MECHANISMS OF SEED TRANSMISSION OF BACTERIAL BLIGHT OF COWPEA (*Xanthomonas axonopodis* pv. *vignicola*) (BURKHOLDER) DYE

BEING A Ph.D. DISSERTATION

BY

Umoru Simeon AMODU
B. Agric., M. Sc. Crop Protection (ABU)
REG NO.; Ph.D. / AGRIC./02022/2008-2009

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AHMADU BELLO UNIVERSITY
ZARIA

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DECLARATION

I hereby declare that the work in this dissertation entitled "Mechanisms of seed transmission of bacterial blight of cowpea *Xanthomonas axonopodis* pv. *vignicola* (Bulkholder) Dye" has been conducted by me in the Department of Crop Protection under the supervision of Prof. (Chief) A.D. Akpa, Dr K.C. Shenge and Dr N.O. Agbenin. The information derived from the literature has been dully acknowledged in the text and a list of references provided; no part of this dissertation was previously presented for another degree or diploma at any university.

_____ _____

Simeon Umoru AMODU Date

Ph. D. Candidate

CERTIFICATION

This dissertation entitled "MECHANISMS OF SEED TRANSMISSION OF BACTERIAL BLIGHT OF COWPEA *Xanthomonas axonopodis* pv *vignicola* (BULKHOLDER) DYE" by Umoru Simeon AMODU meets the award of the degree of Doctor of Philosophy of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

Professor (Chief) A.D. Akpa (Chairman, supervisory committee)	_____ Signature	_____ Date
Dr K.C. Shenge (Member, supervisory committee)	_____ Signature	_____ Date
Dr N.O. Agbenin (Member supervisory committee)	_____ Signature	_____ Date
Professor A. B. Zarafi (Head of Department)	_____ Signature	_____ Date
Professor Adebayo A. Joshua Dean School of Post graduate Studies	_____ Signature	_____ Date

DEDICATION

This thesis is dedicated to my elder brother, **Mr. Lawrence Aku** and to my late mother **Iyawo Omale**.

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ABSTRACT

Understanding transmission mechanisms of cowpea bacterial blight *Xanthomonas axonopodis* pv *vignicola* (*Xav*) from seed to seedling will form the bases for appropriate control measure to be initiated. In order to study the parts of seed where the pathogen usually colonize, three methods were used (seed washing and seed plating) were used to detect the external borne pathogens while the third (separation of seed into components and maceration) were used to detect the internally borne pathogen. The results showed that hilum was the most colonized part of the seeds. The embryo colonization was low and there was no *Xav* detected on cotyledons. In order to study the populations build up *in vivo* and symptom manifestation, seed samples were collected from various sources to reflect the various ways farmers obtain seeds (local markets, seed companies, research institute and treated seeds with fungicides). Fungicides had no significant effect on the pathogen survival and pathogenicity. There was significant difference between the treatments in terms of disease incidence and severity on the vegetative parts. There was steady population build up in all the treatments both in/on the plants. There was no significant difference between all the treatments in terms of *Xav* population build up on the floral parts. Mechanism of transmission of *Xav* was studied by soaking seeds of sorghum, maize, millet and cowpea in bacterial suspension adjusted to $4.7x10^7$cfu/ml for two and four hours respectively. After 2 h of soaking seeds in bacterial suspension, cowpea seeds had higher population of *Xav* attached to it while after 4 h of soaking; all the seeds had *Xav* attached to it. The study of biofilm formation were carried out by growing bacterial in maize, millet, sorghum, cowpea extracts and extracts of cowpea + 0.5 g glucose and nutrient glucose agar (NGA) in a 96 microlitre wells. There was

statistical difference between the biofilm formation induced by the different extracts and NGA. Studies on the route of transmission of *Xav* to seeds indicate that the bacterium was able to transmit systemically and epiphytically from inoculated seeds to harvested seeds and also from inoculated vegetative parts into the harvested seeds. Since adhesion of bacteria to seeds was just on commensal basis, studies were conducted to evaluate the possibility of non-host seeds of cereal crops to transmit the pathogen. Maize, millet and sorghum seeds were collected from cowpea producing states for the isolation of the pathogen. The pathogen (*Xav*) was consistently isolated from millet samples collected from all the states except Kebbi state. In other to determine whether the *Xav* can directly transmit systemically from seeds to harvested seeds or indirect transmission through the floral parts to the non-host seeds (maize, millet and sorghum), seeds were inoculated before sowing and also, the floral parts were inoculated at the flowering stage. The pathogen (*Xav*) was able to systemically transmit from seeds to harvested seeds in millet. Maize and sorghum did not transmit CoBB pathogen systemically. However, millet and sorghum were florally transmitting *Xav* to seeds but maize did not.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 General

The world's food supply, while increasing relies on few crop species of which cowpea is one. Cowpea, *Vigna unguiculata* (L) Walp, is an annual herbaceous legume belonging to the family fabacae order Leguminosae and genus Vigna (Laghetti *et al*., 1990; Singh *et al.,* 1997). Common names of the crop include cowpea, black eye bean, pea, catjang, china pea, cow grain, and southern pea. In Nigeria it is called beans, wake, tsarrariya (Hausa), ewa, ere (Yoruba), akedi, agwa, (Ibo). Cowpea is grown in tropical and subtropical regions of the world, covering parts of Asia and Oceania, the Middle East, Southern Europe, Africa, Southern USA, and Central and Southern America, lying between 35 ^{0}N and 30 ^{0}S of the Equator (Ng and Monti, 1990; Singh and Ajeigbe 1998; Fatokun *et al.,* 2002). The crop shows great variation in growth habit in terms of cultivar (trailing, climbing and erect forms exist), climatic and soil conditions. Most cultivars are indeterminate, producing flowers and seed over a long period. But some are determinate, producing flowers and seeds within a short period (Paino D' urzo *et al*., 1990).

Africa is the center of origin of cowpea (Paino D' urzo *et al*., 1990), where there exists a large diversity among indigenous varieties and wild progenitors. In the West African sub region, Nigeria was postulated as center of origin of cowpea because of the widespread distribution of different varieties and wild prototypes (Laghetti *et al*., 1990; Paino D'urzo *et al*., 1990). However, more recent studies on the genetic exploration of cowpea in Africa suggested that Swaziland may be the primary center of origin of wild progenitors,

because this country has higher species diversity throughout the world (Ng and Monti, 1990; Mongo, 1996).

Cowpea is an important food legume, and an essential component of cropping systems in the tropics. The dominant crop mixtures include millet- cowpea, sorghum-cowpea, maize- cowpea and millet-cowpea - groundnut. Being a fast-growing crop, cowpea curbs erosion by covering the ground, fixes atmospheric nitrogen, and its residues contribute to soil fertility. An important agricultural advantage is that cowpea is able to fix nitrogen in the soil efficiently at about 30-70 kg/ha (Bliss, 1975).

Cowpea is consumed in many forms. The young leaves, green pods, and seed are used as vegetable in Brazil (Laghetti *et al*; 1990; Singh *et al*., 2002). In Southern Italy, the crop is grown essentially for its green pods; which obtain higher prices than *Phaselous vulgaris* (Rachie and Rawal, 1976). Although it is cultivated primarily for its edible seeds, direct consumption of cowpea leaves is also widespread in different parts of Africa (Nielson *et al*., 1997). Dry seeds are used in various food preparations, and the haulms are fed to livestock as nutritious supplement to cereal fodder (Singh *et al.,* 2002). Trading fresh cowpea produce and cowpea processed products and snacks provide rural and urban women with the opportunity for earning an income. The seeds are a major source of protein, minerals and vitamins in the daily diet of people are generally known to positively impact on the health of women and children (Mongo, 1996; Quin, 1997; Singh *et al*., 2002).

Nutritionally, cowpea has excellent food value that include; carbohydrate (55-60 %), protein (23-27 %), fiber (3.9 %), ash (2.77-3.6 %) and fat (1.3 %), depending on genotypes and environmental factors (Olaofe *et al*., 1993; Rajapakse and Van Emden, 1997). The digestibility of cowpea protein was found to be 85 %, compared with 95 % biological value of egg albumen (Bliss, 1975). Nigerians derive substantial portions of their daily plant protein need from cowpea because of shortage of animal protein (Olaofe *et al*., 1993). The per capita consumption is 25 to 30 kg per annum per Nigerian (Fatokun *et al.,* 2002). Part of the popularity of cowpea as a source of protein for many people is the low cholesterol; a major problem associated with consumption of animal protein. Most developed countries with high rates of atherosclerosis, cancer, diabetes, hypertension, and obesity individuals are advised to change diets and consume more vegetables (including legumes) than meat. This will help in the reduction of high fat diet from 35 % to 30 % (Singh, 1990; Timko, 2000). A cup of cooked, dried beans per day lowers the low-density lipid (LDL) cholesterol, regulates blood sugar and insulin, lowers blood pressure and regulates bowels, preventing gastro-intestinal troubles, hemorrhoids and cancer of the gut (Carper, 1988; Timko, 2000).

1.2 Justification of the Studies

The average grain yield of cowpea on farmers' fields in Nigeria ranges from 240 - 400 kg/ha (Singh *et al.,* 1997). However, it has been observed that the potential cowpea grain yield from improved varieties that are adequately protected from insect pests and diseases is up to 2.4 tonnes / ha (Ogunlela *et al.,* 2005). Protection measure can be improved by seed treatment with combined applications of antibiotics (streptocycline) and fungicides

(captan) (Jindal and Thind, 1990).. Efforts to reduce losses in cowpea through breeding and screening for resistance to cowpea bacterial blight (CoBB) have not been successful due to paucity of genetic materials (Yu *et al*., 1998). The quantitative mechanism of inheritance, variations in pathogen races and differential inoculum densities obtained at various locations also contribute to low quality resistance materials available (Webster *et al*., 1980; Beebe, 1989; Tar'an *et al*., 2002, Adipala *et al*., 1995).

World distribution of CoBB is partly associated with the ability of the pathogens to infect seeds of both resistant and susceptible cowpea genotypes (Aggour *et al*., 1989). An assessment of yield loss caused by seed-borne pathogens becomes complicated when information about their biology, epidemiology and pathogenesis is inadequate (Chiarappa, 1988). Cowpea bacterial blight is transmitted by insects, weeds and infected plant debris, but seed transmission is reported to play the most significant role in development of epidemics (Yu *et al*., 1998). The bacterium, after infecting the seed, remains dormant until germination begins (Saettler and Perry, 1972). Epidemiological studies have shown that one infected seed in 10,000 can result in an outbreak of disease in the field (Sutton and Wallen 1970; Opio *et al*., 1993). The significant role played by seed-borne infection calls for more studies on seed transmission of CoBB, since epidemics have been reported on newly opened areas; suggesting seed transmission rather than infected plant debris or soil contamination (Darrasse *et al*., 2010).

Although it is understood that CoBB is seed transmitted, and that seed-borne inoculums play a significant role in the disease epidemiology, the mechanism of pathogen

attachment to seed, location in and on seed, and mode of transmission from seed to emerging seedling has not been fully elucidated . Bacteria are carried in/on seeds by adhering to seed surfaces which preceeds biofilm formation. Over the last two decades, there have been many studies showing the ability of bacteria to form biofilms on biotic and abiotic surfaces. However relatively few studies have been focused on the biofilm-forming ability of (*Xanthomonas axonopodis* pv. *vignicola*) (*Xav*) as a mechanism of transmission. Also information is lacking concerning carriage of the pathogen by nonhost seeds, since farmers grow cowpea in crop mixtures. A major step toward effective management of this disease would require an understanding of bacterial population dynamics in and on seeds, as well as the mechanisms of transmission of the bacterium. Deciphering the mechanisms enabling plant-pathogenic bacteria to disperse, colonize, and survive on their hosts provides the necessary basis to set up new management approach. In view of the forgoing, the current study aims at investigating the seed transmission mechanism of *Xanthomonas axonopodis* pv. *vignicola*.

1.3 Objectives of the Study

The objectives of the study are to determine:

1.3.1 Parts of seed usually colonized by the pathogen.

1.3.2 Correlations between populations build up *in vivo* and symptom manifestation in infected cowpea seedling.

1.3.3 Mechanisms of seed transmission of *Xanthomonas axonopodis* pv. v*ignicola*.

1.3.4 The routes of seed infection by the pathogen.

1.3.5 The transmission of the pathogen by non-host cereal crops.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 General

Cowpea is predominantly a hot-weather crop whose deep-roots are well adapted to the semi-arid savannas and margins of humid forests in tropical lands. The genus *Vigna* consists of more than one hundred different species, widely distributed in tropical and subtropical areas, and characterized by great ecological and morphological diversity (Ng and Monti, 1990; Paino D'urzo *et al*., 1990). The growing season varies between locations and varietal differences are recognized. In Nigeria, the main producing areas are within the Guinea and Sudan savannas where the growing period is from June to November (Mongo, 1996).

FAO (2007) estimated a production of about 4.8 million tonnes of dry cowpea seeds worldwide in the year 2007, with Nigeria accounting for 2.8 million metric tons (55 %); making Nigeria the largest producer of the crop. The major producing region in Nigeria is the northern parts of the country. Production is highest in the North-Eastern axis, where an estimated 703.13 metric tons is produced from 641.03 ha. This is followed by the North West; with about 519.51 metric tons from 1068.02 ha, and North Central with 166.58 metric tons from 307.70 ha (NAERLS and NFRA, 2009). In spite of these comparatively high production levels, Nigeria imports 260,000 tonns of cowpea annually to meet local consumption. Other major producing countries in Africa include Republic of Benin, Ghana, Senegal, Niger, Somalia, Uganda and Burkina Faso (IITA, 1989).

2.2 Constraints to Cowpea Production

The apparent popularity of cowpea may seem paradoxical if the relatively low grain yields on farmer's field are not considered (Fatokun *et al.,* 2002). Low yields of 240-300 kg/ha is considered to be a significant attribute of cowpea production in Africa (Jackai and Singh, 1988; Adipala *et al*., 1995). These low yields have been attributed largely to heavy pest and disease pressure on the crop (Adipala *et al*., 1995; Jackai and Singh, 1998; Alabi and Emechebe, 2004).

Another constraint limiting cowpea production in Africa is that production of the crop has been in the hands of small- scale farmers, whose traditional methods do not produce enough to meet growing demands. This situation has been observed to be because the crop is on the way to being abandoned, and that it continues to be grown as a minor pulse crop (Paino D'urzo *et al*., 1990; Ahmad *et al*., 2010). The crop is therefore under severe genetic erosion due to disappearance of land races and multiple biotic problems. The first cause of genetic erosion may be explained by the fact that farmers hardly buy seeds for growing from specialized shops, except in the large rural centers where improved farming techniques often require the use of selected seeds. In the small centers, where people stick more to tradition, farmers still grow their own seeds, from low yielding verieties (Paino D'urzo *et al*., 1990; Mongo, 1996). The second cause is one of the consequences of general gradual withdrawal from farming the crop; an activity no longer profitable in some areas for the well known reason of pests and diseases (Paino D'urzo *et al*., 1990; Adipala *et al*., 1995; Ahmad *et al*., 2010).

According to IITA (1982), cowpea is attacked by at least 35 diseases, which cause seed rots, seeding mortality, roots and stem rots, foliar diseases and deterioration of seed quality. This report was collaborated by Alabi (1994); who reported that cowpea suffers from many diseases wherever it is grown, and that over 35 diseases (mostly induced by fungi, bacterial, virus, nematodes and parasitic flowering plant) were found associated with the crop. Almost all parts of the plant are attacked by various pests, and more than 85 insect species have been found to cause up to 100 % yield loss to cowpea (Jackai and Singh 1988). There are over 185 insect species and 50 diseases associated with cowpea in Nigeria, with the number varying from region to region (Emechebe and Lagoke, 2002). One of the major constraints to its production is the attack by diseases.

In northern Nigeria, where 80 % of the country's total cowpea production takes place, bacterial blight *Xanthomonas axonopodis* pv. *vignicola* (*Xav*) is the most important bacterial disease that limits cowpea production. According to Emechebe and Soyinka (1985) CoBB is the most important bacterial disease that limits cowpea production in the Nigeria Savannas. The genus *Xanthomonas* consists of 27 plant-associated species and they cause disease on at least 124 monocot species and 268 dicot species (Hayward, 1993).

Xav is one of the most important bacterial diseases in bean producing areas, especially in tropical and subtropical regions, where it can be severe because of high temperature and frequent rainfall (Yoshii, 1980; Silva *et al*., 1989; Ferreira *et al*., 2003). Yield losses caused by cowpea bacterial blight range from 68 % in Nigeria (Okechukwu *et al*., 2010), 38-40 % in Canada (Wallen and Jackson, 1975; Opio *et al*., 1996), 40 % in Uganda (Opio

et al., 1996), and 45 % in Columbia (Serracin *et al*., 1991; Singh and Munoz, 1998). CoBB is primarily a seed-borne pathogen (Cafati and Saettler, 1980; Lawyer, 1984; Sengooba, 1985; Umesh *et al.,* 1996' Sahin and Miller, 1997; Singh and Munoz, 1998). The worldwide distribution of this disease is partly associated with the ability of the pathogen to infect seeds of both resistant and susceptible genotypes (Aggour *et al.,* 1989; Saetler, 1989; Alabi and Emechebe, 2004).

2.3 Economic Importance of Cowpea Bacteria Blight

Cowpea bacterial blight, also known as bacterial canker, remains one of the most damaging diseases that significantly reduce yield of cowpea wherever the crop is grown (Allen, 1991; Saettler, 1989; Gopal, 1999). Up to 60 % seedling mortality has been reported in cowpea due to infected seeds (Gopal, 1999). Some losses may be attributed to pod lesions, which can expand to the seed, if infection occurs during pod development and can result in rotting and shriveling of the seed (Yoshii, 1980). Most yield losses, however, presumably result from leaf lesions and an attendant reduction in photosynthesis (Goodwin 1992). CoBB-infected seeds with visible symptoms can lose their colour or are stained, and their value is thus lowered (IITA, 1982; Singh and Munoz, 1999). Severity of yield loss varies according to cultivar, levels of infecting environment and stage of growth (Serracin *et al*, 1991)

2.4 Symptoms of Cowpea Bacteria Blight

Symptoms of CoBB first appear on leaves as small, water-soaked spots and or light green areas. These spots enlarge, and the tissue in the centers dies and turns brown. These

irregularly-shaped spots are bordered by a lemon yellow ring, which is a diagnostic symptom of CoBB (Davis, 2007). The spots or lesions can develop on the edges or in interveinal areas of leaves.

