failed to produce symptoms of the disease on inoculated seedlings.

Table 3: Characteristics of fungi isolated from various onion parts

Tissue Used	CABI Identification	Colony colour on PDAs*	Conidia septations (No.)	DAI*
Root	*Fusarium* clade V. (IMI 504847)	Pink	3-5	8-9
	Fusarium chlamydosporum. (IMI 504851)	White	3-5	8-9
	Rhizoctonia sp.	Brown	No conidia produced	4-5
Pseudostem	*Fusarium* clade V. (IMI 504848)	White	3-5	8-9
	Fusarium clade V. (IMI 504850)	Pinkish white	3-5	8-9
Leaf	*Thielavia* sp. (IMI 504849)	White	2-3	6-7
	Aspergillus sp.	Dark green	1-2	4-5

PDAs* = Potato dextrose agar amended with streptomycin sulfate

DAI*= Days after inoculation



Plate II: Upper view of 10 day-old culture of *Fusarium* clade V. isolated from onion pseudostem

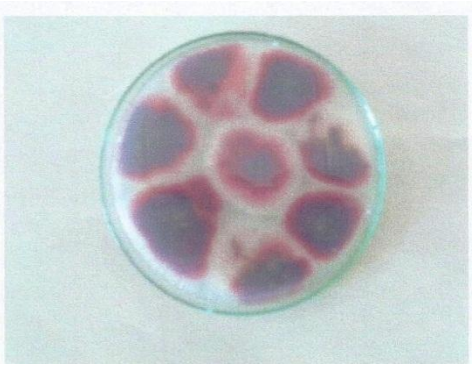


Plate III: Reverse view of 10 day-old culture of *Fusarium* clade V. isolated from onion pseudostem



Plate IV: Macro-and micro-conidia of *Fusarium* clade V. (mag×100)

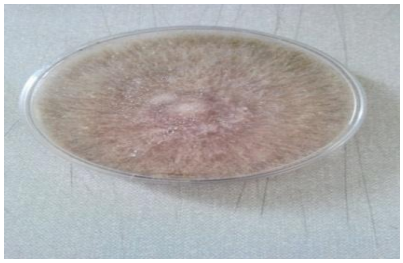


Plate V: Upper view of 5 day-old culture of *Rhizoctonia* sp. isolated from onion root

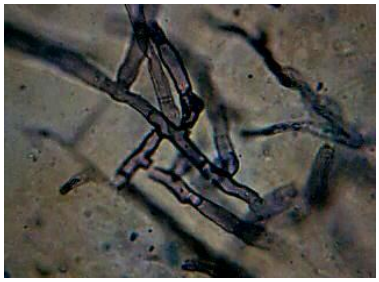


Plate VI: Hyphae of *Rhizoctonia* sp. (mag x100)



Plate VII: Upper view of 5-day old culture of *Aspergillus* sp. isolated from onion leaf



Plate VIII: Upper view of 10-day old culture of *Thielavia* sp. isolated from onion leaf

4.1.3 Effect of method of inoculation on disease development

Table 4 shows that at 14, 21 and 28-day after inoculation (DAI), disease incidences were significantly different at 5 % level with method of inoculation of seedlings. Plants inoculated by root dip method had higher disease incidence: 24.89, 28.24, and 29.49 % at 14, 21, and 28 DAI respectively compared with plants inoculated by soil drench (19.23, 19.59 and 21.43 %) at 14, 21, and 28 DAI respectively. Similar trend was observed for disease severity as plants inoculated by root dip had higher disease severity, (4.23, 5.21, and 6.71 %) at 14, 21, and 28 DAI respectively compared with plants with soil drench treatments which had (2.88, 3.38 and 4.63%) at 14, 21, and 28 DAI respectively. At 7 DAI, both disease incidence and severity of root dip and soil drench inoculation did not vary from each other.

The effect of inoculum concentrations on disease incidence and severity were significantly different at 5 % level. Plants inoculated with inoculum concentration of 1×10^6 conidia/ml had higher disease incidence, 16.43, 17.69 and 19.78 % at 14, 21 and 28 DAI respectively compared with plants inoculated with the concentration of 1×10^3 conidia/ml which had 11.78, 12.86 and 13.97 % at 14, 21, and 28 DAI respectively. For disease severity, there were significant differences at 5 % level with inoculum concentration of 1×10^6 conidia/ml which had higher disease severity, 4.79 %, 5.91 % and 7.81 % at 14, 21 and 28 DAI respectively compared with plants inoculated with the inoculum concentration of 1×10^3 conidia/ml which had 3.06, 3.82 and 4.56 % at 14, 21, and 28 DAI respectively. The control plants did not show symptoms of the disease.

Table 4: Effect of methods of inoculation and conidial concentrations of *Fusarium* clade V. on the incidence and severity of 'Danzazzalau' disease of onion at Samaru, 2015

Treatments	Incidence (%) at DAI				Severity (%) at DAI			
	7	14	21	28	7	14	21	28
Inoculation method								
Root dip	21.57	24.89[a]	28.24[a]	29.49[a]	4.13	4.23[a]	5.21[a]	6.71[a]
Soil drench	19.55	19.23[b]	19.59[b]	21.43[b]	3.67	2.88[b]	3.38[b]	4.63[b]
Concentration conidia/ml								
10^3	8.45	11.78[b]	12.86[b]	13.97[b]	2.73	3.06[b]	3.82[b]	4.56[b]
10^6	10.84	16.43[a]	17.69[a]	19.78[a]	3.69	4.79[a]	5.91[a]	7.81[a]
Control	0.00	0.00[c]	0.00[c]	0.00[c]	0.00	0.00[c]	0.00[c]	0.00[c]
SE±	0.43	0.67	0.81	0.83	0.15	0.18	0.22	0.26

Means with the same superscript along a column are not significantly different at 5 % level of probability ($P \leq 0.05$) using Student Newman Keuls (SNK) Test.
DAI=Days after inoculation

4.2 Effect of some Cultural Practices on Yield Components and 'Danzazzalau' Disease of Onion

4.2.1 Effect of some cultural practices on 'Danzazzalau' disease incidence and severity

There were significant differences at 5 % level as water regime decreased. Decreasing the water application regime from 10 to 21 days led to disease incidence reduction from 54.95 % to 36.02 % (Table 5). Disease severity was highest, at 10-days water regime followed by 3- and 21-day water regimes respectively though the differences were not statistically significant from each other.

As the rate of manure increased from 40 to 80 Kg/plot, disease incidence reduced from 44.77 % to 42.13 %. However, disease severity was highest at 0 Kg/plot followed by 40 kg/plot and 80 kg/plot manure rates but not significantly different.

Mulch treatments had no significant effect on disease incidence (Table 5). Highest disease incidence was obtained on white polyethylene sheet treatment followed by no mulch and rice straw treatment respectively. There was no significant difference ($P \leq 0.05$) in the disease severity for the various mulch materials used.

In Table 6, the interaction effect of water regime by mulch was significant for disease incidence but not for disease severity while water regime x manure application; mulch x manure application; and water regime x manure application x mulch were not significant on disease incidence and severity. Disease incidence was significantly highest on plants mulched with white polyethylene sheet at 10-day water regime followed by 21-and 3-days water regime, although these did not differ statistically. For rice straw mulch, there were no significant differences amongst the water regimes, although 3-days water regime recorded the lowest disease incidence. For the control plots (no mulch) there was also no significant difference.

Table 5: Effect of water regime, manure and mulch on the incidence and severity of 'Danzazzalau' disease of onion at Samaru, 2015

Treatments	Disease Incidence (%)	Disease Severity (%)
Water regime (days) (I)		
3	43.02[b]	35.20[b]
10	54.95[a]	37.92[a]
21	36.02[c]	32.93[b]
SE±	2.317	1.594
Manure (Kg/plot) (C)		
0	43.09	36.73
40	44.77	35.26
80	42.13	34.97
SE±	2.317	1.594
Mulch (M)		
Polyethylene	45.41	36.65
Rice straw	40.94	36.44
No mulch	43.65	33.88
SE±	2.059	0.982
Interactions		
I x C	NS	NS
I x M	*	NS
C x M	NS	NS
I x C x M	NS	NS

Means with the same superscript along a column are not significantly different at 5 % level of probability ($P \leq 0.05$) using Student Newman Keul's (SNK) Test.
*=Significant at 5 % level of significance.
I=water regime C= manure M= mulch
NS= Not significant

Table 6: Effect of water regime x mulch interaction on the incidence of 'Danzazzalau' disease of onion at Samaru, 2015

Water regime (days)	Mulch		
	Polyethylene Sheet	Rice Straw	No mulch (Control)
3	29.96[b]	27.61[b]	38.49[b]
10	66.33[a]	47.92[ab]	51.24[ab]
21	39.94[b]	47.28[ab]	41.22[b]
SE±	8.28		

Means with the same superscript along a column and row are not significantly different at ($P \leq 0.05$) using Student Newman Keul's (SNK) Test.
SE= Standard error

4.2.2 Effect of some cultural practices on yield parameters of onions

Table 7 shows that the number of bulb harvested did not vary significantly ($P \leq 0.05$) with water regime. Highest number of harvested bulbs (54,633/ha) was obtained with 10-day water regime and least (52,533/ha) with the 21-day regime (Table 7). Bulb diameter was significantly different at 5 % level with water regime, largest bulbs (6.18 cm) were harvested on plots with 10-day water regime which did not differ with 21-day water regime while the smallest bulbs (5.72 cm) was recorded for 3-day water regime. Bulb yield significantly ($P \leq 0.05$) varied with water regime. Yield from 21- and 10-days water regime was highest and were not statistically different from each other. The 3-day water regime yielded statistically the lowest bulbs.