The spots may later coalesce; resulting in the death of the entire leaf and defoliation of the plant. Infected pods first show small, water-soaked spots that develop into large, dark-red irregular spots. Under favourable conditions, these spots may show yellow slimy ooze. Seeds in infected pods can become infected. Heavily infected seeds may be shriveled and germinate poorly (Dillard and Legard, 1991; Howard, 1994; Davis, 2007). CoBB causes seed rots, seedling mortality and, root and stem rots (Adipala, 1995).

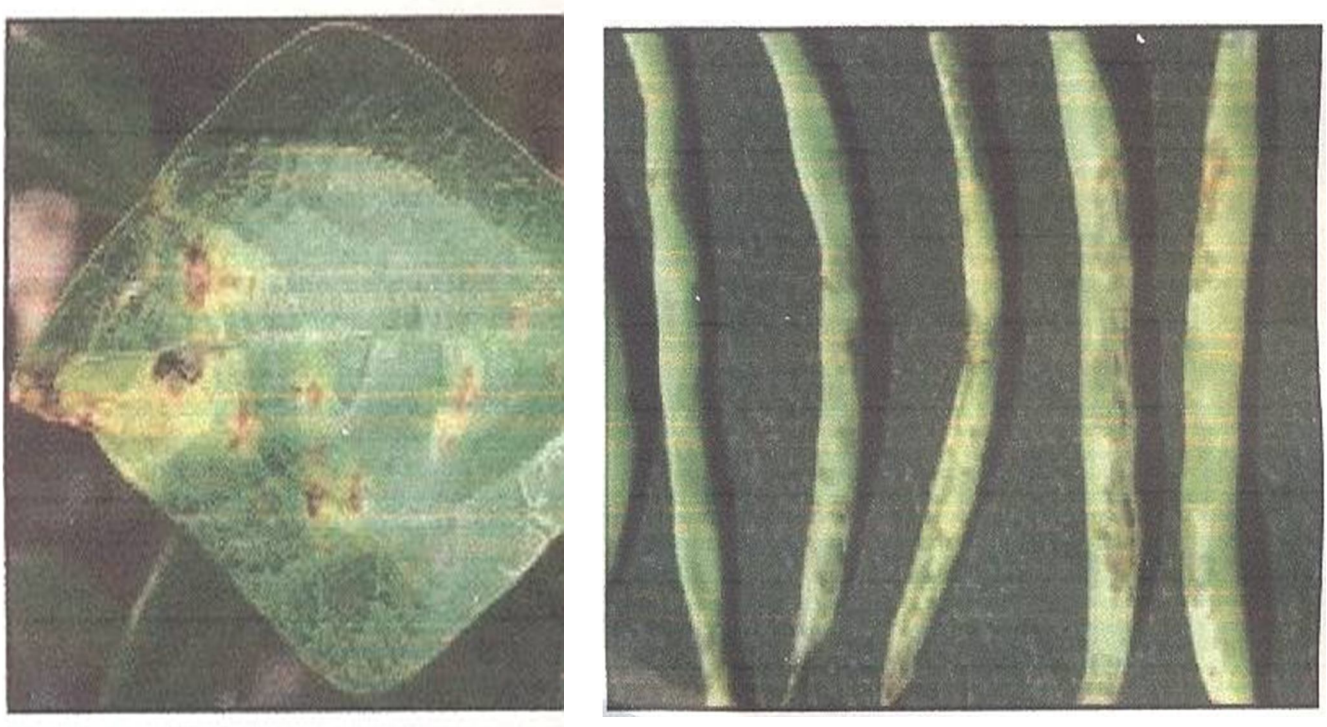


Plate I. Symptoms of Cowpea Bacterial Blight on Cowpea Leaf and Pods.

2.5 Host Range of Cowpea Bacteria Blight

Xanthomonas axonopodis pv. v*igicola* has been found to survive in some weeds such as *Euphorbia heterophyla (*Spurgeweed or Wild poinsettia*), Digitaria horizontalis (*Digitgrass or Crabgrass*), Talium triangulare* (waterleaf), *Euphorbia hirta* (snakeweed or garden spurge) and in the African yam bean (*Sphenostylis stenocarpa)* as well as *synedrella nodiflora* (Node weed or starwort), and *Panicum subalbidum.* The alternative host may form a bridge for primary inoculum within and between seasons (Sikiro and Wydra, 2004).

2.6 Epidemology of Cowpea Bacteria Blight

Xanthomonas axonopodis pv v*ignicola* is a warm climate pathogen, favoured by a temperature range of 28-32 ^{0}C and high relative 0-80 % (Gilberston *et al*., 1990). Infection occurs naturally through stomata, hydathodes and wounds; resulting in the disease symptoms (Dillard and Legard, 1991). *Xav* can survive in residues of infested cowpea leaves and stems (Gilberston *et al*., 1990), and can survive for up to 60 days in plant residues left on the surface of the soil, and up to 45 days when buried at depths of 10-20 cm (Sikiro and Wydra, 2004). Crop residues and weeds are likely sources of primary inoculum when planting two consecutive cowpea crops per year, and they probably play a role in dissemination of the pathogen during the cropping season. The alternate host may form a bridge for primary inoculum between cropping seasons (Sikiro and Wydra, 2004; Zandjanakou *et al*., 2006). In the studies to quantify the population of *Xav* in organs of insects, the pathogen was isolated from organs and faeces of grasshoppers (*Pyrgomorpha congnata*), the Senegalese grasshopper (*Oedalus*

senegalensis), Bees (*Apismellifera* spp.*)* and three coleopterans (*Ootheca mutabilis, Mylabris* spp, and *Exchomus trobertis)* collected in bacterial blight-infected cowpea fields (Zandjanakou *et al*; 2006). Although *Xav* can be transmitted by insects, weeds or infected plant debris, seed transmission plays a significant role in the development of an epidemic (Yu *et al.,* 1998). The movement of infected seed through international trade is also a major means of pathogen dispersal (Beebe and Pastor Corrales, 1991; Singh and Munoz, 1999).

2.6.1 Exit of Pathogen

Infection chain comes to an end either due to end of crop season or due to death of host organ. To ensure the recurrence of disease, the pathogen has two options:

- Survive with the infected plant parts (seed and debris) to bridge the time gap between successive crops.

- Come out of host tissue before or after the hosts' life cycle and exploit the alternatives to continue activities with other host/vector (Chaube and Pundhir, 2005).

Survival and dissemination are important phases in epidemiology of plant diseases. The inoculum density eventually encountered by the host is determined by the survival characteristics of the organism. Thus, a successful pathogen must bridge the discontinuities in the infection chain/disease cycle occurring due to gaps in time (Chaube and Pundhir, 2005).

According to evolutionary medicine, virulence of a pathogen increases with horizontal transmission (between non relative hosts) and decrease with vertical transmission (within the same host) (Horia and Matsumotob, 2010). Some pathogens survive in plants that belongs to the same botanical family to which cultivated host belongs (collateral host), while some survive in plants that do not belong to the same botanical family of the cultivated host (alternate host) (Chaube and Pundhir, 2005). There are three main hypotheses about why a pathogen evolves as it does. These hypotheses help to explain the life activity of bacteria including reproduction, survival, transmission and virulence. These are Trade-off, the Short-Sighted Evolution and Coincidence evolution Hypothesis.

Trade-off hypothesis: At one time, some biologists argued that pathogen would tend to evolve toward ever decreasing virulence because the death of host (or even serious disability in host) is ultimately harmful to the pathogen living inside. For example, if the host dies, the pathogen population inside may die out entirely. Therefore, it was believed that pathogen that does not kill host or interact with other host should have higher success reproducing and dispersing.

Short-sighted evolution hypothesis: this suggests that the traits that increase reproduction rate and transmission to a new host will rise to high frequency within the pathogen population. These traits include the ability to reproduce in higher numbers, live longer, and survive against adverse environmental conditions. These traits typically arise due to mutation, which occur more frequently in pathogen populations than in host populations, due to the pathogens' rapid generation time and immense numbers. After only a few generations, the mutations that enhance rapid reproduction and dispersal will increase in

frequency. As long as transmission continues despite the virulence, virulent pathogen will have the advantage.

Coincidental evolution hypothesis: Some forms of pathogenic virulence or pathogenesis did not co-evolve with the host. For example, tetanus is caused by the soil bacterium *Clostridium tetani.* After *C. tetani* enter a human wound, the bacteria may grow and divide rapidly, even though the human body is not their normal habitat. The bacterium finds itself inside human body by mere happenstance, while dividing, *C. tetani* produce a neurotoxin that is lethal to humans. It is the bacterium normal life cycle in the soil that leads to the production of the toxin, not any evolution with a human host (Bruce and Carl, 2000; Scott and Jon, 2007, Encyclopedia Britannica, 2009; Horia and Matsumotob, 2010).

2.7 Factors Affecting Infection and Multiplication of Cowpea Bacteria Blight

Heavy and early infection, high humidity, temperatures fluctuating between 20 – 25 ^{o}C, and alternately dry and wet weather can cause more than 40 % yield loss in susceptible cultivars (Serracin *et al.,* 1991). Other factors influencing disease severity are photoperiod and stage of crop maturity at infection (Arnaud- Santana *et al*., 1993), and inoculum level (Aggour *et al.,* 1989). In tropical and subtropical regions, where the severity of the disease is very high, the most important factors appeared to be high temperatures and frequent rainfall (Yoshii, 1980). CoBB, like most bacterial diseases, is favored by conditions of moisture and humidity. Although it can over-winter in infected debris, survival and dissemination with seed is more important (Davis, 2007).

2.8 Survival Strategies of Phytopathogenic Bacteria in the Host

Bacterial pathogens device several mechanisms for survival both in the host and in non host; these are:

a. Bacteria survive by invading and remaining confined in regions inaccessible to plant defense metabolite.

b. Avoid provoking an overwhelming inflammatory response. Some bacterial pathogens induce minimal or no inflammation required inducing plant defense reaction like hypersensitive reaction (h.r) or other phytoalexins.

c. Molecular mimicry. If a bacterial antigen is very similar to normal host "antigens", the immune responses to this antigen may be weak giving a degree of tolerance. Resemblance between bacterial antigens and host antigen is referred to as molecular mimicry or antigenic disguise. In this case the antigenic determinants of the bacterium are so closely related chemically to host "self" components that the immunological cells cannot distinguish between the two and an Immune Response (I.R) cannot be raised. Some bacteria capsules are composed of polysaccharides so similar to host tissue polysaccharides that are not immunogenic (Todar, 2011).

2.9 Seeds as Vehicles for Pathogen Movement

Seeds are regarded as a highly effective means for transporting plant pathogens over a long distance. Numerous examples exist in agricultural literatures for the international spread of plant disease as a result of the importation of seeds that were infected or contaminated with pathogens (Fedrich, 1996; Agrawal and Sinclair, 1996). Since the

1900s, consumer demand for new plant products gave opportunity for many plant pathogens to disseminate to new areas on imported seeds. New markets for plant commodities encouraged plant breeders to begin collecting seed stocks from abroad. The birth of new seed companies extends their markets to new area (Elmer, 2011). Most of these seed borne pathogens could have severe economic and ecological consequences if they are introduced and become established in regions where they are not native. A pathogen may penetrate parts of the seed and in turn infect them. The infestation/ contamination of the seed may occur during harvesting, threshing and processing.

The pathogen may thus, be carried with the seeds in three ways:

(i) Admixture: pathogens are independent of seeds but accompany them. *Ergot sclerotia* are mixed with healthy seeds during threshing.

(ii) External: the pathogen may be present on seed surface.

(ii) Internal: pathogens establish within the seed with definite relationship with seed parts.

The seed borne pathogen may result in loss of germination, discoloration and shriveling, development in plant diseases and toxin production in infected seeds (Fraedrick, 1996; Agrawal and Sinclair 1996; Elmer, 2011).

With changing food production and eating habits, new pathogens and newly recognized vehicles of infection have emerged. In the last decades, multiple outbreaks linked to raw seed sprouts have occurred in countries throughout the world (Taormina *et al*., 1999). Seed sprouts have been implicated as vehicles of transmission in out breaks of food borne

illness. Soy, mustard and cress sprouts submitted by a patient with gastrointestinal illness were found to contain large numbers of aerobic spore forming bacteria. Bacteriologic examination of seeds in previously unopened sprouting kits revealed that the soy seeds were contaminated with *Bacillus cereus* in pure culture, while the mustard and cress seeds had *B. cereus* as a minor part of their flora. After germination, all the sprouts were contaminated with the pathogen (Taormina *et al*., 1999). In 1988, raw Mung bean sprouts were implicated in an epidemiologic study as the cause of an outbreak of Salmonella in Saint Paul infection in the United Kingdom. In Finland, eight sprout-borne Salmonella outbreaks occurred from 1980-1997 (Puohiniemi *et al*., 1997). In 1994, two large outbreaks of Salmonellosis were linked to alfalfa sprouts (282 cases in Sweden and 210 cases in Finland). Both outbreaks were caused by *Salmonella*, the implicated seeds were grown from Australian alfalfa seeds. In 1995, a large international outbreak of *S. stanley* infections in Finland and 17 states in the United States was caused by alfalfa sprouts grown from contaminated seeds suggesting the seeds were contaminated at some point during growing, harvesting or processing (Mahon *et al*., 1997).

2.10 Infection and Transmission of Seed borne Pathogens

The seed consists of three basic parts: (a) embryo, (b) storage tissue / cotyledons and (c) seed coat. The embryo has a reproductive function; being capable of initiating cellular division and growth. It is an axis that originates growth in two directions with objectives of originating a root and a shoot (Schaad *et al*., 2001). It consists of a radicle, plumule, one or two cotyledons, and hypocotyle or epicotyle, depending on the type of plant. The episperm (seed cover) consists of two layers; the testa, hilum, scar or point of attachment

of the seed to the funiculus, microphyle, the point upon the seeds at which was the orifice of the ovule through which the pollen tube enters. Endosperm is the inner layer. The establishment of a pathogen in any part of the seed described above is referred to as seed infection. The process of seed infection is influenced by the conditions under which the crop grows (Darrasse *et al*., 2010). Seed borne micro-organisms can be considered in four ways:

a. The first consists of pathogen for which the seed is the main source of inoculum.

b. The second class consists of important pathogens in which the seed borne phase of the disease is of minor significance as a source of inoculum.

c. The third and largest group of seed borne microorganisms consists of those that have never shown to cause disease as a result of their presence on seeds.

d. The fourth class is a group of micro organisms that can infect the seed either in the field or in storage and reduce yield and seed quality (Chiarappa, 1988; Darsonval *et al*., 2009; Darrasse *et al*., 2010).

Seeds provide an efficient means of inadvertently disseminating plant pathogens. However, pathogen transmission is one of the important parameters for pathogen fitness (Fenton *et al*., 2002). An efficient pathogen combines the ability to survive outside the host prior to infection, multiply on the host, and disperse and transmit to new ecological niches, including host seeds (Darrasse *et al*., 2010). Pathogen transmission through seeds occurs when pathogenic microorganisms spread from seeds to seedlings. A developing infected plant can show symptoms within a few days or develop asymptomatically for a variable period of time. In some cases, pathogens present in infected plants survive,

spreading from seeds to plants and from plants to seeds without causing any external symptom and inoculum production (Edreva, 2005). Moreover, seeds harbour a diverse microbial community, including human pathogens such as *Salmonella enterica* and *Escherichia coli* (Hu *et al*., 2004; Darsonval *et al*., 2009). Some human pathogen established themselves endophytically in plants and may be inherited from generation to generation (Taylor and Triplett, 2008). The interactions of seed-borne pathogens with seeds can be divided into (i) endophytic stage (the colonization of seedling xylem) and (ii) a symptom-inducing stage (Chalupowicz *et al*., 2010).

2.10.1 Endophytic Stage

Bacteria physically interact with surfaces to form complex multicellular populations that are often multi -species assemblies; including biofilms and smaller aggregates (Danhorn and Fuqua, 2007). Attachment is required for biofilm formation (i e., aggregation of cells to each other after cells had attached to a surface). Bacteria interact with plant tissues through adhesins; including polysaccharides and surface proteins, with initial contact often mediated by active motility (Danhorn and Fuqua, 2007). The bacterial surface structures important for adhesion cover a broad group of fimbrial and non fimbrial structures commonly known as adhesins (Barnhart and Chapman, 2006; Latasa *et al*., 2006). The mechanism for adherence may involve two steps: (i). Nonspecific adherence: reversible attachment of the bacterium to the eucaryotic surface (sometimes called "docking"). Nonspecific adherence involves nonspecific attractive forces which allow approach of the bacterium to the eukaryotic cell surface. Possible interactions and forces involved are: hydrophobic interactions, electrostatic attractions, atomic and molecular

vibrations resulting from flunctuating dipoles of similar frequencies, Brownian movement and recruitment and trapping by biofilm polymers interacting with the bacterial glycocalyx (capsule) (Todar, 2011).

(ii). Specific adherence: Reversible permanent attachment of the microorganism to the surface sometimes called “anchoring”. Specific adherence involves permanent formation of many specific lock-and-key bonds between complementary molecules on each cell surface. Complementary receptor and adhesin molecules must be accessible and arranged in such a way that many bonds form over the area of contact between the two cells. Once the bonds are formed, attachment under physiological conditions becomes virtually irreversible. Specific adherence involves complementary chemical interactions between the host cell or tissue surface and the bacterial surface. In the language of medical microbiologist, a bacterial "adhesin" attaches covalently to a host "receptor" so that the bacterium "docks" itself on the host surface. The adhesins of bacterial cells are chemical components of capsules, cell walls, pili or fimbriae. The host receptors are usually glycoprotein located on the cell membrane or tissue surface (Todar, 2011). Below is how bacteria pathogen attaches to the surface of the eukaryotic cells.

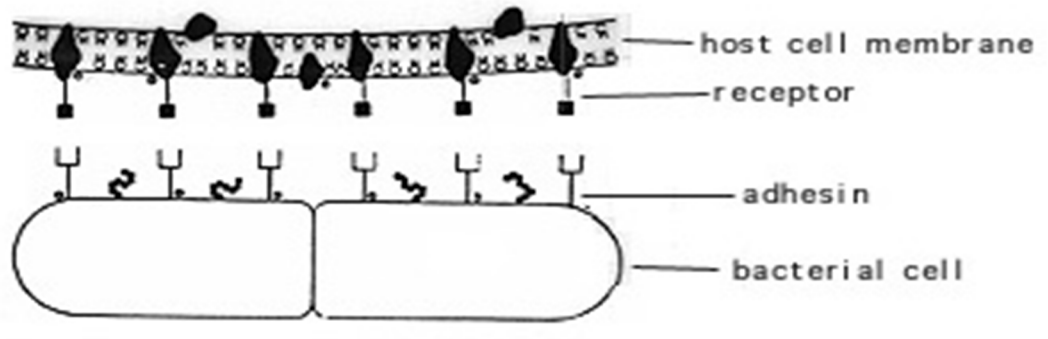


Fig. I. Mechanism of Adherence to Eucaryotic Cell or Tissue surfaces by Bacterial pathogen

Adhesins are cell surface components or appendages of bacteria that facilitate bacterial adhesion or adherence to other cells or to inanimate surfaces. Sequenced genomes of phytopathogenic Xanthomonads contain many genes coding surface adhesion structures, and comparative genomic studies suggested that they may play key roles in pathogenecity of strains on plants (Lee *et al.,* 2005; da Silva *et al*., 2002). Adhesion is an essential step in bacterial pathogenesis or infection required for colonizing a new host (Coutte *et al*., 2003). Some adhesins identified in some pathogenic bacteria are pilA, Fhab, xada1, xadA2, and yapH. Darsonval *et al.* (2009) demonstrated that pilA was involved in adhesion and transmission to seed, and mutation of pilA led to lower pathogenicity on bean by *X. fuscans* subsp. *Fuscans,* while YapH was required for adhesion to seed, leaves and abiotic surfaces but not in planta transmission to seed or aggressiveness on leaves. If a bacterium is to multiply and cause infection, it must have some way of fixing its position and becoming established. This adherence is produced by bacteria's adhesins or ligards that bind specially to host molecules known as receptors. These adhesins can be proteins found on the bacteria cell wall / membrane or they can be collected together on structures that project from the cell surface (for example fimbriae and pili), which seem to be expressly aimed at increasing the chance of adherence (Langermann *et al*., 2000; Coutte *et al*., 2003). The fimbrial proteins include the type IV pili, which are polymorphic assemblies of the protein pilin expressed by many gram-negative bacteria (Craig *et al*., 2004; Pizarro-Cerda and Cossart, 2006). Unlike other types of pili, type IV pili not only participate in adhesion to host cells alone but are also involved in a surprisingly high number of functions, including bacterial aggregation, microcolony formation, and more complex biological functions such as DNA transformation and a

form of surface translocation known as twitching motility (Tobe and Sasakawa, 2002; Merz *et al*., 2000; Kaiser, 2007). The non fimbrial adhesions are widely represented in α-, β- and γ-proteobacteria and contain different classes of proteins implied in bacterial attachment (Gerlach and Hensel, 2007; Pizarro-Cerda and Cossart, 2006). The non-fimbrial adhesion XadA is required for optimum virulence in *Xanthomonas oryzae* pv *oryzae* (Ray *et al*., 2002). *Xanthomonas citri* subsp *citri* and *X. campestris* pv. *campestris* have genes for type IV fimbrial pili for several proteins could act as non-fimbrial adhesions (Thieme *et al*., 2005). Bacteria may also secrete viscous substance onto their surface such as alginate capsule and polysaccharide slime which increase adherence in a non-specific fashion (Danhorn and Fuqua, 2007).

In natural settings, bacteria prefer a sedentary lifestyle in communities to a nomadic existence (West *et al*., 2006; Danhorn and Fuqua, 2007). Aggregation followed by biofilm formation is a strategy used by pathogenic bacteria during colonization of plants phyllosphere for its protection from stresses and maintenance of inoculum reservoirs (Jacques *et al*., 2005; Darsonval *et al*., 2009)

2.10.2 Symptom-inducing stage

Bacteria need to be able to remain in, or in close contact to the plant, and to multiply there, before it can do harm. Host plant has developed several strategies to make life difficult as possible for pathogenic bacteria to enter and multiply (Wassenaar, 2009). An infection can be seen as a miniature battle between bacteria and host, the first trying to remain present, and to feed and multiply, while the host is trying to prevent this. The

resulting infection is a process with three possible outcomes: the host wins and the bacteria are removed (possibly with h.r.) so that the host can recover. The bacteria win the ultimate battle and kill the host; or an equilibrium is reached in which host and bacteria live involuntarily together and damage is not done or is minimized (Wassenaar, 2009).

Following infection, pathogens grow, multiply, or both within the plant tissues and invade and colonize the plant to a lesser or greater extent. Successful infection result in the appearance of symptoms i.e., discoloured, malformed or necrotic areas in the plant. Some infections however, remain latent; i.e. they do not produce symptoms right away but at a later time when the environmental conditions or the stage of maturity of the plant become more favourable (Agrios, 2005).

During compatible host-pathogen interactions, phytopathogenic bacteria must overcome the immune response of their host plants (Jones and Dangl, 2006).Two phases of plant immune systems are recognized: the first, the basal defense phase, uses transmembrane pattern recognition receptors that respond to microbe or phytopathogen-associated molecular patterns (eg.,MAMPS or PAMPS) such as flagellin or lipopolysaccharide; the second phase acts largely inside the cell, using polymorphic resistance genes. This includes induction of pathogenesis-related proteins (PR) such as chitinases, glucanases, and protease (Chalupowicz *et al*., 2010). These are believed to play an important role in plant defense, in pathogenic constraints and in general adaptation to stressful environments. These basal defense responses do not discriminate between pathogenic and

non-pathogenic microbes (Nomura *et al*., 2005; Nurberger *et al*., 2004; Edreva, 2005). Hence, there are two broad qualities of pathogenic bacteria that underline the means by which they induce disease: invasiveness and toxigenesis.

a. Invasiveness is the ability of a pathogen to invade host tissues, and encompasses:

(i) Mechanisms for colonization (adherence and initial multiplication)

(ii) Production of extracellular substances (invasins), that promote the immediate invasion of tissues and

(iii) Ability to bypass or overcome host defense mechanisms which facilitate the actual invasive process.

b. Toxigenesis: Toxigenesis is the ability to produce toxins. Toxic substances produced by bacteria, both soluble and cell-associated, cause cytotoxic effects at tissue sites remote from the original point of invasion or growth (Coutte *et al*., 2003; Danhorn and Faqua, 2007). Bacterial pathogens produce a variety of cell wall degrading enzymes (e.g., xylanase, pectate lyases, polygalacturonase), which might be involved in canker production and tissue maceration (Gartmann *et al*., 2008).

Most gram-negative bacteria pathogens habour a T3SS (typed III secretion system) that allow the delivery of effectors directly into the plant cytoplasm, leading to a hypersensitive reaction (HR) in incompatible interactions and symptoms development in compatible ones (Bruning and Gabrial, 2003; Gumlebeck *et al*., 2006). In compatible interactions, defense responses are suppressed by T3SS effectors (Boureau *et al.,* 2006; DebRoy *et al*., 2004). The type III secretion system operates somewhat like a syringe and plunger to transport pathogen-produced proteins that cause disease or trigger a defense

reaction (Ponciano *et al*., 2003). These mechanisms have sometimes shown surprising and unexpected similarity to those found in animal and human bacterial pathogens (Coa *et al*., 2001; Darasse *et al*., 2010).

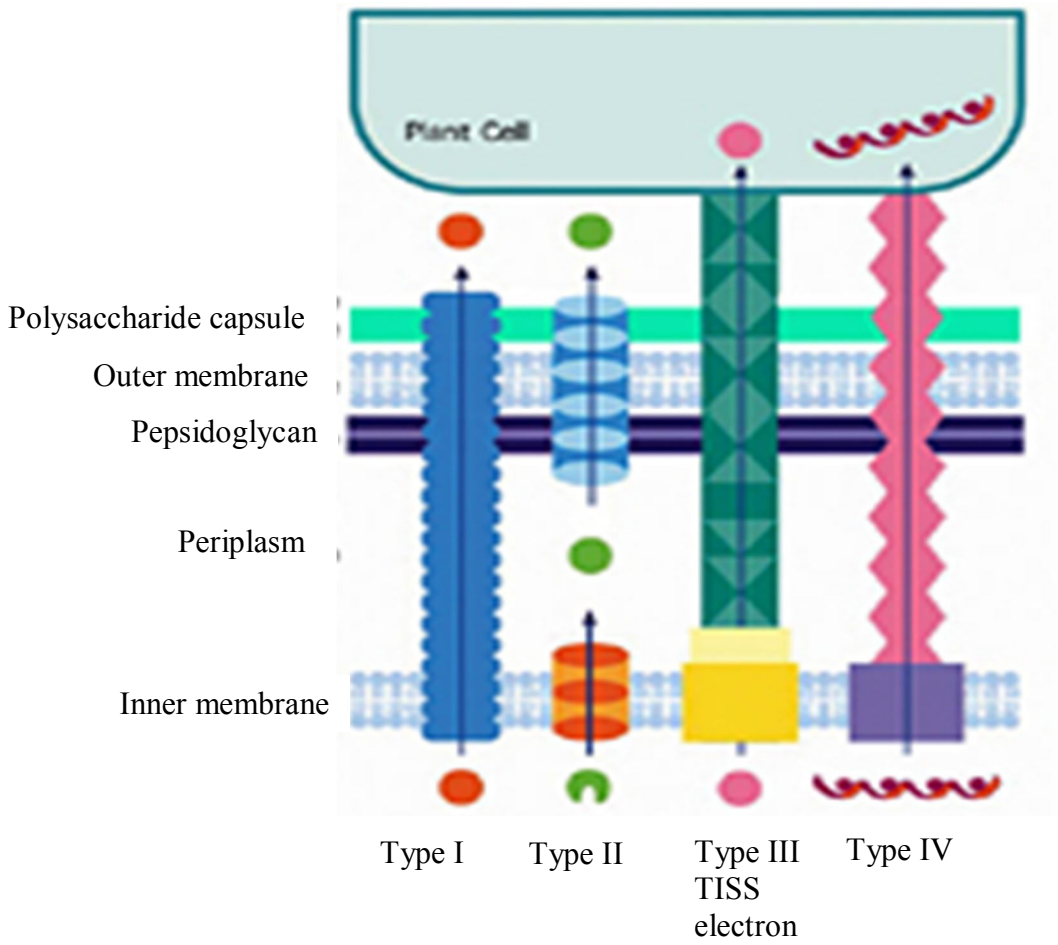


Fig. II. Different Types of Secretion Systems (SS) Produced by Bacteria Pathogens

The colonization of germinating seeds and seedlings represents a critical step in the establishment of bacterial disease. One observation made previously by Hirano *et al*. (1999) was that *Pseudomonas syringae* pv. *syringae* does not behave as a parasite during germination, as its type III secretion system (T3SS) does not seem to play a major role

during seed germination and seedling colonization. Indeed, similar dynamics of population sizes were observed for wild-type *P. syringes p*v. *syringae* and its T3SS mutants on seedlings in field trials. In the experiments transmissions from seed to seedlings were compared for *Xanthomonas citri* pv. *Phaseoli* var *fuscans* in compatible interactions with beans, *Xanthomonas campestris* pv. *campestris* in incompatible interactions and for *Escherichia coli*, a human pathogen, in a null interactions with bean. Results showed that the dynamics of bacterial population sizes were similar on seedlings, and no defense responses were induced evidencing bacterial colonization of seedling without any associated defense response induction (Darrasse *et al*., 2010). Bacteria associated with the spermosphere multiplied in this rich environment, suggesting that the colonization of seedlings relies mostly on commensalism. The transmission of pathogenic bacteria to and by non host seeds suggested a probable role of seeds of no host plants as an inoculum sources (Darsonval *et al*., 2008; Darrasse *et al*., 2010).

The floral transmission of bacteria to seeds is a permissive pathway compared to transmission through the vascular system. Floral transmission allows the contamination of seeds by a cohort of diverse bacteria, including biocontrol agents (Fasshair and Walcott, 2005; Darsonval *et al*., 2008). Plant pathogenic bacteria are transmitted to seed during cultivation of mother plants. Transmission of pathogens to seed has been reported to occur through three pathways: (i) seed can be internally contaminated via the host xylem, as occurs for virus transmission, some fungi and other bacteria. This usually results in the invasion of seed through the hilum. (ii) Seed may also become infested via the pistil, where pathogen move from the stigma through the stylar tissues down to

embrayo, Finally (iii) an external contamination of seed occurs via fruit as a consequence of contact of the seed with fruit tissues showing symptoms, or at harvest and during threshing with residues carrying large population of pathogens (Maude, 1996). Although there is accumulating data concerning plant-pathogen interactions during vegetative growth, the interactions of bacterial pathogens with reproductive organs have not yet received much attention and, therefore, almost nothing is known of the mechanisms underlying the transmission of bacterial pathogen to seed (Darsonval *et al*., 2009).

Biofilm is a complex aggregation of microorganisms growing on a solid substrate. Bacteria adhere to environmental surfaces in multi-cellular assemblies during pathogenesis, symbiosis and in commensally relationships (Ramey *et al*., 2004). Formation of biofilm begins with the attachment of free floating microorganisms to a surface through weak, reversible Van der waals forces. If the colonies are not immediately separated from the surface, they can anchor themselves more permanently using adhesion molecules such as pilli and polysarcharides and surface proteins (Danhorn and Fuqua, 2007). The first colonists facilitate the arrival of other cells by providing more diverse adhesion sites and beginning to build the matrix that holds the biofilm together. Biofilm forms when bacteria adhere to surfaces in aqueous environments and begin to excrete a slimy, glue-like substance that can anchor them to all kinds of materials. A biofilm can be formed by a single bacteria species, but more often biofilms consist of many species of bacteria, as well as fungi, algae, protozoa, and debris. Essentially biofilm

may form on any surface exposed to bacteria and some amount of water (Mah and O'Toole, 2001).

Biofilm development and the resulting intimate interactions with plants often require cell-cell communication between colonizing bacteria called "quorum sensing". Most bacteria probably communicate using secreted chemical molecules to coordinate the behavior of the groups. Gran-negative bacteria use quorum sensing communication circuits to regulate a diverse array of physiological activities such as symbiosis, virulence, competence, conjugation, antibiotic production, mobility, sporulation and biofilm formation. In general, Gram-negative bacteria use acylated homoserine lactose as autoinducers or to communicate, and Gran-positive bacteria use processed oligopeptides to communicate (Miller and Bassler, 2001).

Although the nature of chemical signals, the signal relay mechanisms, and the target genes controlled by bacterial quorum sensing systems differ, in every case the ability to communicate with one another allows bacteria to coordinate the gene expression, and therefore the behavior of the entire community. Presumably, this process bestows upon bacteria some of the qualities of higher organisms (Miller and Bassler, 200**1).**

2.10.3 Biofilm formation

Biofilm are common in nature, as bacteria commonly have mechanisms in which they can adhere to surfaces and to each other. Observation of bacteria associated with plants increasingly reveals biofilm-type structures that vary from small clusters of cells to

extensive biofilm. The surface properties of plant tissue, nutrient and water availability, and the proclivities of the colonizing bacteria strongly influence the resulting biofilm structure (Ramey *et al*., 2004).

Essentially, biofilm may form on any surface exposed to bacteria and some amount of water. Once anchored to surface, biofilm micro-organisms carry out a variety of detrimental or beneficial reactions depending on the species and on the surrounding environmental conditions (Danhorn and Fuqua, 2007).

Most of the bacteria that cause us problems are sessile – attached to a surface and they live in biofilm. Most researchers study extensively on planktonic cells (Free moving cell), while the actual problems involve biofilm bacteria. So new strategies based on a better understanding of how bacteria attach, develop biofilms and detach (spread) are urgently needed so as to develop affective control strategies (Ceri *et al*., 1999).

2.10.4 Biofilms in disease pathogenesis

The diversity of bacteria host interactions is remarkable. This interaction ranges from mutualistic/symbiotic relationships that benefit both parties to explosive infection in which the pathogens rapidly kill the host (Hoiby *et al*., 2001). Chronic infection lies somewhere between these two extremes. Increasing evidence suggests that biofilm mode of growth may play a key role in both of these adaptations (Parsek and Singh, 2003).

Bacterial mechanisms of pathogenicity (virulence factors) are as follows:

a. Attachment to host. The hair like structure on the surface of the bacterial body is called fimbriae or pili. These hairs are able to attach themselves to certain sites of the host, and in this way the bacteria cannot be washed away. Also bacteria can adhere to the host by means of matrix of extracellular polymeric substance (EPS). For bacterial pathogens, successful adherence or attachment is usually a necessary prerequisite for virulence and even infection.

b. Motility. Bacterial pathogen can move from one place to another by means of flagella. These are long tails with which bacteria can swim. The motion helps bacteria reach the site where they can survive, and for that reason flagella can have a function in pathogenicity or virulence.

c. Damage to host. During infection there are three general mechanisms by which a pathogen can induce damage to the host.

    i. Direct damage: This is the consequences of pathogen growth. During pathogen growth, pathogen utilizes the host's resources to adhere, grow and evade host defenses.

    ii. Via hypersensitive reaction. A hypersensitive reaction is an immune response that is excessive beyond the bounds of normalcy, to a point where it leads to damage of the host self.

    iii. Toxigenicity. Some bacteria produce toxic compounds that cause harm to their host. Bacteria often produce substances for various reasons, generally referred to as toxins that cause host damage and diseases. In some cases bacteria produce toxins whenever they grow, and that what happens during certain types of food poisoning. About 220 bacterial toxins are

known and about 40 % disrupt vascular system and inhibit protein synthesis.

d. Surviving the host defense. This could be a way in which pathogenic bacteria can hide, evade, or even destroy immunological reactions by the host, in order to cause disease. These characteristics are also seen as pathogenic. Example is phytopathogenic bacteria and hosts of other microbial organisms in flower due to the fact that flower do not produce defense response. (Ngugi and Seherm, 2006; Wessenaar, 2009).

The following criteria are proposed to define infections caused by biofilms: (a) the infecting bacteria are adherent to some substratum or are surface associated (b) direct examination of infected tissue shows bacteria living in cell clusters or in micro colonies, encased in an extracellur matrix (c) the infection is generally confined to a particular location, although dissemination may occur. (d) The infection is difficult or impossible to eradicate with antibiotics despite the fact that the responsible organisms are susceptible to killing in the planktonic state (Parsek and Singh, 2003). There are limitations to these criteria; however, they provide general characteristics with which to consider the role of biofilm in plant diseases.

Biofilm can be thought of as “bacterial cities or home”. Within these cities are groups of bacteria consisting multiple species. Individual bacteria coalesce by linking extracellular polysaccharides on their cell walls. Polysaccharide chains exhibit chemical properties that make them polar and thus very “sticky” This polarity is what leads to surface

adhesion and cell cohesion (Ramey *et al.,* 2004). This is one of the properties that make biofilms tough to remove.