Bulb number harvested increased with increase in manure rate although the increases were not significant ($P \leq 0.05$); highest number of harvested bulbs (56,166/ha) was obtained where 80 kg/plot of manure was applied. As the rate of manure increased from 0 to 40 kg/plot, number of bulb harvested increased from (51,666/ha) to (52,166/ha) respectively. Bulb diameter was not affected by manure rate treatments; largest bulbs (6.13 cm) were harvested on plots with 40 kg/plot of manure and smallest bulbs (5.94 cm) where 0 kg/plot of manure were used. Bulb yield did not significantly ($P \leq 0.05$) varied with manure rate treatments; highest and lowest bulb yields (4.45 t/ha and 4.12 t/ha) were obtained where 80 kg/plot and 40 kg/plot of manure were applied respectively.

Mulch treatments had significant ($P \leq 0.05$) effect on number of bulbs harvested.

Highest and lowest number of bulbs harvested (57,467/ha and 48,583/ha) were obtained on polyethylene sheet and no mulch treatments respectively. Bulb diameter was not significantly affected at 5 % level of significance by mulch treatments; largest bulbs (6.16 cm) were harvested on plots with polyethylene sheets and smallest (5.89

cm) where no mulch was applied. Bulb yield significantly (P≤0.05) varied with mulch treatments; highest and lowest yields (4.80 t/ha and 3.95 t/ha) were obtained where rice straw and no mulch treatments were used respectively.

Interaction effect of water regime x manure, water regime x mulch application, mulch x manure application and interaction of water regime x manure application x mulch were not significant on all the yield components of onion.

4.3 *In vitro* Evaluation of Aqueous and Methanolic Crude Leaf extracts of some Plants on *Fusarium* clade V.

4.3.1 Effect of aqueous and methanolic crude leaf extracts of some plants on morphological characteristics of *Fusarium* clade V.

Table 8 shows that the reactions of *Fusarium* clade V. isolate to aqueous crude leaf extracts were significantly different ($P \leq 0.05$) from each other in respect to percentage conidial count and mycelial growth inhibition but not for conidial size. Aqueous crude extracts of tested plant leaves at 2-day after inoculation (DAI) showed that highest percentage mycelia growth inhibition (42.96 %) was by 10 g Jatropha amended media which was followed by 5 g Moringa amended media (30.33 %) while the least (11.08 %) mycelial growth inhibition was produced on 7.5 g Jatropha amended media which was significantly lower than the standard check. The trend was different at 4-DAI where 10 g Moringa amended media had highest (16.26 %) mycelial growth inhibition which was followed by 5 g Eucalyptus amended media (13.28 %), while the least (6.23 %) mycelial growth inhibition was produced on 5 g Jatropha amended media which was significantly lower than the standard check. The percentage mycelial growth inhibition for 6, 8, 10, 12 and 14-DAI were 41.64 %, 57.64 %, 44.32 %, 47.83 % and 39 % for 10 g Moringa amended media.

Table 7: Effect of some cultural practices on yield and some yield components of onion at Samaru, 2015

Treatment	Bulb (No./ha)	Bulb diameter (cm)	Yield (t/ha)
Water regime (days) (I)			
3	52,833	5.72[b]	3.48[b]
10	54,633	6.18[a]	4.62[a]
21	52,533	6.13[a]	4.67[a]
SE±	1.389	0.089	0.207
Manure (Kg/plot) (C)			
0	51,666	5.94	4.22
40	52,166	6.13	4.12
80	56,166	5.96	4.45
SE±	1.389	0.089	0.207
Mulch (M)			
Polyethylene	57,467[a]	6.16	4.02[ab]
Rice straw	53,950[ab]	5.98	4.80[a]
Control	48,583[b]	5.89	3.95[b]
SE±	1.361	0.092	0.171
Interactions			
I x C	NS	NS	NS
I x M	NS	NS	NS
M x C	NS	NS	NS
I x M x C	NS	NS	NS

Means with the same superscript along a column are not significantly different at 5 % on the Student Newman Keul's (SNK) Test

SE=standard error

NS = Not significant

I = water regime C= manure M = mulch

Aqueous plant extracts produced higher number of conidia with highest (265 x 10^4) number of conidia produced by 10 g Jatropha amended media compared to other media. This was followed by 7.5 g Eucalyptus amended media (229 × 10^4) and 5 g Eucalyptus amended media (199 × 10^4), while the least conidia (16 × 10^4) were produced on 10 g Moringa amended media though it was not statistically different from the standard check. Longest conidia (4.51 µm) was produced on 7.5 g Jatropha amended media while the least (4.24 µm) was produced on 5 g Eucalyptus amended media. Largest conidia width (2.57 µm) was produced on 5 g Eucalyptus amended media while the least (2.40 µm) was produced on 5 g Jatropha amended media which was not statistically different from the standard check.

Table 9 shows that the reactions of *Fusarium* clade V. isolate to methanol extracted crude leaf extracts were significantly different ($P \leq 0.05$) from each other in respect to percentage conidial count and mycelial growth inhibition but not for conidial size. Mycelial growth inhibition was highest at 2-day after inoculation in 10 g Eucalyptus amended media extracted with methanol which had (63.17 %) followed by 7.5 g Moringa amended media which had (57.34 %) and the least was by 10 g Moringa amended media which had (0 %) mycelial growth inhibition which was significantly lower than the standard check. The percentage mycelial growth inhibition for 4, 6, 8, 10, 12 and 14-DAI were 75.83 %, 81.67 %, 80.67%, 74.48 %, 68.99% and 61 % respectively for 10 g Moringa amended media, while control had 0 % inhibition from 2 to 14 DAI. Highest (409 × 10^4) number of conidia was obtained from 7.5 g Jatropha amended media compared to other media. This was followed by 5 g Jatropha (254 × 10^4) amended media, while the least conidia (32 × 10^4) were produced by 10 g Eucalyptus amended media which was significantly lower than the standard check. Generally, methanol extracted plant extracts produced significantly ($P \leq 0.05$) higher

number of conidia, higher percentage mycelia growth inhibition and conidial size than aqueous extracted plant extracts. Longest conidia (4.53 µm) was produced by 10 g Moringa amended media while the least (4.26 µm) was produced by 5 g Jatropha amended media which was not statistically different from the standard check. Conidia produced on 10 g Moringa amended media had the widest width (2.59 µm) followed by 10 g Eucalyptus amended media (2.58 µm) and 5 g Moringa amended media (2.52 µm). The lowest conidial width (2.42 µm) was recorded from 5 g Eucalyptus amended media which was significantly lower than the standard check.

Table 8 Effect of aqueous crude extracts of *Eucalyptus*, *Moringa* and *Jatropha* leaves on mycelial growth, sporulation and conidia size of *Fusarium* clade V.