2.10.5 Why do bacteria form biofilm?

It is very important to note that biofilms are simply a survival mechanism of bacteria cells (Miller and Bassler, 2001) individual bacterium comes together as a whole in order to become stronger. As the cliché goes, there is strength in numbers, with bacteria being no exceptions. Biofilms are found on the surface of and inside plants. They can either contribute to crop disease or as in the case of nitrogen-fixing Rhizobium on roots, exist symbiotically with plant (Andersen-nsissen *et al*; 2007; Wilson, 2003). For example, a biofilm can be composed of aerobic (requiring oxygen) and anaerobic (not requiring oxygen) bacteria, oxygen requiring bacteria actually form a layer over the non-oxygen requiring bacteria. Thus the anaerobic bacteria exist in a low-oxygen microclimate while the aerobic bacteria have a neighbor that's not competing for the oxygen they require. Meanwhile, they both benefit from the protective nature of a biofilm (Ramey *et al.,* 2004). These clusters of individual bacterium frequently inhabit both inert and living surfaces in a non-pathological manner. The first major variable seen in biofilm formation concerns the ability of a microorganism to adhere to a surface for adequate period of time. This period must be long enough for the cell(s) to become irreversibly attached. The rate at which the biofilm grows beyond the initial attachments is influenced by both the type and amount of cells that are present at any one time, the flow rate of water, temperature, surface characteristics, and the amount of nutrient within the aqueous medium (Ramey *et al.,* 2004). Plants are colonized by bacteria on their leaves, roots,

seeds and internal vasculature. Each tissue type has unique chemical and physical properties that represent challenges and opportunities for microbial colonists (Ramey *et al,* 2004).

Well-developed biofilms have been found to contain water channels that help distribute nutrients and signaling molecules as well as waste disposal for each bacterial cell (Ramey *et al.,* 2004). The biofilm coat surrounds the bacteria, thus protecting each cell. One benefit of this environment is increased resistance to detergents and antibiotics. In some cases antibiotics resistance can be increased a thousand fold (Stewart and Costerton, 2001). Biofilms are notoriously resistant to washing and other common antibacterial treatments and may make organisms less conspicuous to the immune system of the host. Also, biofilms are much more resistance than planktonic cells to antimicrobial agents. Fett and Cooke (2005) reported that both *Escherichia coli* and *Salmonella* population on alfafa sprouts required treatments much harsher than simple treatment to reduce the numbers of adherent microns, and full removal was never achieved.

Biofilms have been found to be involved over 80 % of all infections (Rodgers, 2008). The five stages of biofilm development are:

i. Initial attachment (planktonic cell attachment).

ii. Irreversible attachment

iii. Maturation I

iv. Maturation II

v. Dispersal

2.10.6 Colonisation pattern of bacteria pathogens

Internally and externally borne X.a. pv. v*ignicola* inocula are thought to move from the infected germinating seed to the emerging cotyledon, and subsequently to the young seedling, where seedling blight can then ensue (Fig. III).

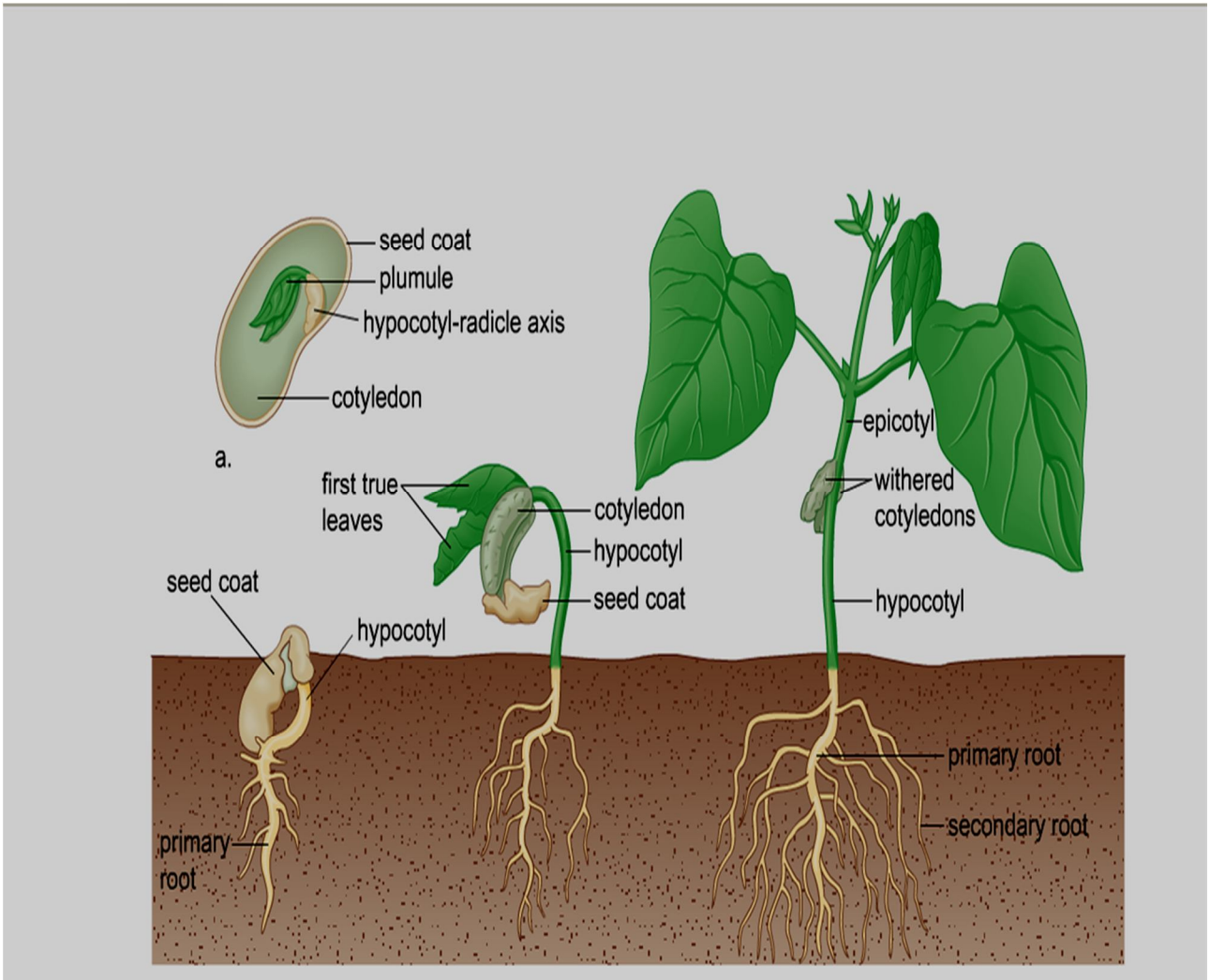


Fig. III. Structure and Germination Process of Cowpea

http://academic.kellogg.edu/herbrandsonc/bio111/animations/0023.swf (2011)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Seed Collection

Seeds were obtained from the open market in nine cowpea producing states (3 states each from North-east, North-central and North-west). The sites where the seed lots were collected are Bama in Borno, Akor in Gombe, Mubi in Adamawa, Duchin Kura in Sokoto, Jega in Kebbi, Saminaka in Kaduna, Keffi in Nasarawa, Mokwa in Niger and Idah in Kogi state. The seed companies were Alheri, Masalaha, and Premier. Seeds were selected on the basis of absence of visible damage and on cleanliness. Seed lots of cowpea (*Vigna unguiculata* L.) were collected from seed companies and open markets in all the states while maize (*Zea mays* L.), sorghum (*Sorghum bicola* L.) and millet (*Setaria italica* L.) seed lots were collected from the open markets only. The seed were clean, with no obvious symptoms of disease, which for farmers form part of seed for planting in the following season.

3.1.1 Objective I. Nature of Seed Infection by *Xav.*

3.1.2 Isolation of bacterial pathogen.

Cowpea seeds were collected from nine cowpea producing states and three cowpea seed companies. Four hundred (400) seeds each of cowpea seed lot were drawn and were thoroughly washed in running tap water to remove soil and debris from the samples. The seed were surface disinfected with 2 % sodium hypochloride for three minutes. Seed were rinsed with sterile distilled water (SDW) in order to remove excess of disinfectant. The presence of *Xav* was detected from ground up 400 seeds each of the seed samples

collected using blender. These were incubated in sterile saline solution (0.85 %) sodium chloride from which serial dilution of up to 10^{-5} were made, two petri dishes containing Yeast Dextrose Carbonate Ager (YDCA) media were inoculated per dilution level and were incubated at 27 ^{0}C for 7 days. Colonies with typical characteristics of *Xav* were selected for pathogenicity testing. Pathogenicity test was conducted on three cowpea varieties. The incidence of *Xav* in seed samples were determined by plating 400 seeds in YDCA. The data collected (incidence and population of the pathogen on samples) were analysed statistically using analysis of variance ANOVA and means were separated using New Duncan's Multiple Range Test (NDMRT).

3.1.3 Experiment I1. Washing assay for externally-borne pathogen

The occurrence of CoBB on the surface of the seeds were determined by randomly selecting 400 cowpea seeds from each of the seed lots from nine cowpea producing states and three cowpea seed producing companies. These were put into a 250 ml flask containing sterile distilled water (SDW) and shake vigorously using electric shaker. After mechanical stirring for 5 mins, the seed suspension was filtered through layers of Cheese-cloth. The filtrate was dispensed in two 50 ml centrifuge tubes and centrifuge at 12,000 g for 10 mins (approx. 10,000 rpm) to pellet bacteria. Extract of 1.0 ml was introduced into a McCartney bottle containing 9 ml of SDW, from which a serial dilution of up to 10^{-5} were prepared. Ten microlitre of the serial dilution were introduced in Petri- dishes containing 20 ml of Yeast Dextrose Carbonate Agar (YDCA) and spread using glass spreaders (Chiarappa, 1988; Mortensen, 1997). The treatments were incubated at 28 ^{0}C for 48 h. Colony enumeration from the incubated Petri dishes were done after 48 h. The

experiment was laid out using a completely randomized design CRD with five replications. Colony forming units (cfu) were taken after 48-72 h. The trial was repeated twice.

3.1.4 Experiment III. Plating seeds in media

Nine seed lots from nine states and three cowpea seed companies were collected as described previously, from which 400 seeds from each seed lots were washed with sterile distilled water. Afterwards, 10 seeds were plated on YDCA and replicated ten times with the hilum upward, and downwards, in an alternating manner (Opio *et al*. 1993). Seeds on which there was *Xav* growth were recorded as positive and those on which there was no *Xav* growth were recorded as negative. Colonies that hydrolyze starch on YDCA were assumed to be *Xav*. Colonies with typical characteristics of *Xav* were selected for pathogenicity testing as decribed in section 3.1.2. Data collected were analyzed statistically using analysis of variance (ANOVA) and means separated using NDMRT. The trial was repeated once.

3.1.5 Experiment IV. Detection of bacteria from different seed parts

Four hundred seeds from each seed lots were randomly selected and washed in a conical flask containing 250 ml of SDW for 5 mins. The seeds were gently pressed using sterilized scalpel and pin to remove the seed coat, embryo from the cotyledons and the helium. The cotyledons, embryo, hilum and the seed coats were separated and put into 5 % sodium hypochlorite solution for 5 mins and rinsed with SDW (Youcef- Benkada *et al.*, 1994). Care was taken to avoid contaminations. After surface sterilization, the samples (seed coat, embryo, hilum and cotyledons) each were put into a conical flask

containing 100 ml of SDW for 2 h; after which 1 ml of each of the samples were introduced into 3 McCartney bottles containing 9 ml of SDW, from which a serial dilution of up to 10^{-3} were made. 10 µl of the serial dilutions were introduced into the Petri-dishes containing 20 ml of YDCA and spread thoroughly using sterilized glass spreader. The treatments were incubated at 28 ^{0}C and colony enumeration was done after 48-72 h. The experiments were laid out using CRD with five replications. Data collected were analyzed statistically using analysis of variance (Anova), and means were separated using NDMRT. The trial was repeated once.

3.2 Objective II: Correlation between Population Build-up *in vivo* and Symptoms Manifestation in Infected Cowpea Seedling

Three Ife brown seed lots from different cowpea seed companies and were treated with different fungicides. Three seed lots of local varieties were purchased from open markets within Zaria, two seed lots (IT86D-721 and IT98-503-1) were obtained from International Institute of Tropical Agriculture (IITA) and SAMPEA-7 (divided into 3 seed lots) was obtained from Institute for Agricultural Research (IAR). Fungicides used for treatment of seeds were the same used by the seed companies, two artificially inoculated seed lots with *Xav* and untreated SAMPEA-7 as control making a total of fourteen seed lots, were used in the trial. These were carefully selected to reflect the various sources farmers usually obtained seeds. Fungicides used were the same with those used by the different seed companies. The different fungicides used by the seed companies were Apron-star (tiamethoam 20 % + metalaxyl-m 20 % + difenocoazole 20 % w/w), Dress force (imidacloprids 20 % + metalaxyl-m 20 % + tebuconazoles 20 %) and Team (carbendazin 12 % + mancozeb 63 %). The cowpea varieties were Ifebrown,

SAMPEA-7, IT86D-721, and IT98K-503-1, Details of the treatment are shown in appendix 22. The initial populations of *Xav* were quantified by ground up four hundred seeds each of the seed lot and plated on YDCA media before sowing from which population build were monitored. Seeds from each seed lot were planted in plastic pots of 25 cm diameter filled with sterile soil. Each seed lots were planted at the rate of 5 seeds per pot, with 5 replications. The seeded pots were placed randomly in the screen house and observed for germination. After which the plants were observed for a typical blight symptoms on cotyledons, stem, leaves and general seedling mortality for two weeks. Before inoculation, leaf and stem discs measuring about 2 mm were taken from the plants using cork borer. The discs were teased in drops of SDW and a loopful of the resulting suspensions was mixed in 10 ml of SDW from which a serial dilution of up to 10^{-5} were prepared. 10 µl of the suspensions were placed on YDCA in Petri-dishes and spread using glass spreader and incubated at ca. 28 ^{0}C for 48 h. (Opio *et al.,* 1993).

3.2.1 Experiment II: Distribution of *Xav* in plant parts

Two weeks after, the plants in the pots were inoculated with the suspension of bacterial blight pathogen adjusted to 4.5 x 10^{7} cfu/ml. 2 mm^{2} discs of leaf and stems were taken at 14, 28, 42 and 56 days after inoculation (DAI). The discs (stem/leaves) taken were washed with SDW. The washed suspension were serially diluted and plated accordingly to determine phyllosphere population of *Xav*. The plants were observed for typical blight symptoms on the leaves and stems. Disease incidences were taken by counting the number of infected plants and severity were scored using a modified CIAT 1-9 scale (Opio *et al*., 1993). Disease severity was measured by three parameters both on leaves

and on pods, namely percentage of infected leaves, percentage of defoliated leaves and percentage of aborted pods using scale shown in Fig IV. To determine the number of infected leaf and pods and consequently the fallen leaves and aborted pods, ten plants were randomly chosen in pots and tagged. The number of leaves with blight and the number of fallen leaves (indicated by blight on the nodes) on the tagged plants were counted. Also the number of flower fallen without the pod initiation was counted (aborted flower). The total number of leaves, flowers and pods produced by the tagged plants were recorded, from which, the percentage of infected leaves, percentage of aborted flower and pods were calculated. The experiments were laid out using complete randomized design (CRD). Data collected were analyzed statistically using ANOVA and means ware separated by NDMRT. The trial was repeated once.

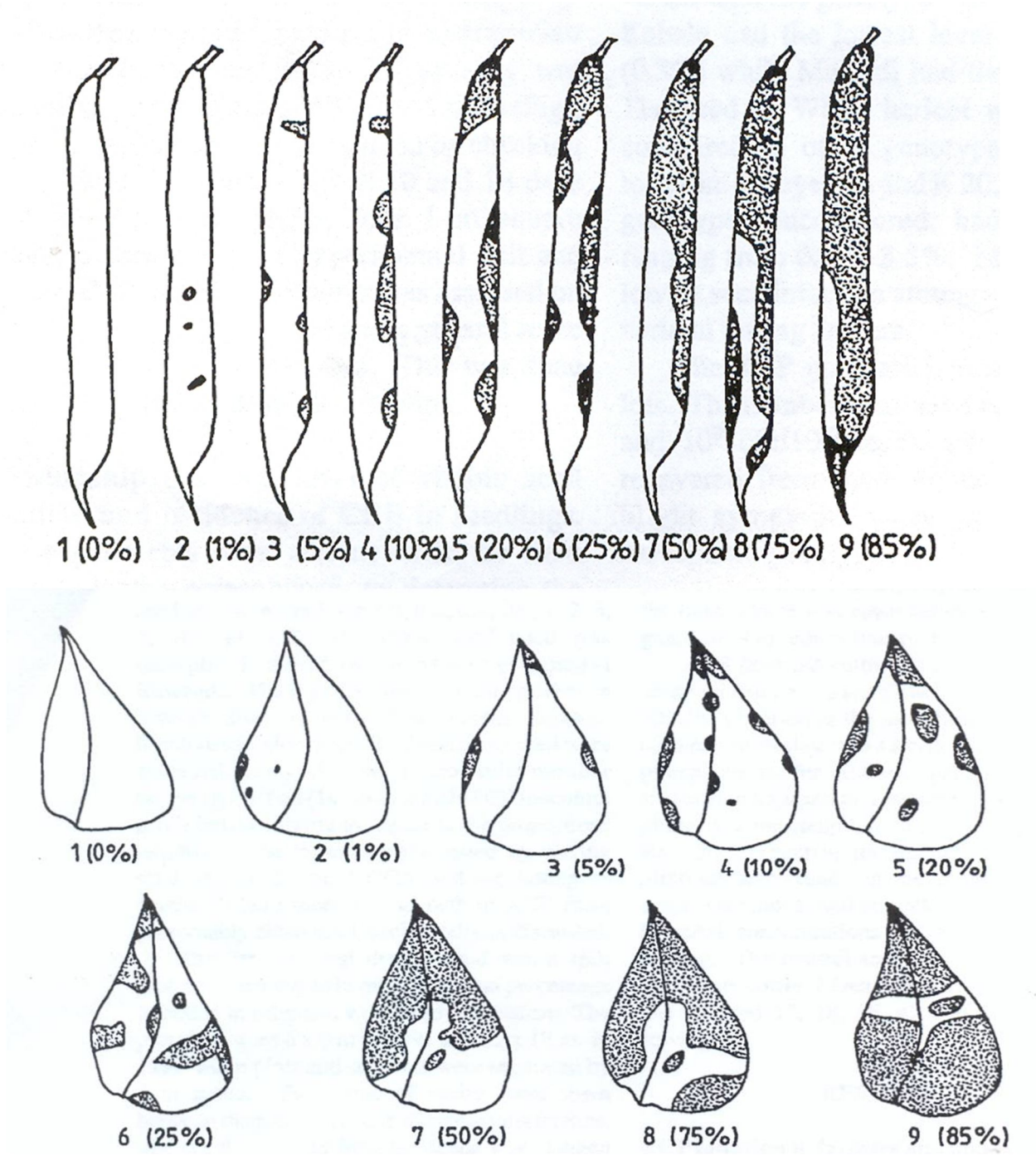


Fig.IV. Diagrammatic Representation of the Scale used to Rate Bacterial blight of Common Beans on Leaf and Pods (Opio *et al*., 1993).

3.3 Objective III: Seed Transmission Mechanism by *Xav* from the Germinating Seed to the Seedling

3.3.1 Experiment I Bacteria adhesion to seeds

Twenty seeds of cowpea (Ife Brown), Maize, Millet and Sorghum were surface disinfected by washing in 3 % sodium hypochloride for 5 minutes. Ten seeds each of the grains were put into a sterilized sample bottles containing 10 ml of bacterial suspension adjusted to *ca*.4.5x10^7 cfu/ml. Seeds soaked in SDW was used as control. These were left on the laboratory bench for 2 h and 4 h contact time between the culture and the seeds. After 2 h the suspension were drained away and the seeds washed twice with SDW and the other one after 4 h. Bacteria population sizes adherent on seeds were monitored by seed maceration and making serial dilutions to quantify the bacteria population. The percentage number of attached cells to the number of inoculated cells indicated irreversible adhesion capacities (Darrase *et al*., 2010). Experiments were repeated twice.

3.3.2 Experiment II: Biofilm formation

One hundred seeds each of Ife brown, millet, sorghum and maize were surface disinfected in 3 % sodium hypochloride. These were put in a 250 ml flask containing 100 ml SDW and incubated for 20 h. (Barbour *et al*., 1991). The resulting extracts were filtered under sterile conditions. X. *axonopodis* pv. *Vignicola* were grown on NGA until a log phase (Hamon and Lazazzera, 2001). Bacterial suspension adjusted to *ca*. 4.5 x 10^7 cfu/ml was suspended in each of the extracts and Ife brown exudates + 0.5 g glucose. Biofilm formations were determined using 96 – well microtiter plates filled with 200 µl bacterial suspension/ well. The control plates were their corresponding extract without inoculation. The plates were incubated at 27 ^{0}C without shaking for 72 h. After which the

planktonic cells were removed by rinsing the well with SDW five times and the cells biomass attached to the surface of the well were washed with physiologic solution and allowed to dry overnight at 25 ^{0}C. The plates were stained with 1 % crystal violet (CV) solution in 33 % (V/V) acetic acid for approximately 20 minutes. Excess CV was washed with SDW. The bound CV were solubilized with 200 µl of 33 % acetic acid or acetone – ethanol and quantified spectrophotometrically using Well Reader (GF 3000 microplate Reader –Bran scientific and Instrument Company England). Specific Biofilm formations (SBF) was calculated using the formula: SBF = B –NC/BG, Where B is the amount of CV bound to the cells attached to the surface of the wells, NC is the negative control, and BG is the OD_{630} of bacterial growth (Niu and Gilbert,2004).

3.3.3 Experiment III: Colonization pattern of the pathogen

Seeds of Ife brown were surface disinfected before inoculation. The bacterial inocula were obtained as described in section 3.3.1 previously. One hundred seeds of cowpea were soaked in 100 ml of SDW containing *Xav* suspension adjusted to *ca*. 4.5 x 10^7cfu/ml^{-1}. After 4-5 h the seeds were removed from the suspension and dried at room temperature. Five seeds were planted in a petri-dish containing SDW and filter papers. Seeds soaked in SDW were used as control. At different times after inoculation (24, 48 and 72 h) seeds were collected at random and ground up, from which serial dilution were made to quantify the population of bacterial colonizing the seeds (cfu seed^{-1}). At 5, 7, and 9 days after inoculation, plants were withdrawn from the petri dishes and were divided into: seed coat, cotyledons, hypocotyle, radicle, side roots, primary root and the emerging

leaves as shown in Fig. IV, each were ground and serial dilution was made to quantify the bacterial population colonizing the various parts (Yaryura *et al*., 2008).

3.3.4 Experiment IV: Relationship between nutrient status of media and biofilm formation

Media that were used for this investigation were: Nutrient glucose Agar (NGA), Ife brown seed extract, Ife brown seed extract plus 0.5 g glucose, maize extract, sorghum extract, and SAMPEA-7 extract. Nutrient glucose Agar was prepared as follows: glucose 1 g, yeast extracts 0.5 g and peptone 1 g /L. Seeds extracts were prepared as described previously. Bacteria suspension adjusted to *ca* 4.5 x 10^7cfu/ml was suspended in each of the media while their corresponding extracts without inoculation were used as control. Biofilm formations were determined using 96 well microtiter plates as described previously at 72 h, 96 h and 120 h. Result obtained were subjected to statistical analysis using analysis of variance ANOVA and means separated by Least Significant Difference (LSD).

3.4 Objective IV: Determination of Routes of Infection

3.4.1 Experiment 1: Direct infection of seeds by the pathogen through the vascular system

Direct systemic infection of seed by the pathogen was investigated using artificial inoculation method. Two cowpea varieties, Ife brown and SAMPEA-7 were used. The seeds were divided into 3 seed lots of 40 seeds each of the varieties. One seed lot was soaked in bacteria suspension adjusted to *ca*. 4.5 x 10 cfu/ml^{-1} before planting. Other seed lots were spray-inoculated at vegetative stage (25 days after sowing) with bacteria

suspension adjusted to *ca*. 4.7 x 10^7cfu ml^{-1} while one seed lot soaked in sterile distilled water was used as control. Five seeds per pot were planted in 25 cm diameter pot filled with sterilized soil. These were thinned to two plants per pot after seedling establishment. Harvested pods were sorted into symptom categories using scale 1-9 (Fig. III) and threshed accordingly. Hundred seeds each of the harvested seeds were plated on YDCA media and planted in the screen house to examine the presence of *Xav* and percentage infected seedlings were calculated. While the population of *Xav* in/on was determined by seed destruction assays. Data collected were analyzed statistically using analysis of variance (ANOVA) and means were separated by means of LSD.

3.4.2 Experiment II: Indirect systemic infection via floral parts

In order to determine the indirect systemic infection in the floral parts, twelve pots each of the two cowpea varieties were raised in the screen house. Bacterial suspensions adjusted to *ca*.4.5x10^7 cfu/ml were prepared. The suspensions were sprayed by hand held atomizer sprayer at flowering stage. After spray inoculated 2 mm^2 of floral parts (flower stalk, calyx, receptacle, corolla, carpel and ovary) were harvested at random and were macerated and serially diluted and plated on YDCA after 24, 48 and 72 h of inoculation. At maturity pods were harvested. The harvested pods were grouped according to disease severity rating scale 1-9 (Fig III). Percentage aborted flower and pod were calculated accordingly as described previously. Hundred seeds each from symptom groups were plated on YDCA media and planted in the screen house to examine the presence of *Xav* and to calculate the rate of transmission and percentage infected seedlings. While the population of *Xav* was determined by seed destruction assays as described in section

3.1.2. Data collected were analyzed statistically using analysis of variance (ANOVA) and means were separated by means of LSD.

3.4.3 Experiment III: Indirect seed infection via pod inoculation at flat pod and matured pod Stages

Five seeds per pot each of cowpea varieties (Ife brown and SAMPEA-7) were planted in a 25 cm diameter pot filled with sterilized soil. These were watered and fertilized as required. Cowpea plants were spray inoculated at flat pod and when the pod was ripped for harvest, with bacterial suspension adjusted to *ca*.4.7 x 10^7 cfu ml^{-1}. The control seedlings were sprayed with sterile distilled water. Polyethylene bags were used to cover areas that were not inoculated and all inoculated parts were tagged accordingly. The experiments were laid out in CRD with 10 replications. The experiments were repeated once. In order to determine the distribution of *Xav* in material tissues that leads to internal and external seed colonization, fertilized young pod and matured pods were randomly collected a 2 mm^2 section of fruit coat, fruit stalk, dorsal suture, funicle, and testa were macerated and were serially diluted and plated on YDCA for colony enumeration. The relevance of the work was to demonstrate the colonization pattern of the immature and mature pod during the various stages of seed development. Harvested pods and seeds were processed as described in section 3.4.1.

3.5 Objective V. Transmission of the Pathogen by Non host Cereal Crops

3.5.1 Experiment I: Detection of *Xav* on cereal grains

Since the colonization of seeds and seedling by pathogens is reported to be mostly commensality, the possibility of the pathogen being carried on the seeds of a non-host like maize, sorghum and millet which were usually cultivated crop in mixtures with cowpea was examined. Seeds that were used were purchased from the nine (9) cowpea producing states earlier stated in section 3.1.1. The occurrences of *Xav* on the seeds were determined by three methods (seed washing, seed plating and seed destruction assay). Four hundred seeds each from the various seed lots collected were washed, ground and serially diluted as described in section 3.2.1. The seed plating in media were done as described in section 3.1.4. While the seed destruction assays were carried out as described in section 3.1.2.The biochemical and pathogenicity test were done as in section 3.1.2. The trial was repeated once.

3.5.2 Experiment II: Comparing the dynamics of *Xav* population build-up *in vivo* in Cowpea and cereal seedlings following seed inoculation

Four hundred (400) seed each of the seed lots were inoculated by soaking the seeds in bacterial suspension adjusted to *ca*. 4.5 x 10^7 cfu ml^{-1} for 4 h. After 4 h of soaking, the bacteria suspensions were drained out. Ten seeds per pot were planted in a 25 cm diameter pot filled with heat sterilized soil and were fertilized and watered as required. The seeds that were soaked in distilled sterile water for 4 h were used as control. Leaf and stem disc measuring about 2 mm^2 were taken from the plants using cork borer at 7, 14 and 21 DAI. The discs were teased apart in drops of SDW and serial dilution were made and plated accordingly. There after the population build up in the shoot were taken at 35

and 49 DAI. The experiments were laid out using CRD. Data collected were analyzed statically using ANOVA and means were separated by means of (LSD). The trials were repeated once.

3.5.3 Experiment III: Transmission of *Xav* to non-host seeds via vascular system and floral system

Transmission through vascular and floral system was done as decribed previously in section 3.4.1 and 3.4.2.

CHAPTER 4

4.0 RESULTS

4.1 Detection of *Xav* in/on Seed Samples

4.1.1 Incidence and population of *Xav* on collected seed samples

Table 1 shows the result of incidence and population of *Xav* on the collected seed samples. There was high incidence and population of *Xav* in the seed samples collected across the nine (9) cowpea producing states and the three (3) cowpea seed companies. The incidence of *Xav* was higher on the seed samples collected from Kogi and Niger (3.3 and 3.1 %) respectively) followed by Nassarawa and Kaduna States. There was no statistical difference between the incidence of *Xav* on samples from Kebbi, Adamawa, and Gombe ($P \leq 0.05$). Also, there was no statistical difference between the incidence of *Xav* on seed samples collected from seed companies and that of Borno, but were however the lowest incidence had. Similar results also were observed in the population of *Xav* on seeds samples collected where samples collected from Kogi and Niger had the highest *Xav* population (4.1×10^3), followed by samples collected from Nassarawa , Kaduna and Kebbi States. There was no significant difference between the population of *Xav* on samples collected from Borno and that collected from seed companies which were statistically lower than the samples collected from Gombe and Adamawa states.

Table 1: Incidence and Population of *Xav* on Seed Samples in 2010 Combine Analysis of two trials

Isolate/Strain	Incidence	Population
Borno (Bama)	1.0e	$7.0x10^3$e
Gombe (Akor)	1.3ed	$8.0x10^3$d
Adamawa (Mubi)	1.3ed	$8.0x10^2$d
Sokoto (Duchin Kura)	1.0e	$7.0x10^2$e
Kaduna (Saminaka)	1.9c	$2.8x10^3$b
Kebbi (Jega)	1.4d	$1.6x10^3$c
Kogi (Idah)	3.3a	$4.1x10^3$a
Nassarawa (Keffi)	2.7b	$2.8x10^3$b
Niger (Mokwa)	3.1a	$4.1x10^3$a
Alheri	1.0e	$7.0x10^2$e
Masalaha	1.0e	$7.2x10^2$e
Premier	1.0e	$7.1x10^2$e
S.E	0.04	0.06

Means with the same letter within a column are not significantly different at $p \leq 0.05$ level of significance using NDMRT test.

4.1.2 Washing assay

In Table 2, the population of *Xav* on seed samples collected from Kogi, Nassarawa and Niger States were the highest followed by Kaduna State and were not statistically different from samples collected from other states and the seed companies. The lowest *Xav* populations were recorded on samples collected from the seed companies though not statistically different from samples collected from other states except Niger, Nassarawa and Kogi. In plating the seeds hilum up (Table 2), however, there was no significance difference between *Xav* dectected owing to low incidence on the part of the seed, while there was high significant difference in *Xav* detected on seeds plated hilum down. The seed samples collected from seed companies had lowest incidence of *Xav* (0.00- 0.01 %) though not statistically different from seeds samples collected from Borno, Gombe, Adamawa Sokoto, and Kebbi states. Seed samples collected from Kogi had the hightest incidence (0.12 %) and was statistically different from samples collected from Niger, Nassarawa, and Kaduna States. Table 3 shows relative distribution of *Xav* in internal parts of seeds. There was no significant difference in the population of *Xav* isolated from seed testa across the seed lots tested. Similar results were also recorded on seed hilum and embryo. There was no *Xav* detected on the cotyledons. The hilum, however, had the highest population of *Xav* (10^3) compared to testa and embryo which had 10^1 cfu.

Table 2: Bacterial Population from Washing Assay (cfu) and Incidence of X*av* on Seed Surface in 2010 Seed Plating

Seed Source/Variety	Bacterial population (cfu) in Washing assay	Incidence of *Xav* on seed plating (%)	
		Hilum up	Hilum down
Borno (Bama)	0.33 x 10^2bc	0.00e	0.07f
Gombe (Akor)	0.30 x 10^2bc	0.00e	0.07f
Adamawa (Mubi)	0.45 x 10^2bc	0.00e	0.06g
Sokoto (Duchin kura)	0.47 x 10^2bc	0.00e	0.05h
Kaduna (Saminaka)	0.48 x 10^2bc	0.05c	0.12e
Kebbi (Jega)	0.45 x 10^2bc	0.00e	0.13d
Kogi (Idah)	1.83 x 10^2a	0.12a	0.29a
Nassarawa (Keffi)	0.50 x 10^2a	0.10b	0.18c
Niger (Mokwa)	1.67 x 10^2a	0.10b	0.25b
Alhari	0.20 x 10^2bc	0.01d	0.05h
Masalaha	0.30 x 10^2c	0.00e	0.05h
Premier	0.33 x 10^2c	0.00e	0.05h
S.E	0.25	0.33	0.42

Means with the same letter within a column are not significantly different at $p \leq 0.05$ level of significance using NDMRT test.

Table 3: Relative Distribution of *Xav* in Internal Parts of Seeds in 2010

Source/Variety	Testa	Hilum	Embryo
Borno (Bama)	$1.0x10^1$a	1.5×10^3a	$0.3x10^1$a
Gombe (Akor)	$1.3x10^1$a	2.0×10^3a	$0.4x10^1$a
Adamawa (Mubi)	$1.1x10^1$a	1.8×10^3a	$0.3x10^1$a
Sokoto (Duchin kura)	$1.1x10^1$a	1.7×10^3a	$0.3x10^1$a
Kaduna (Saminaka)	$1.2x10^1$a	2.0×10^3a	$0.5x10^1$a
Kebbi (Jega)	$1.0x10^1$a	2.2×10^3a	$0.3x10^1$a
Kogi (Idah)	$1.4x10^1$a	2.5×10^3a	$0.5x10^1$a
Nassarawa (Keffi)	$1.3x10^1$a	1.9×10^3a	$0.5x10^1$a
Niger (Mokwa)	$1.3x10^1$a	2.1×10^3a	$0.5x10^1$a
Alhari	$1.0x10^1$a	1.5×10^3a	$0.5x10^1$a
Masalaha	$1.0x10^1$a	1.4×10^3a	$0.5x10^1$a
Premier	$1.0x10^1$a	1.4×10^3a	$0.4x10^1$a
S.E	0.33	0.42	0.40

Figures with the same letter within a column are not significantly different at $p \leq 0.05$ level of significance using NDMRT test.

4.1.3 Biochemical/morphological tests (phenotypic characteristics)

The characteristics of the twelve (12) isolates had been identified as *X.* a. *pv vignicola*. All isolates produced colonies of mucoid texture on YDCA (Table 4). All the isolates were gram negative (-) and produced copious amount of extracellular polysaccharide (EPS), hydrolyzed starch, but not all developed typical yellow pigmentation on YDCA. Also variations occur in the number of days various colonies took to developed yellow pigment and others were creamy yellow. It took some three (3) days to develop typical yellow, while some seven (7) days. Also observed under the microscope is the possession of one polar flagellum, produce no fluorescence under UV light, oxidase reaction (nagetive), catalyse reaction (positive) and oxidative metabolism. Typical yellow colonies of *Xav* were observed on Ife brown samples (Plate II) and subcultured colonies outlined in Plate III.

Table 4: Biochemical/Morphological Test in 2010

Isolate/Strain	Gram reaction	Pigmentation	EPS[c]	Starch hydrolysis	Inoculation period (days)
Borno (Bama)	-	T	+	+	5
Gombe (Akor)	-	LY	+	+	3
Adamawa (Mubi)	-	T	+	+	3
Sokoto (Duchin kura)	-	CY	+	+	4
Kaduna (Saminaka)	-	T	+	+	4
Kebbi (Jega)	-	LY	+	+	3
Kogi (Idah)	-	T	+	+	3
Nassarawa (Keffi)	-	T	+	+	4
Niger (Mokwa)	-	CY	+	+	7
Masalaha	-	T	+	+	7
Premier	-	T	+	+	7

Key: T= Typical yellow, LY = Light Yellow, CY = Creamy yellow, c = extracellular polysaccharide.