Materials	Quantity (g)	Mycelial inhibition (%)							Conidia count ($\times 10^4$/ml)	Conidia size (µm)	
		2 DAI	4 DAI	6 DAI	8 DAI	10 DAI	12 DAI	14 DAI		Length	Width
Eucalyptus	10.0	20.09^{cd}	8.04^{bc}	9.33^{c}	7.36^{de}	4.20^{d}	9.49^{bc}	5.33^{b}	163^{bcd}	4.28	2.42
	7.5	22.15^{cd}	11.18^{b}	5.15^{cd}	5.46^{de}	5.64^{d}	13.22^{bc}	4.55^{b}	229^{ab}	4.29	2.39
	5.0	22.20^{cd}	13.28^{b}	9.99^{c}	7.77^{de}	7.38^{cd}	11.22^{bc}	2.89^{b}	199^{bc}	4.24	2.57
Moringa	10.0	20.79^{cd}	16.26^{b}	41.64^{b}	57.64^{a}	44.32^{a}	47.83^{a}	39.00^{a}	16^{f}	4.37	2.48
	7.5	15.24^{cd}	9.00^{bc}	7.46^{cd}	17.27^{c}	20.93^{bc}	22.75^{b}	11.22^{b}	84^{e}	4.47	2.47
	5.0	30.33^{bc}	6.76^{bc}	7.48^{cd}	7.19^{de}	7.41^{cd}	11.79^{bc}	7.22^{b}	146^{cd}	4.39	2.51
Jatropha	10.0	42.96^{b}	10.82^{b}	10.40^{c}	10.80^{d}	3.42^{d}	13.44^{bc}	4.11^{b}	265^{a}	4.36	2.47
	7.5	11.08^{cd}	6.84^{bc}	7.20^{cd}	4.40^{de}	2.85^{d}	12.45^{bc}	4.33^{b}	169^{bcd}	4.51	2.45
	5.0	14.42^{cd}	6.23^{bc}	4.62^{cd}	5.41^{de}	4.41^{d}	9.00^{bc}	4.44^{b}	186^{bcd}	4.26	2.40
Mancozeb	2.0	69.98^{a}	59.89^{a}	48.26^{a}	40.26^{b}	25.00^{b}	17.78^{bc}	13.22^{b}	12^{f}	4.29	2.53
Control	0.0	0.00^{d}	0.00^{c}	0.00^{d}	0.00^{e}	0.00^{e}	0.00^{c}	0.00^{c}	189^{bcd}	4.38	2.49
SE±		4.407	2.860	2.524	2.191	3.583	3.659	3.321	0.559	0.002	0.039

Means with the same superscript along a column are not significantly different at 5 % on the Student Newman Keul's (SNK) Test
DAI= Days after inoculation
SE= Standard error

Table 9: Effect of methanolic crude extracts of *Eucalyptus*, *Moringa* and *Jatropha* leaves on mycelial growth, sporulation and conidia size of *Fusarium* clade V.

Materials	Quantity (g)	Mycelial inhibition (%)							Conidia count ($\times 10^4$/ml)	Conidia size (µm)	
		2 DAI	4 DAI	6 DAI	8 DAI	10 DAI	12 DAI	14 DAI			
										Length	Width
Eucalyptus	10.0	63.17^{ab}	62.74^{b}	73.03^{b}	74.20^{b}	69.94^{a}	65.84^{a}	61.00^{a}	32^{d}	4.33	2.58
	7.5	52.84^{b}	46.71^{c}	55.93^{c}	56.84^{c}	48.60^{b}	46.61^{b}	41.00^{b}	88^{bc}	4.48	2.43
	5.0	42.07^{c}	24.29^{d}	38.10^{d}	40.25^{d}	31.44^{c}	28.78^{cd}	24.44^{c}	87^{bc}	4.28	2.42
Moringa	10.0	0.00^{f}	78.53^{a}	81.67^{a}	80.67^{a}	74.48^{a}	68.99^{a}	55.44^{a}	67^{bc}	4.53	2.59
	7.5	57.34^{b}	45.82^{c}	53.01^{c}	51.97^{c}	29.24^{c}	33.14^{c}	23.89^{c}	75^{bc}	4.29	2.51
	5.0	40.53^{c}	20.69^{de}	30.24^{de}	28.86^{e}	21.01^{d}	21.21^{def}	17.00^{cd}	164^{ab}	4.48	2.52
Jatropha	10.0	23.39^{d}	14.26^{def}	27.73^{e}	28.58^{e}	21.24^{d}	25.78^{cde}	20.56^{cd}	151^{ab}	4.41	2.49
	7.5	13.77^{e}	7.98^{fg}	10.21^{f}	6.22^{f}	8.29^{e}	13.92^{f}	12.00^{d}	409^{a}	4.36	2.47
	5.0	12.42^{e}	11.03^{efg}	10.06^{f}	6.14^{f}	8.20^{e}	13.77^{f}	10.44^{d}	254^{ab}	4.26	2.48
Mancozeb	2.0	69.98^{a}	59.89^{b}	48.26^{c}	40.26^{d}	25.00^{d}	17.78^{ef}	13.22^{cd}	119^{b}	4.29	2.53
Control	0.0	0.00^{f}	0.00^{g}	0.00^{g}	0.00^{g}	0.00^{f}	0.00^{g}	0.00^{e}	189^{ab}	4.38	2.49
SE±		4. 407	2.860	2.524	2.191	3.583	3.659	3.321	0.559	0.002	0.039

Means with the same superscript along a column are not significantly different at 5 % on the Student Newman Keul's (SNK) Test
DAI= Days after inoculation
SE= Standard error

CHAPTER FIVE

5.0 DISCUSSION

5.1 Isolation and Identification of Fungi Associated with 'Danzazzalau' Disease

Differences in altitude, source of seeds and cropping patterns practiced by farmers in the study areas might play crucial roles in Danzazzalau disease expression. The farmers visited during sample collection in Kebbi State attempted managing the infection of onion by 'Danzazzalau' disease through reduced frequency of irrigation for 14-21 days and rouging out infected plants. Only farmers at Jandutsi reported the use pesticides such as neonicotinoid 20 % + phenylamide 20 % + triazole 2 % (Dress force®, Jubaili Agrotec). *Fusarium* clade V., *Rhizoctonia* sp., *Thielavia* sp. and *Aspergillus* sp. were isolated and identified from diseased samples. These fungi are widespread with large host range (Agrios, 2005). *Thielavia* sp. belongs to the family Chaetomiaceae and is not reported to be pathogenic on onion plants (Chauhan, 1991). Inoculating 6-week old onion seedlings with *Thielavia* sp., *Aspergillus* sp. and *Rhizoctonia* sp. in the glasshouse, typical symptoms of 'Danzazzalau' disease were not produced. This agrees with Schwartz and Mohan (2008) who listed these as general and non-specific fungi of the onion plant. Seedlings inoculated with 10^6 conidia/ml *Fusarium* clade V. suspension developed symptoms such as central leaf elongation, loss of vigour, curling and tip dieback within five days of treatment. This indicates that *Fusarium* clade V. is a possible cause of 'Danzazzalau' disease observed and at reported Aliero and Maiyama Local Government Areas of Kebbi State. This contrasts with the report of Pablo *et al.* (2014) who reported a complex of phytopathogenic species of *Fusarium proliferatum* and *Fusarium oxysporum* which have been indicated as the causal organisms of *Fusarium* basal rot which are affecting the yield of irrigated onion in Argentina.

Evaluation of inoculation methods showed that seedlings inoculated via root dip and soil drench became infected. This may be due to higher inoculum load of the organism whose inoculum build-up or multiplication is fast in the host plant because inherent genetic characteristics of host tissue could not produce more inhibitory substances at that period of its growth that mitigated the presence of the pathogen (Shukla and Chand, 1975). Trillas and Segarra (2009) also reported the presence of non-pathogenic *Fusarium* strains in the soil and their antagonistic activities in relation to the virulent isolates may also play an important role in disease expression, as shown in other *Fusarium* patho systems such as in tomato, watermelon and pea.

5.2 'Danzazzalau' Disease Incidence and Severity as Influenced by some Cultural Practices

On the fields of Institute for Agricultural Research, Samaru, when seeds obtained from farmers in Kebbi State were sown, highest disease incidence and severity were obtained where 10-day water regime was used. Incidence was lowest at 21-day water regime which might be attributed to the amount of available oxygen been optimum to soil borne pathogens, while the microclimate of root growth zone ensures constant water availability at 10 to 15 cm depth compared to 3-day water regime (Gwari *et al*., 2014). The 3-day water regime had soil almost becoming water logged and unfavourable for growth and development of soil borne pathogens due to low Oxygen tension (Kumar *et al*., 2007). Incidence of the disease at 21-day regime was lower because infrequent water led to soil becoming more friable, deeper water table level and nutrient availability reduced. These are not favourable to most soil-borne pathogens like *Fusarium* species; except for opportunistic pathogens such as *Macrophomina phaseoli* which take advantage of weakened/stressed plants and being fast in development, quickly complete their life cycle (Chauhan, 1991).

Everts *et al.* (1985) reported that incidence and severity of basal rot induced by *Fusarium* sp. increased in fields cropped frequently to onions season after season in Colarado, United States of America. The pathogen is known to survive as dormant hyphae and chlamydospores on decaying plant debris with ability to infect rapidly where susceptible plants become stressed due to high soil temperatures. This might explain the insignificant effect of 'Danzazzalau' disease incidence and severity on seedlings treated with polyethylene sheet, rice straw and cow dung. The reduced vegetative growth of onions observed seems to be from the water deprivation coupled with the disease infection by *Fusarium* clade V. Lowest disease incidence was obtained where rice straw was used as mulch followed by polyethylene sheet. This may be that rice straw being organic in origin changes the Carbon-Nitrogen ratio in the soil environment (Inusah *et al*., 2013). This change enhances Oxygen tension within the soil favouring rapid development of roots as against the fungus development while treatment with polyethylene sheet causes solarisation of the soil leading to reduced availability of utilizable Oxygen to competing antagonistic soil myco-flora from the enhanced slight increase in soil temperature (Katan, 1981; Porter and Merriman, 2003).