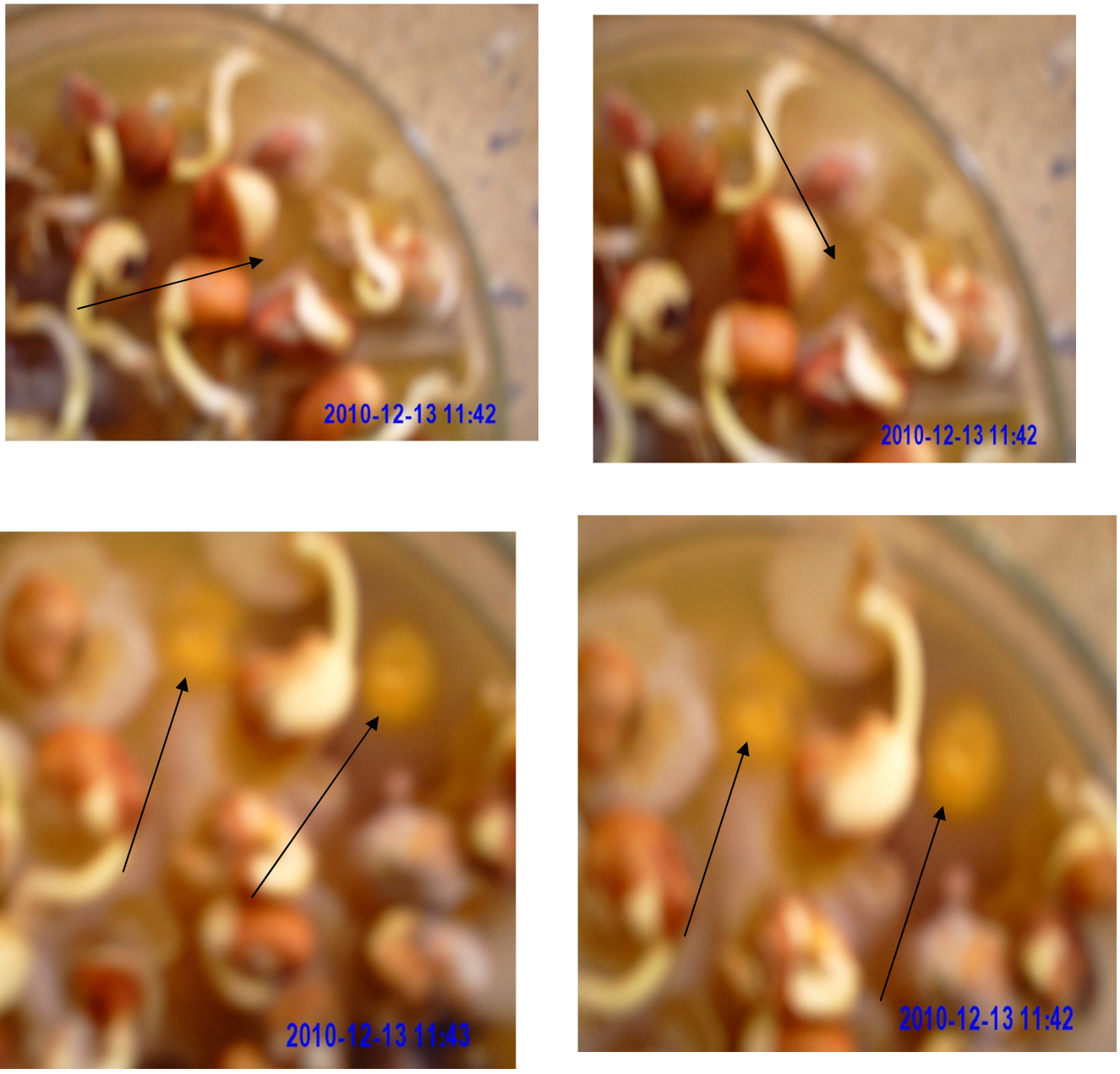


Plate II. Colonies of *Xav* on Ife Brown samples



Plate III. Sub-cultured colony of *Xav* on YDCA isolated from cowpea seeds

4.2 Correlation between Population Build up *in vivo* and Symptom Development

Table 5 shows the incidence; severity and population build-up on germinating and emergigng seedling at 14 DAS. There was significant difference ($P \leq 0.05$) between the treatments in terms of germination though the control treatments appeaed to be the lowest (60 %). There was significant difference between the treatments in terms of seedling establishment. While the control treatments were recording 100 % establishment Masalaha seed treated with Team fungicide had 50% seedling establishment. There was high disease incidence (67 %) in local variety obtained from Sabon Gari market compared to the control seedlings (0.00). There was significant difference between the treatments. The disease severity was higher on all the SAMPEA-7 varieties (both treated and untreated) which were statistically higher than other treaments and there was however, significant difference between all other treatments and the control. The population build up *in vivo* was higher in SAMPEA-7 seeds treated with Apron star ($4.5x 10^8$) and was statistically difference from other SAMPEA-7 treatments, variety IT98K-503-IT and IT86D-721A. IT98K-503-I T Masalaha T, and AlheriA had lower *in vivo* population build up ($2.0x10^8$) but were statistically difference from Premier D.f, MasalahaT, IT86D-721A, local W, Local SG and Local S. Although initial *Xav* population on the artificially inoculated seeds, was higher ($4.7x10^7$), there was no statistics difference in terms of the effect of pathogen on the seedling parts and the population build up *in vivo* compared to other initial populations and their effects on seedling parts and the resultant population build up *in vivo.*

Table 5: Incidence, Severity and Population build-up of *Xav* on Germinating Cowpea Seedling 14 DAS in 2011 Two Trials Combine Analysis

Source/Variety	Initial Population	Germination %	Establishment %	Incidence %	Severity	Population cfu/ml
Alheri A	$1.3x10^3$	65.0c	56.30f	27.7j	2.0i	$2.0x10^8$j
Premier D.F	$1.0x10^3$	70.0b	58.40c	50.0i	2.4e	$2.0x10^8$j
Masalaha T	$1.3x10^3$	65.0c	50.0h	50.0i	2.1h	$2.9x10^8$g
IT86D-721A	$1.3x10^3$	70.0b	51.0g	49.8j	2.4e	$3.2x10^8$f
IT98K-503-1T	$1.0x10^3$	75.0a	57.0e	52.1g	2.1h	$3.4x10^8$e
Local W	$1.9x10^3$	70.0b	58.3d	64.0b	2.3f	$3.5x10^8$d
Local SG	$1.4x10^3$	70.0b	57.0e	63.4c	2.3f	$2.3x10^8$h
Local S	$3.3 x10^3$	70.0b	57.0e	67.3a	2.2g	$2.2x10^8$i
SAMPEA-7A	$2.7 x10^3$	70.0b	43.0f	52.4f	4.6b	$4.5x10^8$a
SAMPEA-7T	$3.1 x10^3$	70.0b	58.3d	50.0i	4.8a	$3.3x10^8$e
SAMPEA-7D.F	$1.0 x10^3$	65.0c	57.0e	50.3h	4.3d	$3.2x10^8$g
SAMPEA-7I	$4.5 x10^7$	65.0c	57.3e	62.4e	4.4c	$3.8x10^8$b
IFbI	$4.5 x10^7$	70.0b	64.0b	62.9d	4.3d	$3.6x10^8$c
SAMPEA-7C	0.00	60.0d	100a	0.00k	0.0j	0.00k
S.E		0.74	0.93	0.84	0.92	0.91

Means in a column followed by the same letter are not significantly different at $p \leq 0.05$ level of significance using NDMRT test.

Table 6 shows the incidence of CoBB on seedling parts 14 DAS. The result shows that all SAMPEA-7 seed lots had high incidence of CoBB on roots except SAMPEA-7I which had (4.50 %). On the seed cotyledon there was statistical difference between all the treatments except SAMPEA-7DF and SAMPEA-7I that are statistically similar. The incidence of CoBB on the stem was higher on SAMPEA-7Df and SAMPEA-7I (5.30 %), this was followed by IFbI. There was no statistical difference between Local S and Local W treatments, between IT98k-503-IT, IT86D-721A and Alheri A and also between Premier DF and MasalahaT. The incidence of CoBB on the leaf was higher on SAMPEA-7I followed by IfbI. There was however statistical difference ($P \leq 0.05$) between all the treatments except Premier D.F and IT98-503IT which are statistically similar. The leaf incidence was lower (9.50 and 9.90 %) respectively on AlheriA and IT86D-721.

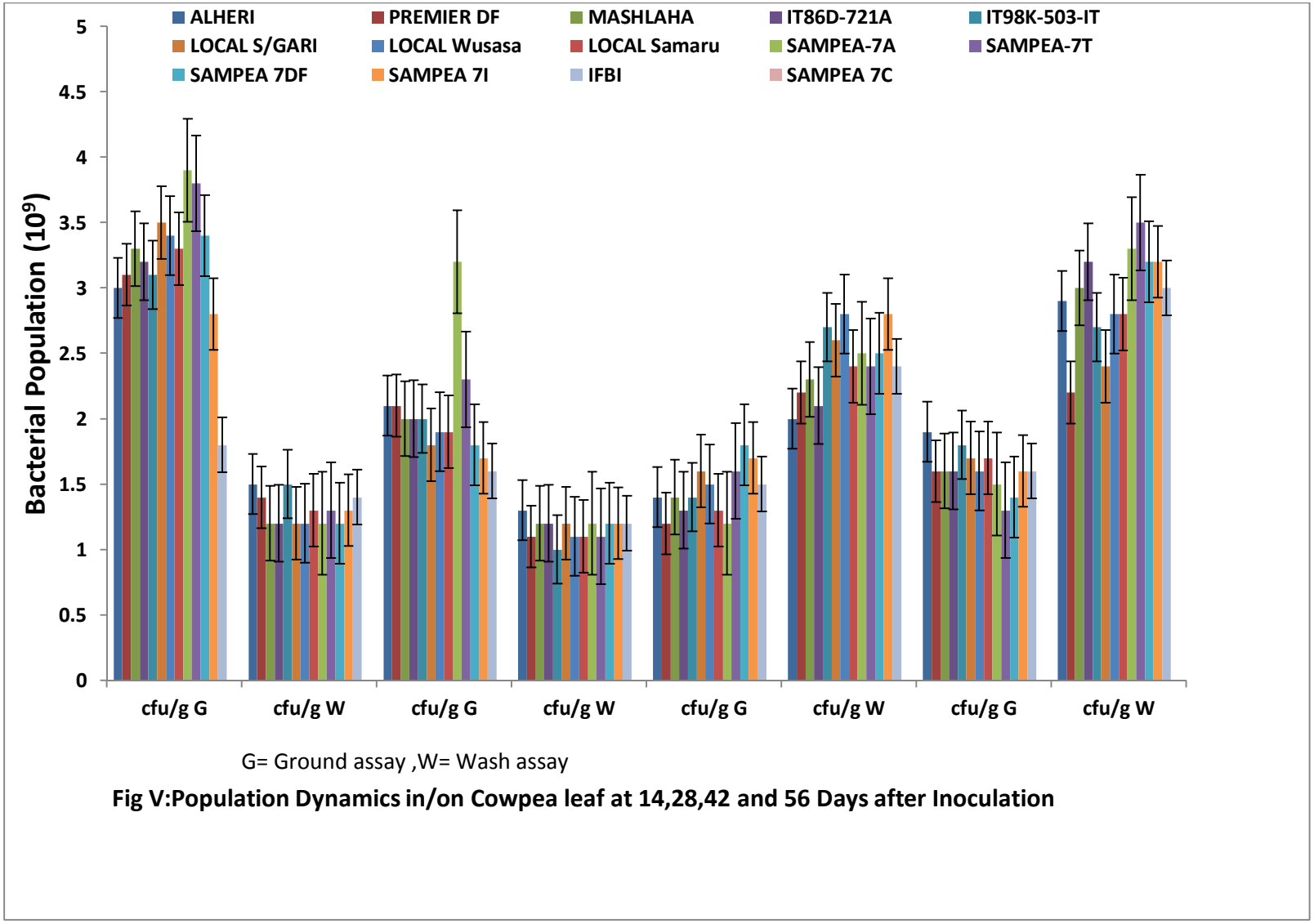
Table 6: Incidence of CoBB on Seedling Parts (%) 14 DAI in 2011 combine analysis of two trials

Source/Variety	Root	Cotyledon	Stem	Leaf
Alheri A	4.50h	2.00f	1.15g	9.45l
Premier D.F	4.25i	1.65g	1.10h	10.25i
Masalaha T	4.50h	2.05f	1.10h	9.90j
IT86D-721A	4.50h	1.00j	1.25g	9.65k
IT98K-503-1T	6.60g	2.20d	1.15g	10.25i
Local W	6.75e	2.25c	1.30f	11.75f
Local SG	6.65f	2.00f	1.45e	10.95h
Local S	7.00d	2.20e	1.40f	11.40g
SAMPEA-7A	10.15c	3.20b	2.10d	12.20e
SAMPEA-7T	10.50b	3.45a	2.35c	12.25d
SAMPEA-7D.F	10.75a	3.50a	5.30a	12.35c
SAMPEA-7I	4.50h	1.25h	5.30a	19.75a
IFbI	4.20k	1.15i	3.50b	17.40b
SAMPEA-7C	0.00k	0.00k	0.00i	0.00m
S.E	0.92	0.20	1.3	0.41

Means in a column followed by the same letter are not significantly different at $p \leq 0.05$ level of significance NDMRT test.

Fig V. Show the population dynamics of *Xav* in /on cowpea plant after spray inoculation. At 14 DAI, the population of *Xav* build up in cowpea plant was higher in SAMPEA-7A but statistically similar to SAMPEA-7T, SAMPEA-7Df and Local W. The population of *Xav* was lower in SAMPEA-7I and was statistically similar to other treatments and the lowest population of *Xav* was recorded in IfebI. There was no statistical difference between all the treatments in terms of phyllosphere population of *Xav*. After 28 DAI, the population of *Xav* in vivo was higher in the ground assay on SAMPEA-7A; this was followed by SAMPEA-7T but was statistically similar to all other treatments. There was no statistically difference between the phyllosphere populations of *Xav* on all the treatments 42 DAI. Similar results also were obtained from the ground assay 56 DAI where the *in vivo* population of *Xav* was higher in SAMPEA-7 D.f but was statistically similar to all other treatments except the control. The phyllosophere Population of *Xav* was higher in SAMPEA-7I and Local SG followed by IT98K-503T which was statistically similar to other treatments except Alheri A which had the population of *Xav*. At 56 DAI, the population of *Xav in vivo* was higher in Alheri A followed by IT98K-503-IT which was statistically similar to all other treatments except SAMPEA-7T. There was high phyllosophere population of *Xav* on SAMPEA-7T but was statistically comparable to all the treatments except the control.

Fig.VI. shows the disease incidence and severity after inoculation. At 14 DAI the disease incidence was higher in SAMPEA-7I followed by SAMPEA-7 D.f, SAMPEA-7T and SAMPEA-7A. There was no statistical difference between all other treatments except the control. Similar results were also obtained in the disease severity, where the severity was



G= Ground assay ,W= Wash assay

Fig V:Population Dynamics in/on Cowpea leaf at 14,28,42 and 56 Days after Inoculation

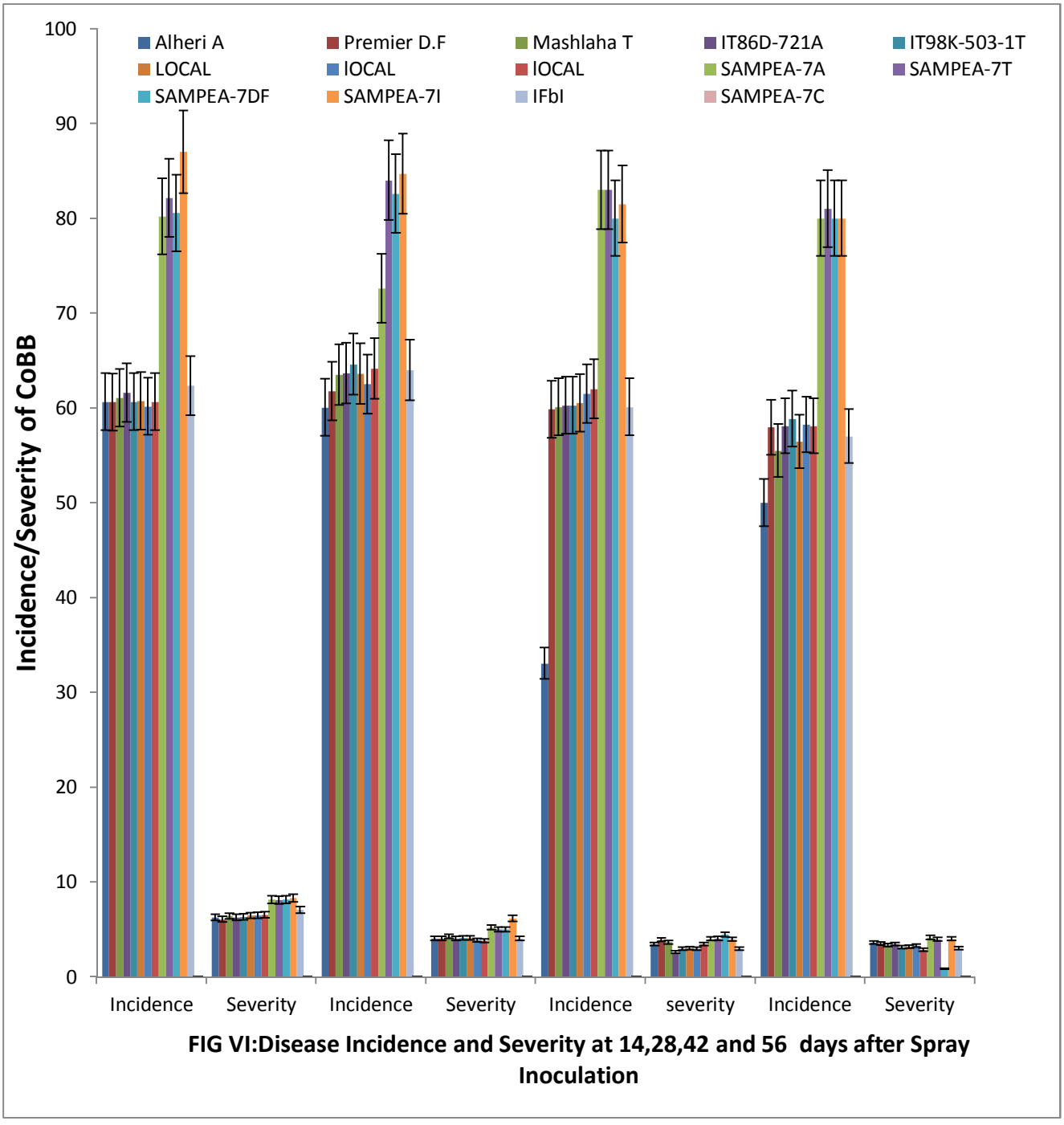


FIG VI:Disease Incidence and Severity at 14,28,42 and 56 days after Spray Inoculation

higher in all SAMPEA-7 varieties except the control, LIfbI which is statistically comparable to IT98K-503-IT, IT86D-721A, MasalahaT, Premier D.f and Alheri A. There was no statistical difference between Local W, Local SG, and Local S. At 28 DAI disease incidence was higher in SAMPEA-7I, SAMPEA-7 D.f and SAMPEA-7T followed by SAMPEA-7A. There was no statistical difference between all other treatments except the control. The disease severity was however high in SAMPEA-7A followed by Masalaha T, IT98K-503-IT and Local W and was statistically comparable to all the other treatments except the control. At 42 DAI, there was no statistical difference between all the treatments except Alheri A. In disease severity however, SAMPEA-7D.f had the highest severity and are comparable to SAMPEA-7I, SAMPEA-7T, SAMPEA-7A, Local S, MasalahaT, Premier D.f and Alheri A. The lowest severity was obtained in IT86D-721A but was comparable to IT98K -503-IT, Local W, Local SG and IfbI. At 56 DAI, the disease incidence was higher on all SAMPEA-7 variety. There was no statical difference between all other treatments except Alheri A. The disease severity was higher in SAMPEA-7A which is comparable to SAMPEA-7T, SAMPEA-7D.f and SAMPEA-7I followed by AlheriA which was comparable to Premier D.f Masalaha T, Local W and Local SG. The lowest severity was obtained in Local S and IFbI.