Interaction effect of the various factors on disease incidence and severity were not significantly different at 5 % level of significance. This might be due to the soil organic matter content and quality which affect many functions that are related to soil health such as moisture retention, infiltration, release, and also plant health (Stone *et al*., 2004). Field-applied organic residues (crop residues, cover crops and organic wastes) can affect soil borne pathogens and disease expression (Stone *et al*., 2004). Practices such as addition of green manures and animal manures have been shown to

produce suppressive soils on which pathogens do not establish or persist and do not affect the crop plants (Hu *et al*., 1997).

However, interaction effect of water regime and mulch treatments on disease incidence was significantly different ($P \leq 0.05$). Incidence of disease at 10-day water regime increased as the manure rates increased from 40 to 80 Kg/plot, which might be due to synergistic effects of the applied mulch materials leading to improved performance of crop by suppressing weed growth, ameliorating soil temperature, conserving soil moisture, reducing soil erosion, improving soil structure and enhancing organic matter content (Stone *et al*., 2004).

The interaction for disease severity was however not significantly different from each other. Highest bulb yield, 4.67 t/ha was obtained with 21-day water regime which was at par with 10-day water regime, while the lowest yield 3.48 t/ha was obtained with 3-day water regime. This is in contrast with Gwari *et al*. (2014) who reported that irrigation at 3-day and 6-day regime gave highest bulb yield of 5.06 t/ha. The highest bulb yield, 4.80 t/ha was obtained from mulch treatment in which rice straw was used and this is significantly different at 5 % level than polyethylene sheet. Bulb yield for polyethylene sheet 4.02 t/ha was at par statistically with the no mulch 3.95 t/ha. Cecil (1989); Inusah *et al*. (2013) and Bashir *et al*. (2015) showed that different mulch materials including polyethylene sheets and rice straw do improve yield of crops like yam, cowpea, onion and other vegetables. Mulch generally improves growth, vigour, yields and productivity of irrigated onion by minimizing the effect of disease on the performance and yield of onion (Dilipkumar *et al*., 1990).

5.3 *In vitro* Evaluation of Aqueous and Methanolic Crude extracts of some Plants on Growth and Development of *Fusarium* clade V.

Several workers have reported the antifungal properties of several plant parts and their phytochemicals on many phytopathogenic fungi without the extracts being

phytotoxic (Lyon *et al*., 1995; Satish *et al*., 2007; Raj *et al*., 2011; Moyo *et al.*, 2012). Media amended with mancozeb, both methanol and aqueous crude leaf extracts individually reduced mycelial growth starting from 2-day after inoculation, which was similar to the report by Cycon *et al.* (2010), who found that mancozeb inhibited energy production within *Fusarium* clade V. and inactivated the –SH groups in amino acids, proteins and enzymes. The effect of mancozeb observed in this study could be attributed to the depletion of energy production and protein utilization in *Fusarium* clade V. isolate. Osunlaja and Alamutu (1999) also reported that mancozeb used in the control of brown spot of maize (*Physoderma maydis*) significantly inhibited mycelial growth and there was complete inhibition of sporangia germination at 10,000 ppm a.i. However, from 6-14 DAI, Moringa amended media had highest inhibitory effects on mycelial growth and sporulation and was followed by media amended with Eucalyptus. The fungicidal effect of Moringa extract on soil-borne fungi including *Rhizoctonia, Pythium* and *Fusarium* has been reported by several workers to be promising (Chuang *et al*., 2007; Al-Asker and Rashed 2010; Raj *et al*., 2011; Moyo *et al.*, 2012). Dwivedi and Enespa (2012) reported that leaves, bark and seed extracts of *Moringa oleifera* 75 % (v/v) had significant inhibitory effect on mycelial growth of *Fusarium solani* and *Fusarium* clade V. f. sp. *lycopersici*. Batish *et al*. (2008) reported that leaf extracts of *Eucalyptus globulus* significantly inhibited mycelial growth of wood rot fungi.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

A field trip was made to Kebbi State, North-Western Nigeria to some onion farms in October, 2014. The fungus inducing 'Danzazzalau' disease was isolated from onion root and pseudostem, identified and its pathogenicity confirmed. The fungus was initially identified at the Department of Crop Protection, ABU Zaria as *Fusarium* sp. and was later confirmed at Centre for Agriculture and Bioscience International (CABI), Egham, United Kingdom as *Fusarium* clade V. and given the International Mycological Institute (IMI) number 504847 (Appendix A). The pathogenicity test carried out involved inoculation of six weeks old onion seedlings with the identified organism using two inoculation methods (root dip and soil drench), at inoculum concentrations of 1×10^6 and 1×10^3 conidia/ml showed that the seedlings were susceptible using both methods. The incidence and severity of 'Danzazzalau' disease induced by *Fusarium* clade V. as influenced by water regime, mulching and cow dung rates in the field varied significantly from each other.

The incidence and severity of 'Danzazzalau' disease was assessed on an onion variety called 'Barke'. Highest disease incidence 54.95 % and severity 37.92 % were obtained for plants with 10-day water regime while the least disease incidence 36.02 % and severity 32.93 % were obtained for 21- day water regime. The results of this study indicate that the combined effects of 21-day water regime that had the least disease incidence and severity, and rice straw mulch that had the highest bulb yield, the two treatments were significant in reducing the incidence and severity of the 'Danzazzalau' disease as they both enhanced the overall

performance of the onion crop, thus providing a better management option.

The effect of aqueous and methanol crude extracts of *Moringa oleifera*, *Jatropha curcas* and *Eucalyptus globulus* leaves on mycelia diameter and number of conidia/ml produced was studied *in vitro*. Aqueous extract of Moringa at 5 % (w/v) when used to amend culture media led to increase in conidia population by 91.53 %, however when Jatropha and Eucalyptus extracts at 5 % (w/v) were used conidia populations were reduced by 42.3 % and 19.04 % respectively. Conidial dimensions were reduced by 6.32 %, 6.05 % and 0.64 % when 2.5 % (w/v) aqueous extracts of Jatropha, Eucalyptus and Moringa leaves were used to amend the culture media respectively.

Methanolic extract of Eucalyptus at 5 % (w/v) when used to amend culture media reduced conidia population by 88.3 % followed by 64.5 % and 20.10 % reduction when Moringa and Jatropha leaves extracts were used respectively. Conidial dimensions were reduced by 5.31 %, 3.20 % and 1.28 % when 2.5 % (w/v) when methanolic leaf extracts of Eucalyptus, Jatropha and Moringa were used to amend the culture media respectively.

6.2 Conclusions

In the study carried out during the 2015 dry season at Samaru, *Fusarium* clade V. was isolated and identified as the fungus that induces the 'Danzazzalau' disease of irrigated onion in Kebbi State, Nigeria. 'Danzazzalau' disease was wide spread at the locations visited in Kebbi State.

The effect of some cultural practices such as water regime, cow dung and mulch on bulb yield as influenced by 'Danzazzalau' disease was significant and showed potentials for use in an integrated management of the disease. Infection of onion by *Fusarium* clade V. can be effectively managed when mulched with rice straw

and 21-day irrigation schedule were implemented.

This study indicates that amending culture media with methanolic leaf extracts of Moringa and Eucalyptus at 10 g/ml significantly inhibited percentage mycelial growth and reduced spore production of *Fusarium* clade V. *in vitro*.

6.3 Recommendations

Based on the findings of this study, the following recommendations are suggested:

1. Seedlings were affected by the 'Danzazzalau' disease 2-4 weeks after transplanting which may necessitate prophylactic spray of fungicides immediately after transplanting;
2. Twenty one (21)-day water regime is recommended to be adopted by onion farmers to reduce incidence and severity of 'Danzazzalau' disease;
3. Use of rice straw as mulch should be encouraged in irrigated onion production especially in areas where onions are prone to infection by *Fusarium* clade V., as it recorded the least incidence and severity of the disease;
4. Further studies on the mode of action of different plant extracts on the disease should be explored.

REFERENCES

Abdulaziz, A. A. and Younes, M. R. (2010). Efficacy of some Plant Extracts against *Rhizoctonia solani* on Pea. *Journal of Plant Protection Research*, 50 (3): 239-243.

Ado, P. O. (2001). Onion Cultivation. *Onion Newsletter*, 20: 30-34.

Agrios, G. N. (2005). *Plant Pathology*. 5th Edition. Elsevier Academic Press, California, USA, 988 pp.