Table 7 shows CoBB severeity on the leaf. There was statistical difference in disease severity between all the treatments at 21 DAI except SAMPEA-7D.F and IT86D-.721A which were statistically similar. However at 35 DAI the blight severity was higher on Sanpea7I followed by IFbI. There was however, no statistical difference between Local S and Local W and there was statistical difference between all other treatments. At 49

Table 7: Severity of blight on the leaf part at 21, 35, 49, 63, 77, and 91 DAS (% Defoliation) in 2011 two trials combine analysis

Source/Variety	21	35	49	63	77	91
Alheri A	2.90f	13.95f	14.70i	14.95k	37.35k	63.00a
Premier D.F	2.65i	13.85g	14.70i	14.70l	39.20h	59.50i
Masalaha T	3.00e	12.90k	14.60j	15.10i	40.80d	56.90j
IT86D-721A	3.20d	13.50j	15.00f	15.45e	37.35k	59.50i
IT98K-503-1T	2.70h	13.80h	14.70i	15.05j	39.90f	61.30c
L-IfbW	2.40k	13.70i	14.95g	15.15h	38.90	59.65g
L-IFb-SG	2.80g	12.60l	14.90h	15.05j	38.90i	59.55h
L-Ifb.S	3.50a	13.70i	14.95g	15.25g	37.80j	60.50d
SAMPEA-7A	3.30c	15.10d	16.00d	17.20c	39.60g	60.05f
SAMPEA-7T	3.40b	15.11c	16.20b	16.85d	54.25b	59.65g
SAMPEA-7D.F	3.20d	15.00e	16.15c	17.50b	52.95c	60.20e
SAMPEA-7I	3.00e	15.25a	16.40a	18.30a	55.10a	62.50b
IfbI	2.95h	15.15b	15.15e	15.40f	40.60e	61.30c
SAMPEA-7C	0.00j	0.00m	0.00k	0.00m	13.00l	15.00k
S.E	0.04	0.01	0.03	0.04	0.004	0.03

Means in a column followed by the same letter are not significantly different at 5 % level of significance NDMRT test.

DAI, the severity of blight was higher on SAMPEA-7I (16.40) and was statistically not similar to SAMPEA-7D.f, SAMPEA-7T, SAMPEA-7A and IT86D-721A. The severity of blight was lowest on Alheri A (14.70) and was comparable to

PremierD.F and IT98K-503-IT. At 63 DAI, CoBB severity was higher in SAMPEA-7I (18.30) followed by SAMPEA-7D.f, SAMPEA-7T and SAMPEA-7A; this was followed by IFebI and IT86D-721A (15.40 and 15.45 respectively). The lowest severity was obtained in Premier D.f (14.70). The percentage defoliation was higher on SAMPEA-7I (55.10 %) and was statistically

higher than other treatments at 77DAI. At 91 DAI, the percentage defoliation was higher on Alheri A (63.00 %) which was significantly different ($p \leq 0.05$) from IT98K-93-IT, SAMPEA-7I and IfBI. There was statistical diference between all other treatments. The distribution of *Xav* population on plant part at flowering (70 DAS) is outlined in Fig.VII. The population of *Xav* was highest on flower cusion and flower of all the varieties while the lowest population was observed on stem and on the root. There was however no statistical difference between population of *Xav* on the stalk and leaf. There was relative varietal resistance observed in the distribution of *Xav* in plant parts except in the flower cusion and the flower.

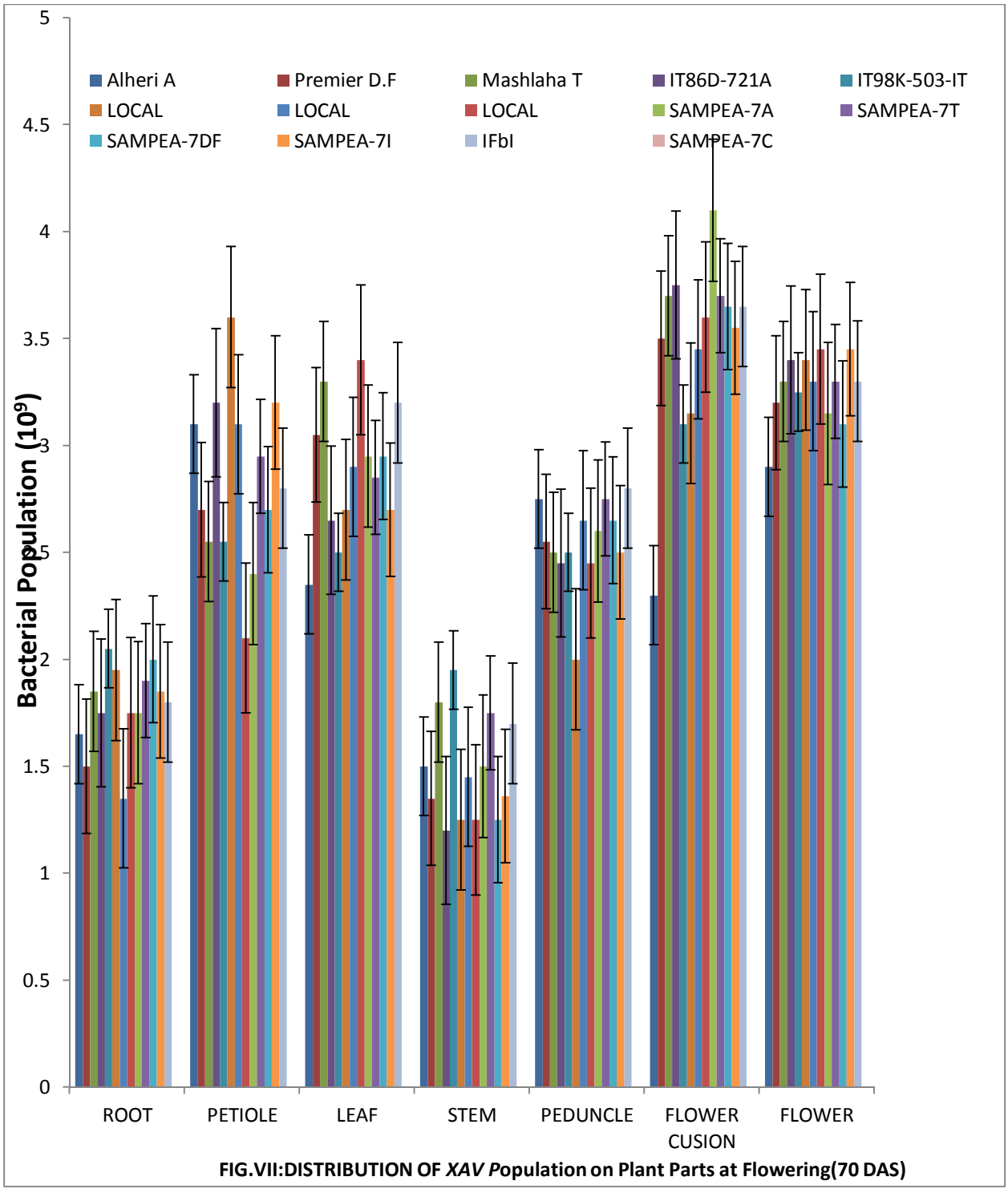


FIG.VII:DISTRIBUTION OF *XAV P*opulation on Plant Parts at Flowering(70 DAS)

Table 8 shows the severity *Xav* on floral parts. The percentage of flower aborted at 70 DAS was higher on IT98K-503-IT (29.85 %), the least was SAMPEA-7C which was the control. There was statistical difference between all the treatments. There was statistical difference between the percentage of flower aborted on all the treatments and the control at 77DAS, similar trend of results were obtained at 84, and 91 DAS. At 91 DAS, however, the highest pod abortion was recorded on IT86D-721A (46.40 %) and there was statistically difference between all other treatments and the control (10.40 %). At 98 DAS, the severity on pod was higher (4.65 %) on MasalahaT and there was statistical difference between all other treatments and the control.

Table 8: Flower and Pod aborted and Severity of *Xav* on Pod in 2011 Two Trails Combine Analysis

% Flower Aborted		% Pod Aborted	Severity on Pod		
Source/ Variety	**70**	**77**	**84**	**91**	**98 DAS**
Alheri A	20.60l	30.65j	53.95j	43.65h	4.05g
Premier D.F	24.85h	30.65j	60.15a	42.95k	4.00h
Masalaha T	29.00c	33.60c	59.10b	45.90c	4.65a
IT86D-721A	23.85 i	32.10g	57.45d	46.40a	4.60b
IT98K-503-1T	29.85a	31.10i	54.45i	45.60d	4.20f
Local W	24.30k	32.20f	54.60h	43.05j	4.25d
Local SG	24.95h	31.40h	55.60g	45.10e	3.85j
Local S	25.45g	32.80d	55.60g	43.30j	4.05g
SAMPEA-7A	29.10b	34.10b	57.75c	44.35f	3.95i
SAMPEA-7T	26.20f	31.40h	55.60g	43.45i	4.21e
SAMPEA-7D.F	26.60e	33.60c	56.15e	46.20b	4.30c
SAMPEA-7I	27.10d	53.10a	55.75f	43.80g	4.00h
IfbI	24.40j	32.45e	54.45i	42.25l	4.20f
SAMPEA-7C	3.50m	5.40k	10.10k	10.40m	0.00k
S.E	0.70	0.67	1.22	1.34	1.37

Means in a column followed by the same letter are not significantly different at 5 % level of significance using NDMRT test.

Table 9 shows *Xav* populations on shed flowers, aborted flower and aborted pods. Population of *Xav* in shed flower ranges from 10^2-10^8 while the aborted flower and aborted pod had high population of *Xav* (10^9). At 70 DAS, the highest population of *Xav* isolated from shed flower was on AlheriA which is statistically similar to SAMPEA-7D.F. Also there was no statistical difference between IT98k-503-IT, Local S and IFbI, PremierD.F and Local W, IT86D-721A and SAMPEA-7T, MasalahaT, Local S and SAMPEA-7I. At 77 DAS the highest population of *Xav* was recorded PremierD.F (10^9) which is statistically similar to SAMPEA-7A and SAMPEA-7D.F. Simillarlly there as no statistical difference beween IFbI.SAMPEA-7I,SAMPEA-7T, Local S, Local SG, Local W, IT86D-721A, MasalahaT and Alheri A. At 84 DAS, however, the highest poplation of *Xav* isolated from aborted pod was on SAMPEA-7T which was statistically similar to Local W. This was followed by SAMPEA-7I which is statistically similar to SAMPEA-7A, Local SG and MasalahaT. There was no statistical difference between all other treatments except the control.

After spray inoculated symptoms of CoBB were recorded on Ife brown (Plate IV), SAMPEA-7 (Plate V) and IT86D-721 (Plate VI). Canker symptoms were recorded on stems (Plate VII) and leaf stalk (Plate VIII). Symptoms of CoBB were also noticed on the pods (Plate IX) and pod suture (plate X).

Table 9: Population of *Xav* (cfu/ g) in Shed, Aborted flower and Pods in 2011 Combines Analysis of Two Trials

	70 DAS	77 DAS	84 DAS
Source	**Cfu/g shed flower**	**Cfu/g arborted flower**	**Cfu/g aborted pod**
Alheri A	$1.5x10^{8}a$	$1.2x10^{9}b$	$1.0x10^{9}c$
Premier D.F	$1.3x10^{6}c$	$1.3x10^{9}a$	$1.1x10^{9}c$
Masalaha T	$1.2x10^{2}f$	$1.1x10^{9}c$	$1.2x10^{9}b$
IT86D-721A	$1.3x10^{4}d$	$1.2x10^{9}b$	$1.1x10^{9}c$
IT98K-503-1T	$1.5x10^{7}b$	$1.3x10^{9}a$	$1.0x10^{9}c$
Local W	$1.3x10^{6}c$	$1.1x10^{9}c$	$1.3x10^{9}a$
Local SG	$1.5x10^{2}f$	$1.2x10^{9b}$	`$1.2x10^{9}b$
Local S	$1.4x10^{7}b$	$1.1x10^{9}c$	$1.1x10^{9}c$
SAMPEA-7A	$1.0x10^{3}e$	$1.3x10^{9}a$	$1.2x10^{9}b$
SAMPEA-7T	$1.2x10^{4}d$	$1.0x10^{9}c$	$1.3x10^{9}a$
SAMPEA-	$1.5x10^{8}a$	$1.3x10^{9}a$	$1.1x10^{9}c$
7D.F	$1.4x10^{2}f$	$1.2x10^{9}b$	$1.2x10^{9}b$
SAMPEA-7I	$1.3x10^{7}b$	$1.2x10^{9}b$	$1.0x10^{9}c$
Ife brown I	0.00g	0.00d	0.00d
SAMPEA-7C	1.03	1.22	1.12
S.E			

Means in a column followed by the same letter are not significantly difference at 5 % level of significance NDMRT test.



Plate IV. Symptoms of CoBB on Ife Brown after Spray Inoculation



Plate V. Typical Hallow border Symptoms of CoBB on SAMPEA-7 after Spray Inoculation