Agyenim-Boateng, S. and Dennis, E. A. (2001). Contribution of Crop Residues to Soil Fertility Restoration. *Journal of the Ghana Science Association*, 3 (1):51-59.

Akpa, A. D., Musa, B. and Poswall, A. T. (1991). Effect of Neem Extract on Mycelia Growth of the Sorghum Anthrachnose Pathogen, *Collectorichum graminicola. Nigerian Journal of Plant Protection*, 11:22-28

Al-Askar, A. A. and Rashad, Y. M. (2010). Efficacy of some Plant Extracts against *Rhizoctonia solani* on Pea. *Journal of Plant Protection Research*, 50:239-243.

Al-Jamal, M. S., Sammis, T. W., Ball, S. and Smeal, D. (1999). Yield-based, Irrigated Onion Crop Coefficients. *Applied Agricultural Engineering* 15(6): 659-668.

Al-Samarrai, G., Singh, H. and Syrahabil, M. (2012). Evaluating Eco-friendly Botanicals (natural plant extracts) as Alternatives to Synthetic Fungicides. *Annals of Agricultural Environment*, 19, 673-676

Amadioha, A. C. (2000). Controlling Rice Blast *in vitro* and *in vivo* with Extracts of *Azadirachta indica*. *Crop Protection*, 19: 287-90.

Anjorin, S. T., Ikokoh, P. and Okolo, S. (2010). Mineral Composition of *Moringa oleifera* Leaves, Pods and Seeds from two Regions in Abuja, Nigeria. *International Journal Agricultural Biology*, 12:431-434

Ashwani, T., Rajesh, K., Nandini, G. and Shailesh, P. (2012). Compatibility of *Trichoderma viride* for Selected Fungicides and Botanicals. *International Journal of Plant Pathology*, 3: 89-94.

AVRDC, Asian Vegetable Research and Development Council, (1990). Vegetables Production Training Manual.Taipei, Taiwan. 64 pp.

Aveling, T., Aveling, S., Snyman, H. G. and Naude, S. P. (1993). Evaluation of Seed Treatment for Reducing *Alternaria porri* and *Stemphylium vesicatorium* on Onion Seed. *Plant Disease*,.77: 1009-1011.

Awodoyin, R. O. and Ogunyemi, S. (2005). Use of Sickle Pod, *Senna obtusifolia* (L) Irwin and Barneby, as Mulch Interplant in Cayenne Pepper, *Capsicum frutescens* L. Production. *Emirate Journal of Agricultural Science*, 17 (10):10-

Awodoyin, R. O., Ogbeide, F. I. and Oluwole, O. (2007). Effects of Three Mulch Types on the Growth and Yield of Tomato (*Lycopersicon esculentum* Mill.) and Weed Suppression in Ibadan, Rainforest-savanna Transition Zone of Nigeria. *Tropical Agricultural Research and Extension*, 5:8-21.

Ayodele, V. D. (1996). Onion (*Allium cepa* L.) Production in Ibadan, Southwest Nigeria: Prospects and Limitation. A paper presented at the 11th Annual Conference of the Horticultural Society of Nigeria (HORTSON), held at Onabisi Onabanjo University (O.O.U) Ago-Iwoye, 1st-4th April, 1996.

Bag, S., Schwartz, H. F., Cramer, C. S., Havey, M. J. and Pappu, H. R. (2015). Iris Yellow Spot Virus (*Tospovirus: Bunyaviridae*): From Obscurity to Research Priority. *Molecular Plant Pathology* 16(3): 224-237.

Bakhru, H. K. (2007). *Nature Cure for Children's Diseases,* "Treatment," from the Reading Room of www.healthlibrary.Com. Accessed 22nd May 2017.

Barnett, H. L. and Hunter, B. B. (2006). *Illustrated Genera of Imperfect Fungi*, 4th Edition. St. Paul (MI): American Phytopathological Society (APS Press). pp 218.

Bashir, A. Y., Liman, Y. M. and Zangoma, I. M. (2015). Effect of Different Sources of Organic Manure on the Growth and Yield of Irrigated Onion in Damaturu Local Government Area of Yobe State, Nigeria. *International Journal of Multidisciplinary Academic Research,* 3(4): 23-29

Batish, R. D., Singh, P. H., Kohli, K. R. and Kaur, S. (2008). Eucalyptus: Essential Oil as Natural Pesticide. *Forest Ecology and Management*, 256:2166-2174.

Booth, C. (1971). *The Genus Fusarium.CMI*, Kew, England. pp 237.

Booth, C. (1977). *Fusarium*: *Laboratory Guide to the Identification of the Major Species*. CMI, Kew, England pp187.

Brewster, J. L. (1994). *Onions and other Vegetable Alliums.* 2nd Edition, CAB International, North America. 236 pp.

Brouwer, C. K., Prins, K. and Heibloem, M. (1989). Irrigation Water Management: Irrigation scheduling. FAO Training Manual No. 4. Rome, Italy. pp 89.

Burgess, L. W. and Liddell, C. M. (1983). *Laboratory Manual for Fusarium Research, Fusarium Research Laboratory*, Department of Plant Pathology and Agricultural Entomology. The University of Sydney, Australia, pp 106.

Carcia, R. P. and Lawas, M. V. P. (1990). Potential Plant Extracts for the Control of Azolla Fungal Pathogens. *Philippine Agriculturist,* 73 (3/4): 343-348.

Cecil, O. (1989). The Yields of Seed Yam Grown from Yam minisetts as Influenced by Mulch. ***In***: Second workshop on improving farming systems in northern interior savanna zone of Ghana. Mensah, J. B., Atsu, P. A. Oumar, M. S.

(Eds.) pp 166-172.

Chauhan, R. K. S. (1991). Occurrence of *Macrophomina phaseolina* on Onion Bulbs. *Indian Phytopathology*, 10:18-25.

Chuang, P. H., Lee, C. W., Chou, J. Y., Murugan, M., Shieh, B. J. and Hueihminchen, M. (2007). Antifungal Activity of Crude Extracts and Essential Oil of *Moringa oleifera* Lam. *Bioresource Technology*, 98:232-236.

Currah, L. P. and Felicity, I. (1990). Onions in Tropical Regions. *Natural Resources Institute, Bulletin,* No 35, 32 pp.

Cycon, M., Piotrowska, Z. and Kozdroj, J. (2010). Responses of Indigenous Microorganisms to a Fungicidal Mixture of Mancozeb and Dimethomorph Added to Sandy Soils. *International Journal of Biodeterioration and Biodegradation*, 64(4): 316-323.

De hoog, G. S., Guraro, J., Gene, J. and Figueras, M. J. (2000). *Atlas of Clinical Fungi*, 2nd edition, vol. 1. Centraa bureau voor Shcimmel cultures, Utrecht, The Netherlands, 122 pp.

Dilipkumar, G., Sachin, S. S. and Rajesh, K. (1990). Importance of Mulch in Crop Production. *Indian Journal of Soil Conservation*, 18: 20-26

Duke, J. A. (2004). Dr. Duke's Phytochemical and Ethno botanical databases. Accessed via http://www.phyto-pag.org 25/3/2017.

Dwivedi, S. K. and Enespa, A. (2012). Effectiveness of Extract of some Medicinal Plants against Soil borne Fusaria Causing Diseases on *Lycopersicon esculentum* and *Solanum melongena*. *International Journal of Pharmacy and Biological Science*, 3(4):1171-1180

Ebele, M. I. (2011). Evaluation of some Aqueous Plant Extracts used in the Control of the Pawpaw (*Carica papaya* L.) Fruit Rot Fungi. *Journal of Applied Biosciences,* 37:2419- 2424

Ebenebe, A. C. (1980). Onion Twister Disease Caused by *Glomerella cingulata* in Northern Nigeria. *Plant Disease*, 64:1030-1032.

Emechebe, A. M., Erinle, I. D., Bos, W. S., Tyagi, P. D., Surdaram, N. V., Manzo, S. K., Ebenebe, A. C. and Subbarayudu, S. (1980). A Check-list of Diseases of Crops in the Savanna and Semi-arid Areas of Nigeria. Institute for Agricultural Research (IAR) Samaru, Zaria, Nigeria. Bulletin No. 13, 60 pp

Everts, K. L., Schwartz, H. F., Epsky, N. D. and Capinera, J. L. (1985). Effects of Maggots and Wounding on Occurrence of *Fusarium* Basal Rot of Onions in Colarado. *Plant Disease,* 69:878-882

FAOSTAT (2014). Available at: http://faostat.fao.org/site/340/default.aspx (Accessed 22nd September, 2016)

FDAE, Federal Department of Agricultural Extension (2015). *Agricultural*

Performance Survey Report of 2015 Wet Season in Nigeria, 233 pp.

Fahey, Sc. D. (2005). *Moringa oleifera*: A review of the Medical Evidence for its Nutritional, Therapeutic and Prophylactic Properties. http://www. tfl.Journal.org. 1(5):1-15. Accessed 3rd March, 2017

Galeone, C., and Pelucchi, C. (2007). "Onion and Garlic Use and Human Cancer." American Society for Nutrition. *American Journal of Clinical Nutrition*, www.ajcn.org/cgilcontentlabstract. Accessed 1st October, 2014

Gent, D. H., Schwartz, H. F. and Khosla, R. (2004). Distribution and Incidence of Iris Yellow Spot Virus and its Relation to Onion Plant Population and Yield. *Plant Disease*.88:446-452.

Ghazanfar, S. A. (2007). *Handbook of Arabian Medicinal Plants*, Boca Raton, FL: CRC Press, Inc., 265 pp., www.ars-grin.gov/duke, Accessed 15th May, 2016.

Grainge, M. and Ahmed, S. (1988). *Handbook of Plants with Pest-control Properties*. John Wiley and Sons, New York. pp 470.

Gugino, B. K., Mansfield, M. A. and Pfeufer, E. E. (2014). Bacterial Rots of Sweet Onion in Pennsylvania: Tracking Sources of Infection and Targeting Critical Management Points. *Phytopathology*: 15(8): 217-229.

Gwari, E. Y., Gambo, B. A. and Kabura, B. H. (2014). Effect of Organic Manures and Irrigation Regimes on the Growth and Yield of Onion (*Allium cepa* L.) in Central and Southern Borno State, Nigeria. *International Journal of Advanced Agricultural Research*, 2(1): 106-111.

Habou, Z. A., Haougui, A., Mergeai, G., Haubruge, E., Toudou, A. and Verheggen, F. J. (2011). Insecticidal Effect of *Jatropha curcas* Oil on the *Aphid aphis* Fabae (*Hemiptera: Aphididae*) and on the Main Insect Pests Associated with Cowpea (*Vigna unguiculata)* in Niger. *Tropicultura*, 29 (4): 225-229.

Habtemariam, K. (2003). *Livestock and Livelihood Security in the Harar Highlands of Ethiopia. An implication for research and development*, Swedish University of Agricultural Sciences, Uppsala, Sweden. 185 pp.

Halim, A. O. and Mehmet, S. (2001). A Study on Irrigation Scheduling of Onion (*Allium cepa* L.) in Turkey. *Journal of Biological Science*, 1: 735-736.

Hamasaki, R., Simmonds, V. H. and George. C. T. (1999). *Irrigation Scheduling for Onions. **In**: Bulb Onion Production in Hawaii*, Tropical Agriculture and Human Resources (CTAHR) Publishers, Hawaii, Valenzuela, H. and Shimabuku, R. (Eds). 52 pp.

Hamilton-Kemp, T. R., Archbold, D. D. and Loughrin, J. H. (2000). Stimulation and Inhibition of Fungal Pathogens of Plants by Natural Volatile Phytochemicals and their Analogs. *Current Topics in Phytochemistry*, 4: 95-104

Hernandez, C. F. D., Castillo, R. F., Gallegos, M. G., Rodriguez, H. R. and Aguilar, G. C. N. (2010). *Lippia graveolens* and *Carya illneesis* Organic Extract and

their *in vitro* effects against *Rhizoctonia solani* Kuhn. *American Journal of Agricultural* and *Biological Science*, (5)3: 380-384

Hillel, D. (1998). *Environmental Soil Physics*. Academic Press, an Imprint of Elsevier, San Diego, CA 921014495, USA.

Hochmuth, G. J., Hochmuth, R. C. and Oslon, S. M. (2001). *Polyethylene Mulching for Early Vegetable Production in North Florida*. University of Florida/Institute of Food and Agricultural Science (UF/IFAS), Florida. A and M University Cooperative Extension Program. http: edis. ifas. ufl. Edu

Hoepting, C. A. (2016a). Efficacy of Fungicide Treatments for Control of Stemphylium Leaf Blight on Onion. *Plant Disease Management*. 10:121. Online publication. doi: 10.1094/PDMR10 Accessed 7thNovember, 2017

Hoepting, C. A. (2016b). Efficacy of Fungicide Treatments for Control of Downy mildew on Onion. *Plant Disease Management*. 10:119. Online publication. doi: 10.1094/PDMR10. Accessed 7th November, 2017

Hu, S., van Bruggen, A.H.C., Wakeman, R. J. and Grunwald, N.J. (1997). Microbial suppression of *in vitro* growth of *Pythium ultimum* and disease incidence in relation to soil Carbon and Nitrogen availability. *Plant and Soil*, 1(5):43-52.

Hussaini, M. A., Amans, E. B. and Ramalan, A. A. (2000). Yield, Bulb Size, Distribution and Storability of Onion (*Allium cepa* L.) under Different Levels of N-fertilization and Irrigation Regime. *Tropical Agriculture* (*Trinidad*), 77 (3): 145-149.

Ibrahim, M. (2014). Economic Analysis of Onion Marketing in Kaduna and Katsina States. A Ph.D thesis Submitted to the Department of Agricultural Economics and Rural Sociology, Ahmadu Bello University, Zaria. 307 pp.

Ibrahim, N. D. and Adamu, T. (2010). Incidence of *Thrips tabaci* on Onion (*Allium cepa* L.) and their control in Sokoto and Kebbi States, Nigeria. *Nigerian Journal of Basic and Applied Sciences*, 16(2): 221-226

I.A.R, Institute for Agriculture Research (2009). Annual Cropping Scheme Meeting Report. 105 pp.

Inusah, B. I. Y., Wiredu, A. N., Yirzagla, J., Mawunya, M. and Haruna, M. (2013). Effects of Different Mulches on the Yield and Productivity Drip Irrigated Onions under Tropical Conditions. *International Journal of Advance*d *Agricultural Research*, 1(3): 133-140.

Jilani, M. S. and Ghafoor, A. (2003). Screening of Local Onion Varieties for Bulb Formation. *International Journal of Agriculture and Biology*, 5 (2):129-133.

Katan, J. (1981). Solar Heating (solarisation) of Soil for Control of Soil borne Pests. *Annual Review of Phytopathology*, 19:211-236.

Kumar, S., Imtiyaz, M. and Kumar, A. (2007). Effect of Differential Soil Moisture and Nutrient Regimes on Postharvest Attributes of Onion (*Allium cepa* L.).

Scientia Horticulturae, 112:121-129.

Kwakye, P. K. (1980). The Effects of Method of Dung Storage and its Nutrient (NPK) Content on Crop Yield in the Northeast Savanna Zone of Ghana. *FAO Soil Bulletin*, 43: 282-288.

Lal, R., De Vleeschauwer, D. and Naganje, R. M. (1980). Changes in Properties of Newly Cleared Tropical Alfisol as Affected by Mulching. *Soil Science Society of America Journal*. 44:827-833.

Lannoy, G. De. (2001). Vegetable Crops in Tropical Africa 395-511. ***In:*** Raemaekers, R. H. (Ed). *Crop Production in Tropical Africa.* Directorate General for International Cooperation, (DGIC) Belgium 1540 pp.

Lucienne, D, Sharon, M., Arbainsyah, A. and Rebecca, H. S. U. (2006). *Moringa oleifera: Medicinal and Socioeconomic Uses*. International Course on Economic Botany. National Herbarium Leiden, Netherlands. pp. 2-6.

Lyon, G. D., Beglinski, T. and Newton, A. C. (1995). Novel Disease Control Compounds: The Potential to "immunize" Plants against Infection. *Plant Pathology*, 44:407- 427.

Malik, M. N. (2000). *Horticulture*. Biotech books. Delhi. 672 pp

Maloy, O. C. (2005). Plant Disease Management. *The Plant Health Instructor.* doi: 10.1094/PHI-I-2005-0202-01, Accessed May 21st 2015.

Marley, P. S. (2013). *Mycology and fungal diseases*. Diligent Publishers Limited Kaduna, Nigeria. 334 pp

Mateen, H. K., Mohammad, I. and Tahir, H. C. (2005). Effect of Irrigation Regimes on Growth and Yield of Onion Varieties Swat-1 and Phulkara. *Journals of Applied Science Research*. 1(2):112-116.

Mermoud, A., Tamini, T. D, Yacouba, H. (2005). Impacts of Different Irrigation Schedules on the Water Balance Components of Onion Crop in Semi-arid Zone. *Agricultural Water Management*. 77: 282-295.

Mishra, R. K., Singh, S., Pandey, S., Sharma, P. and Gupta, R. P. (2010). First Report of Root-Knot Nematodes *Meloidogynne graminicola* on Onion in India. *International journal of Nematology*. 20(2):236-237.

Mishra, R. K., Jaiswal, R. K., Kumar, D., Saabale, P. R. and Singh, A. (2014). Management of Major Diseases and Insect Pests of Onion and Garlic: A Comprehensive Review. *Journal of Plant Breeding and Crop Science.* 6(11):160-170

Moyo, B., Masika, P.J. and Muchenje, V. (2012). Antimicrobial Activities of *Moringa oleifera* Lam. Leaf Extracts, *African Journal of Biotechnology* 11(11):2797-2802.

Musa, A. K., Belewu, M. A., Muhammed-Lawal A., Adekola F., Olanrewaju B. O.

and Ibraheem, S. O. (2011). Costs Analysis and Toxicity of *Jatropha curcas* L. on Maize Weevil, *Sitophilus zeamais* Motsch. *African Journal of Plant Science*, 54 (4): 233-236.

NAERLS, National Agricultural Extension and Research Liaison Services and FDAE, Federal Department of Agricultural Extension (2015). *Agricultural Performance Survey Report of 2015 Wet Season in Nigeria*, NAERLS, Ahmadu Bello University, Zaria. NAERLS Press 233 pp

Nelson, P. E., Toussoun, T. A., and Cook, R. J. (1981). *Fusarium*: *Disease, Biology and Taxonomy*. Pennsylvania State University Press, 457 pp.

Nischwitz, C., Langston, D. J., Sanders, H. F., Torrance, R., Lewis, K. J. and Gitaitis, R. D. (2008). First Report of *Colletotrichum gloeosporiodes* causing 'Twister Disease' of Onion (*Allium cepa*) in Georgia. *Plant Disease*. 92(6):974 -985

Obeng-Ofori, D., Danquar, E.Y. and Ofosu-Anim, J. (2007). *Vegetable Spice Crop Production in West Africa*. The City Publishers Limited, Ghana. pp 77-79

Ofori, C. S. and Sanatanna, R. (1990). Manures and Organic Fertilizers: their potential and use in African Agriculture. pp 67-71. ***In***: Proceedings of the Third Regional Workshop of the AFRICA LAND Programme held at Ministry of Agriculture and Land Resources, Antananarivo, Madagascar, Toudou, A. Y. and Johnson, N. T. (Eds.), 9-15th January, 1990

Okigbo, R. N. and Emoghene, A. O. (2004). Antifungal Activity of Leaf Extract of some Plant species on *Mycopharella fiji*, the Causal Organism of Black Sigatoka Disease in Banana (*Musa acuminata*). *King Mongkut's Institute of Technology Ladkrabang (KMITL) Science Journal*, 4(1):20 – 31

Okigbo, R. N. and Nmeka, I. A. (2005). Control of Yam Tuber rot with Leaf Extract of *Xylopia aethiopica* and *Zingiber officinale*. *African Journal of Biotechnology*, 4(8):804-807

Openshaw, K. (2000). A Review of *Jatropha curcas*: An Oil Plant of Unfulfilled Promise. *Biomass Bioenergy*, 19:1-15.

Osunlaja, S. O. and Alamutu, M. A. (1999). Evaluation of Fungicides for the Control of Brown spot Disease of Maize Caused by *Physoderma maydis* Miyabe. *Nigerian Journal of Plant Protection*, 18:84-95.

Owolade, B. F. and Osikanlu, Y. O. K. (1999). Evaluation of some Plant Extracts for the Control of Brown Blotch Disease of Cowpea in South Western Nigeria, *Journal of Sustainable Agriculture and Environment*, 2:199-202

Pablo, F. C. G., Jorge, G. V., Ricardo, J. P. and Claudio, R. G. (2014). Influence of *Fusarium* spp. Isolate and Inoculum Density on Resistance Screening Tests in Onion. *Tropical Plant Pathology*, 39(1): 19-27

Pfeufer, E. E., Mansfield, M. A. and Gugino, B. K. (2013). Environmental and Management Factors Associated with Bacterial Rots of Onion in Pennsylvania. *Phytopathology*, 103:2 173-185.

Porter, I. J. and Merriman, P. R. (2003). Effects of Solarization of Soil on Nematode and Fungal Pathogens at Two Sites in Victoria. *Soil Biology and Biochemistry*. 15:39-44

Raj, A. J., Gopalakrishnan, V. K., Yadav, S. A. and Dorairaj, S. (2011). Antimicrobial Activity of *Moringa oleifera* (Lam.) Root Extract. *Journal of Pharmacy Research*, 4(5):1426 -1430.

Ratnadass, A. and Wink, M. (2012). The Phorbol Ester Fraction from *Jatropha curcas* Seed Oil: Potential and Limits for Crop Protection against Insect Pests. *International Journal of Molecular Sci*ences, 13: 16157-16171.

Russell, E. J. (2004). *Manuring for Higher Crop Production.* Agrobios, India. Saleem Publishers, 238 pp.

SAS, (2002). *Institute, Inc. SAS User guide: Statistical Version 9*. SAS institute, Carry, NC.27513, USA

Sangeetha, G., Thangavelu, R. and Rani, S. U. (2010). Evaluation of Plant Oils for Suppression of Crown Rot Disease and Improvement of Shelf Life of Banana (*Musa* spp. AAA subgroup, cv. Robusta). *International Journal of Food Science and Technology*, 45: 1024-1032.

Sattar, M. A. and Gaur, A. C. (1989). Effects of VA-mycorrhiza and Phosphate Dissolving Microorganism on the Yield and Phosphorus Uptake of Wheat (*Triticum vulgare*) in Bangladesh. *Bangladesh Journal of Agricultural Research*, 14(3): 233-239.

Satish, S., Mohana, D. C., Ranhavendra, M. P. and Raveesha, K. A. (2007). Antifungal Activity of some Plant Extracts against Important Seed Borne Pathogens of *Aspergillus* spp. *Journal of Agricultural Technology*, 3 (1): 109-119.

Satyaweti, S., Monica, V., Rajendra, P. and Diwakar, I. (2011). Efficacy of Non edible Oil Seed Cakes against Termites (*Ondontotemes opbesus*). *Journal of Scientific and Industrial Research*, 70:1037-1041.

Schroeder, B. K., Waters, T. D. and du Toit, L. J. (2010). Evaluation of Onion Cultivars for Resistance to *Enterobacter cloacae* in Storage. *Plant Disease*, 94:236-243.

Schwartz, H. F. and Mohan, S. K. (1995). *Compendium of Onion and Garlic Diseases*. St. Paul, Minnesota: American Phytopathology Society Press. pp126

Schwartz, H. F. and Mohan, S. K. (2008). *Compendium of Onion and Garlic Diseases and Pests*, 2nd Edition.APS Press. The American Phytopathological Society, St. Paul, Minnesota. pp 198

Shukla, B. N. and Chand, J. N. (1975). Effect of Leaf Age on the Bacterial Blight of *Sesamum* spp. *Indian Phytopathology*, 26:304-305

Stewart, B. A. and Nielsen, D. R. (1990). *Irrigation of Agricultural Crops*. Madison, Wisconsin USA. pp 178

Stone, A. G., Scheuerell, S. J. and Darby, H. D. (2004). *Suppression of Soil borne Diseases in Field Agricultural Systems: Organic Matter Management, Cover Cropping and other Cultural Practices*, ***In***: Magdoff, F. and Weil, R. R. (Eds.), Soil Organic Matter in Sustainable Agriculture, CRC Press, London UK. 278 pp.

Straub, R. W. and Emmett, B. (1992). Pests of Monocotyledon Crops. ***In****:* McKinlay, R. O. (Ed). *Vegetable Crop Pests.* Macmillan Press. U. K. pp 213-262.

Sutton, D. A., Fothergill, A. W. and Rinaldi, M. G. (1998). *Guide to Clinically Significant Fungi*. 1st Edition, Williams and Wilkins, Baltimore, pp 201.

Takahashi, T., Kokubo, R. and Sakainom, M. (2004). Antimicrobial Activities of Eucalyptus Leaf Extracts and Flavonoids from *Eucalyptus maculate*. *Letters in Applied Microbiology*, 39(3), 60-64.

Teklu, E., Stahr, K. and Getachew, T. (2004). Integration of Organic and Inorganic Fertilizers: Effect on Vegetable Productivity. International Research on Food Security, National Resource Management and Rural Development. Humboldt. *Universitat Zu Berlin*. 13(3):245-262

Tello, V., Diaz, L. and Sanchez, M. (2013). Side effects of the Natural Pesticide Spinosad (GF-120 Formulation) on *Eretmocerus paulistus* (Hymenoptera: Aphelinidae), a Parasitoid of the Whitefly *Aleurothrixus floccosus* (Hemiptera: Aleyrodidae), Under Laboratory Conditions. *Journal of Natural Products and Plant* Resources, 40(2):407-417.

Trillas, M. L. and Segarra, O. (2009). Interactions between Non-pathogenic Fungi and Plants. *Advances in Botanical Research*, 51:321-359.

Tripathi, P. and Shukla, A. K. (2007). Emerging Non-conventional Technologies for Control of Post harvest Diseases of Perishables. *Fresh Produce*, 1: 111-120.

Tyagi, S., Dube, V. P., Charaya, M. U. (1990). Biological Control of the Purple Blotch of Onion Caused by *Alternaria porri* (Ellis) Ciferri. *International Journal of Pest Management*. 36(4):384-386.

Usman, I. (2013). Effect of Post- Emergence Herbicides on Weed Control, Yield and Profitability of Onion (*Allium cepa*) at Gombe in Northern Guinea Savanna of Nigeria. *Savannah Journal of Agriculture*. 2(3):42-48

Watanabe, T. (2002). *Pictorial Atlas of Soil and Seed Fungi and Key to Species*. 2nd Edition, CRC Press, New York, 486 pp.

W.F.P. (World Food Programme) (2014). *Annual report of WFP*. Accessed on http: wfp.un 23rd July, 2015.

Winston, C. (2011). *Landscape of Truth: Positive Power*. Accessed on htpp:llrao1181 Opr wordpress.com 9th April 2016

Wohleb, C. H., Waters, T. D, du Toit, L. J. and Schroeder, B. K. (2012). The Washington State University Onion Cultivar Trial: An Important Resource for Washington Onion Growers. *Acta Horticultura*. 969:241-246

Wurdack, K. J. (2008). Molecular Evolution and Phylogenetic of Euphorbiaceae: Beyond the Model Organisms. Plant and Animal Genomes XVI Conference San Diego, CA. Accessed via http://www.intpag.org/16/abstracts 15/03/2015.

APPENDICES

Appendix A: Top Twenty five Onion Producing Countries in the World, 2014

Ranking	Country	Area (×000/ha)	Production (×000MT)	Yield (MT/ha)
1	China	1,001,171	20,817,295	20.76
2	India	804,600	8,178,300	10.16
3	Unitedstates	62,120	3,349,170	53.91
4	Pakistan	153,100	2,015,200	13.16
5	Turkey	75,000	2,007,120	26.76
6	Russia	128,600	1,900,000	14.77
7	Egypt	52,885	1,728,417	32.68
8	Iran	50,000	1,700,000	34.00
9	Brazil	63,639	1,299,815	20.42
10	Mexico	42,998	1,252,441	29.13
11	Japan	24,500	1,165,000	47.55
12	Netherlands	26,200	1,130,000	43.13
13	Spain	21,100	1,098,400	52.06
14	Ukraine	62,000	1,049,200	16.92
15	SouthKorea	15,392	1,035,076	67.25
16	Bangladesh	125,226	889,260	7.10
17	Indonesia	91,780	824,006	48.98
18	Myanmar	60,000	740,000	12.33
19	Uzbekistan	23,000	728,000	31.65
20	Argentina	24,000	700,000	29.17
21	Algeria	38,000	700,000	18.42
22	Morocco	29,900	662,140	23.70
23	Peru	18,879	634,393	33.60
24	**Nigeria**	**42,000**	**1,320,000**	**14.79**
25	Poland	30,187	618,233	20.48

Source:(FAOSTAT, 2014)

Appendix B

CABI IDENTIFICATION REPORT

Our ref.: YN3/15/H62
Your ref:

Your number	IMI number	Identification and comments
SA1	504847	***Fusarium* clade V.** This sample was identified by ITS rDNA sequence analysis using the FASTA algorithm with the Fungus database from EBI. From results of ITS sequencing, >99% matches were obtained and assigned to the genus *Fusarium*, and mainly to members of clade V.
SA2	504848	***Fusarium* clade V.** This sample was identified by ITS rDNA sequence analysis using the FASTA algorithm with the Fungus database from EBI. From results of ITS sequencing, >99% matches were obtained and assigned to the genus *Fusarium*, and mainly to members of clade V.
SA3	504849	***Thielavia* sp.** This sample was identified by ITS rDNA sequence analysis using the FASTA algorithm with the Fungus database from EBI and by examination of morphology. The ITS sequence obtained from this sample produced top matches at >99% identity to 12 sequences from members of the genus *Thielavia*.
SA4	504850	***Fusarium* clade V.** This sample was identified by ITS rDNA sequence analysis using the FASTA algorithm with the Fungus database from EBI. Result: From results of ITS sequencing, >99% matches were obtained to sequences assigned to the genus *Fusarium*, and mainly to members of cladeV, including 100% to *F. oxysporum* strain IHEM 22401 [KP132218] which has been cited in: Irinyi, L. *et al.* (2015).
SA5	504851	***Fusarium chlamydosporum* species group** This sample was identified by ITS rDNA sequence analysis using the FASTA algorithm with the Fungus database from EBI and supported by use of the *Fusarium*-ID online identification tool. Result: From results of ITS sequencing, >99% matches were obtained to sequences assigned to the *Fusarium chlamydosporum* species group. This was supported by the *Fusarium* ID online identification tool which gave 100%.

APPENDIX C

ANOVA Tables

ANOVA Table for Disease Incidence

Source	Df	SS	MS	F-value	Pr≥F
Treatment	44	14742.63	335.06	2.93	0.0006
Error	36	4119.92	114.44		
Total	80	18862.55			

ANOVA Table for Disease Severity

Source	Df	SS	MS	F-value	Pr≥F
Treatment	44	3136.12	71.28	2.74	0.0012
Error	36	937.12	26.03		
Total	80	4073.24			

ANOVA Table for Number of Harvested Bulbs

Source	Df	SS	MS	F-value	Pr≥F
Treatment	44	3628.44	82.47	1.65	0.0625
Error	36	1799.56	49.99		
Total	80	5428.00			

ANOVA Table for Harvested Bulb Yield

Source	Df	SS	MS	F-value	Pr≥F
Treatment	44	66.49	1.51	1.92	0.0232
Error	36	28.31	0.79		
Total	80	94.80			

ANOVA Table for Onion Bulb Diameter

Source	Df	SS	MS	F-value	Pr≥F
Treatment	44	19.44	0.44	1.94	0.0214
Error	36	8.19	0.23		
Total	80	27.63			

ANOVA Table for Spore count

Source	Df	SS	MS	F-value	Pr≥F
Treatment	23	8.6551618E13	3.7631138E12	2.42	0.0022
Error	76	1.1833558E14	1.5570471E12		
Total	99	2.048872E14			

ANOVA Table for Spore length

Source	Df	SS	MS	F-value	Pr≥F
Treatment	23	0.00131	0.000057	2.02	0.0121
Error	76	0.00215	0.000028		
Total	99	0.00346			

ANOVA Table for Spore width

Source	Df	SS	MS	F-value	Pr≥F
Treatment	23	0.18189	0.00790	1.00	0.4801
Error	76	0.60316	0.00793		
Total	99	0.78505			

ANOVA Table for mycelial growth inhibition at 2 DAI

Source	Df	SS	MS	F-value	Pr≥F
Treatment	23	40047.29	1741.19	18.30	<.0001
Error	76	7230.49	95.14		
Total	99	47277.78			

ANOVA Table for mycelial growth inhibition at 4 DAI

Source	Df	SS	MS	F-value	Pr≥F
Treatment	23	49196.45	2138.98	58.44	<.0001
Error	76	2781.71	36.60		
Total	99	51978.16			

ANOVA Table for mycelial growth inhibition at 6 DAI

Source	Df	SS	MS	F-value	Pr≥F
Treatment	23	59011.59	2565.72	89.82	<.0001
Error	76	2170.91	28.56		
Total	99	61182.50			

ANOVA Table for mycelial growth inhibition at 8 DAI

Source	Df	SS	MS	F-value	Pr≥F
Treatment	23	62620.69	2722.64	29.32	<.0001
Error	76	1600.02	21.06		
Total	99	64220.71			

ANOVA Table for mycelial growth inhibition at 10 DAI

Source	Df	SS	MS	F-value	Pr≥F
Treatment	23	48374.39	2103.24	36.09	<.0001
Error	76	4429.56	58.28		
Total	99	52803.95			

ANOVA Table for mycelial growth inhibition at 12 DAI

Source	Df	SS	MS	F-value	Pr≥F
Treatment	23	34749.52	1510.85	22.49	<.0001
Error	76	5105.62	67.18		
Total	99	39855.14			

ANOVA Table for mycelial growth inhibition at 14 DAI

Source	Df	SS	MS	F-value	Pr≥F
Treatment	23	30446.26	1323.76	24.17	<.0001
Error	76	4163.20	54.78		
Total	99	34609.46			