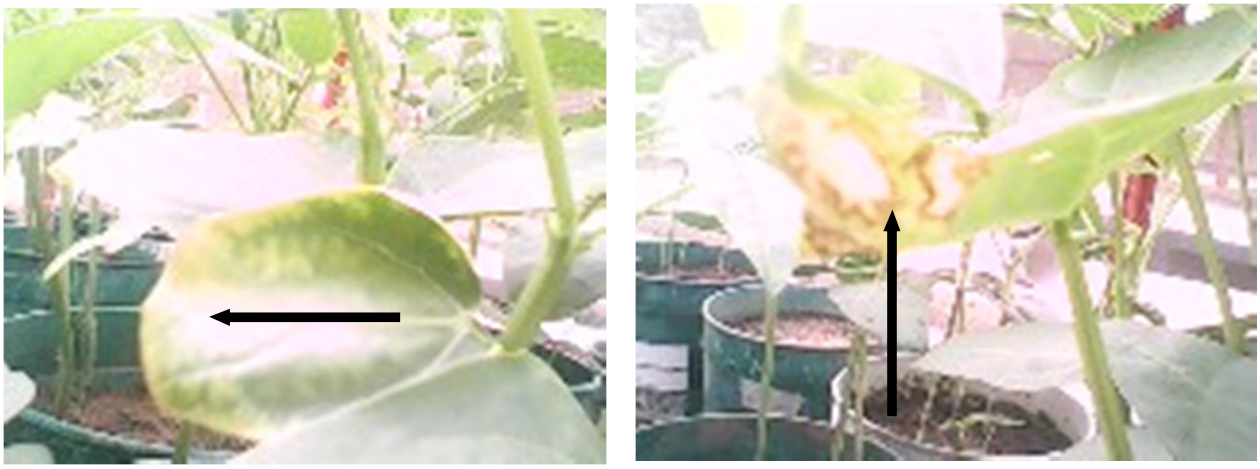


Plate VI. Symptom of CoBB on IT86D-721 after Spray Inoculation



Plate VII. Canker Symptoms on Stems

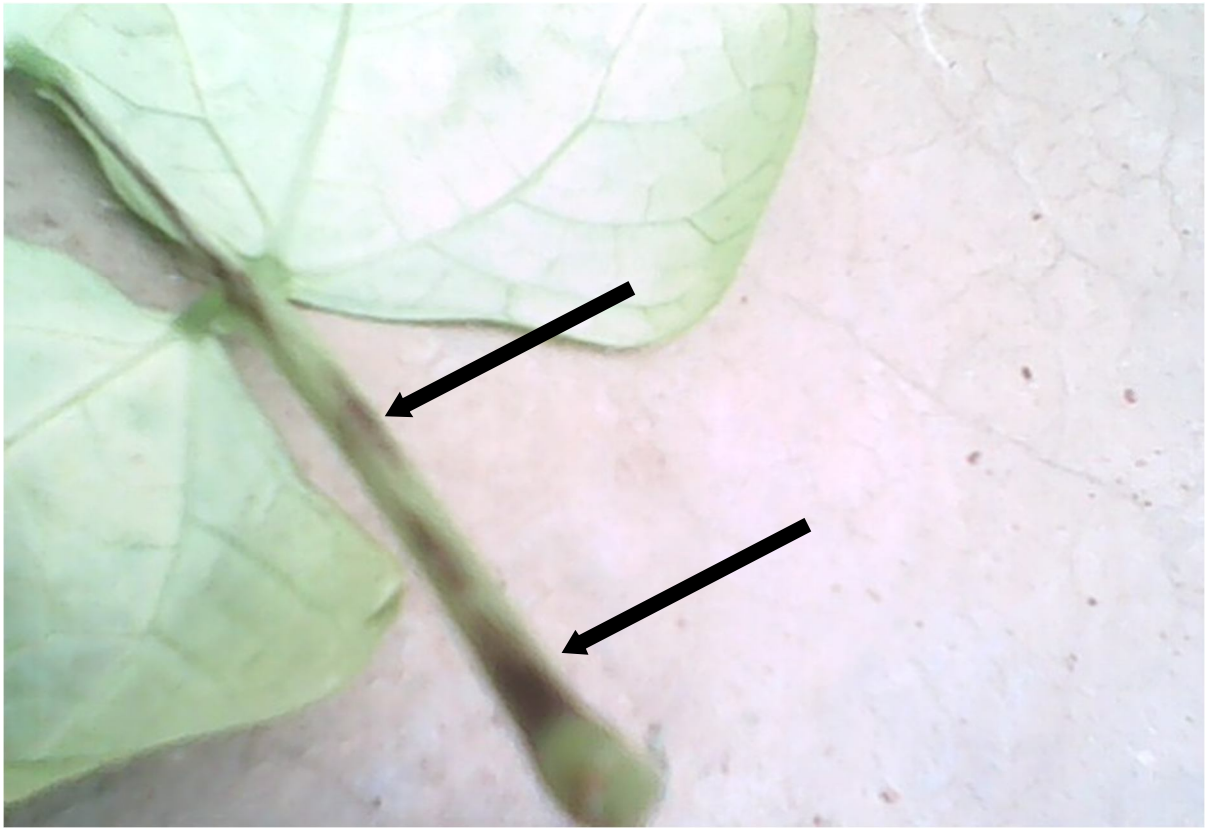


Plate VIII. Canker on the Aborted Leaf stalk



Plate IX. CoBB Spots on Pods

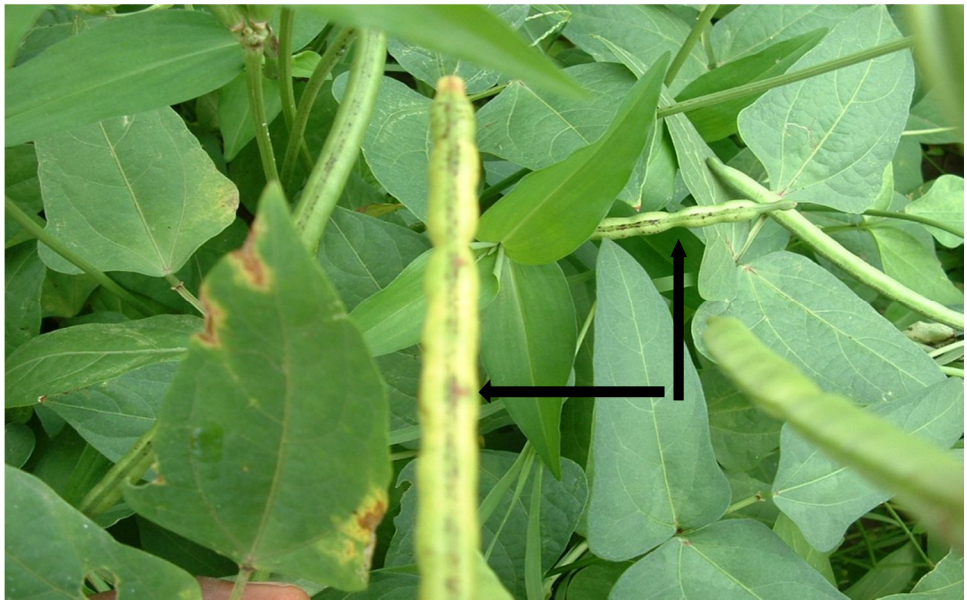


Plate X. Water soaked Legion on Pods suture

4.3 Transmission Mechanism of *Xav*

Table 10 shows the adhesion of *Xav* to seeds. After 2 h of inoculation, cowpea had 100% adhesion, followed by millet (40 %), sorghum (20 %) and maize had no *Xav* adhesion. The *Xav* population was higher in cowpea, followed by millet and sorghum. At 4 h after inoculation all the seeds were attached by *Xav*. The highest adhesion was on cowpea (100%) followed by millet, sorghum and maize. There was however, statistical difference between *Xav* population on the seeds, ($P \leq 0.05$). Table 11 shows the result of colonization pattern of *Xav* on seedling. No *Xav* was detected on seed coat but at 5 DAI only was *Xav* detected on the cotyledons. The population of *Xav* was higher (10^7) on radicle, side roots and primary roots while that of hypocotyle has (10^6). The population of *Xav* was lower on the primary root at 5 DAI but was statistically not similar to population of *Xav* at 7 DAI. The population of *Xav* was lower on hypocotle (10^6) followed by emerging leaves (10^3). Fig.VII shows the result of progressive colonization of seeds/seedling over time. The population of *Xav* was steadily increased and reaches the peak at 72 h. Fig VIII shows biofilm fornation by *Xav*. Cowpea extract + 0.5g glucose induced highest biofilm formation, followed by millet and sorghum extract. There was no significance difference between biofilm induced by NGA and extract of cowpeas. Fig IX show the result of relationship between time and biofilm formation induced by seeds extract and NGA media. Biofilm formation induced by millet and sorghum extract increases at 96 h but remain constant even at 120 h. There was no significance difference in biofilm formation induced by other extract and time.

Table 10: Adhesion of *Xav* to Seeds in 2011 Combine Analysis of Two Trials

	2 h		4 h	
Treatment	% infection	Population	% infection	Population
Maize	0.00d	0.00d	40d	$2.4x10^7$a
Cowpea	100a	$2.4x10^4$a	100a	$2.2x10^7$c
Millet	40b	$1.3x10^4$b	70b	$2.4x10^7$a
Sorghum	20.0c	$0.75x10^4$c	60c	$2.3x10^7$b
Control	0.00d	0.00d	0.00e	0.00d
S.E	0.65	0.05	0.84	0.07

Means in a column followed by the same letter are not significantly different at 5 % level of significance using SNK test.

Table 11: Colonization Pattern of *Xav* on Seed/Seedling in 2011Ccombine Analysis ofTtwoTtrials

DAI	Seed coat	Cotyledons	Radicle	Hypocotyle	Side roots	Primary root	Emerging leaves
5	-	$2.3x10^2$	$2.3x10^7$c	$1.4x10^6$c	$1.1x10^7$c	$2.6x10^7$c	$1.2x10^3$c
7	-	-	$2.5x10^7$b	$1.8x10^6$b	$1.4x10^7$b	$2.8x10^7$b	$1.3x10^3$b
8	-	-	$2.7x10^7$a	$1.9x10^6$a	$1.6x10^7$a	$3.2x10^7$a	$1.5x10^3$a
S.E			0.020	0.024	0.020	0.022	0.20

Means in a column followed by the same letter are not significantly different at 5 % level of significance using SNK test.

DAI = Days after inoculation

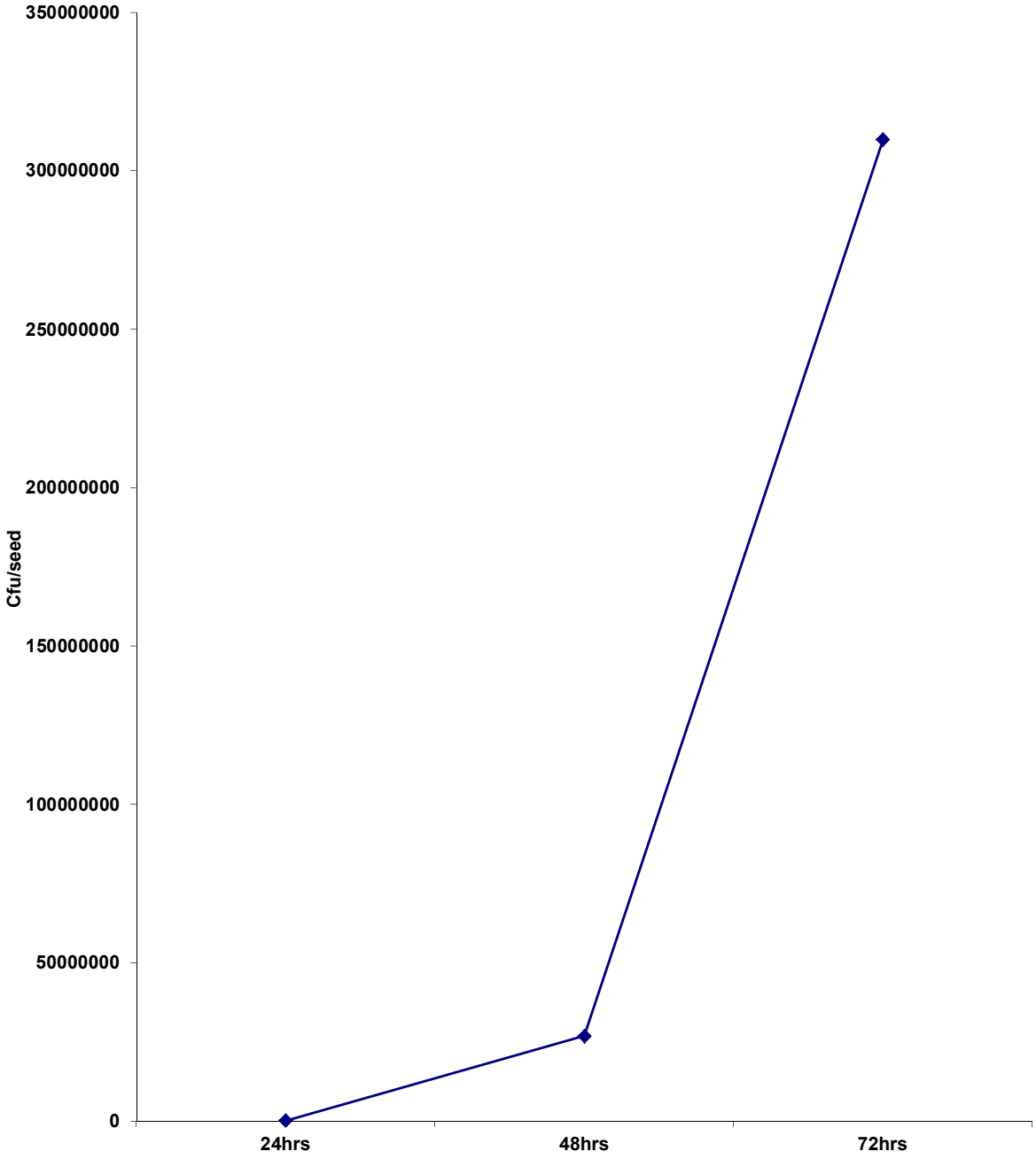


Fig. VIII: Progressive Colonisation of *Xav* on Seed/Seedling over Time

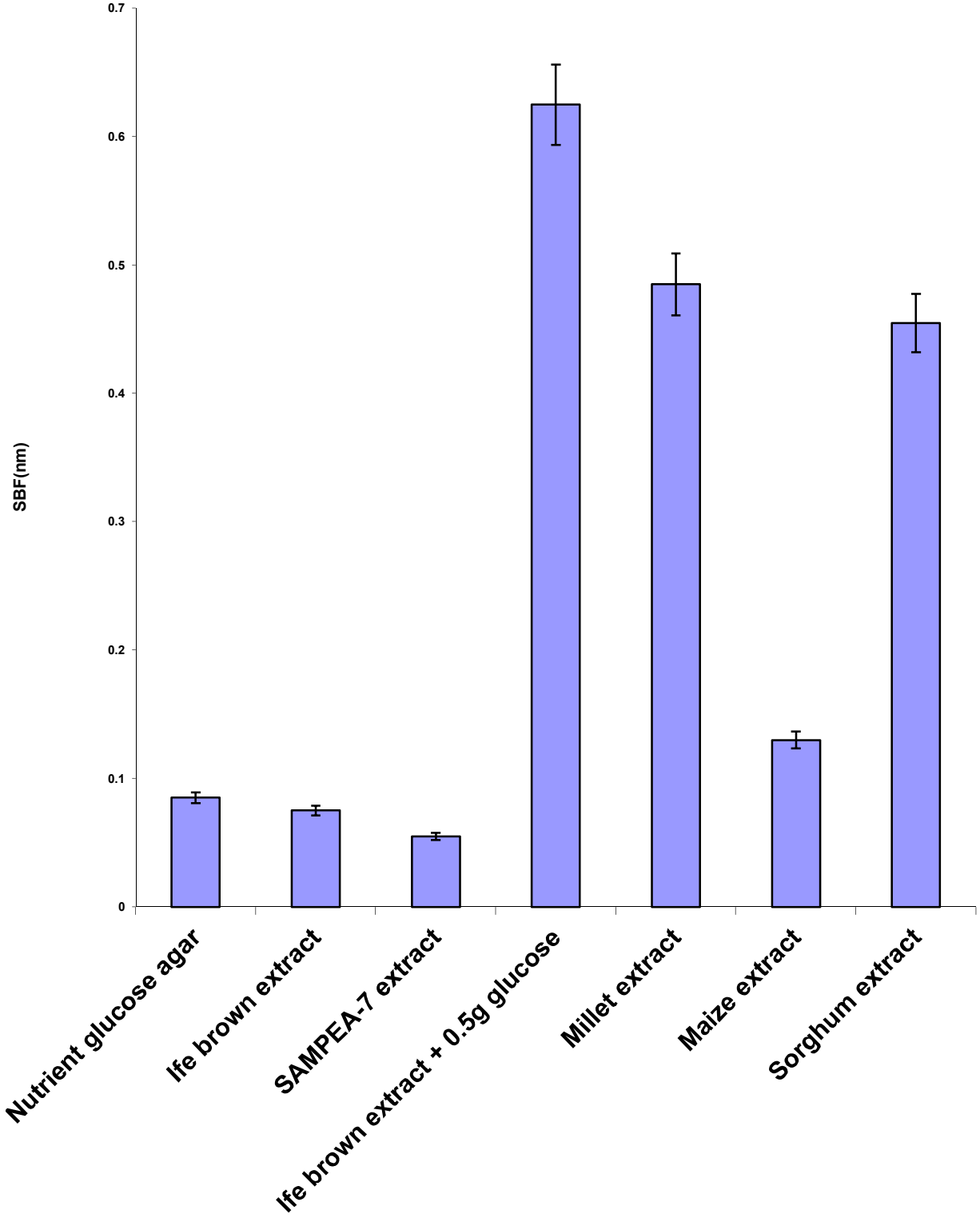


Fig. IX: Biofilm Formation after 72 hours

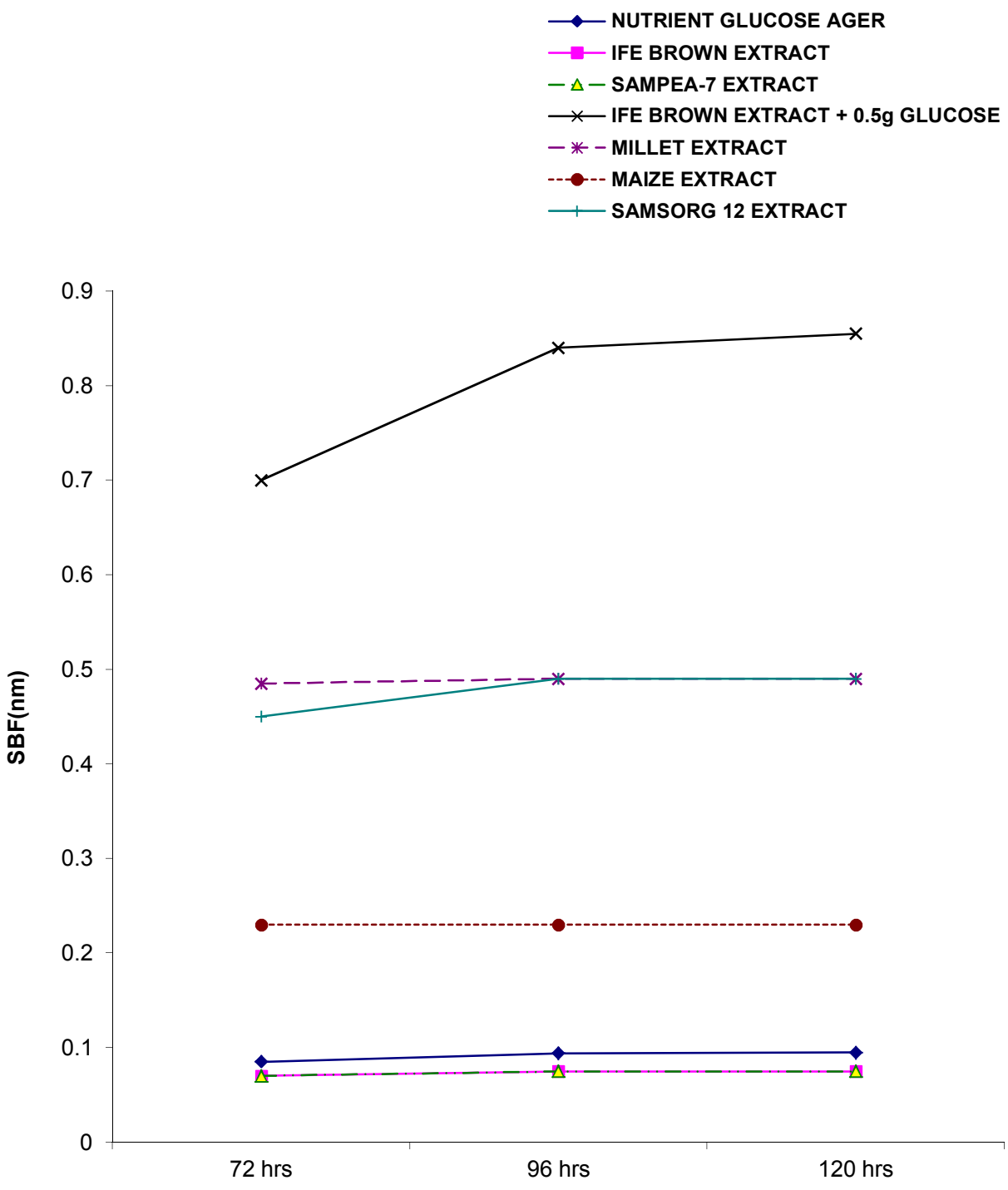


Fig. X: Relationship between Time and Biofilm Formation of the Different Cereals Extracts

4.4 Route of Transmission

Seed inoculation produce more symptomatic pods than vegetative inoculation. In Table 12 however, there was statistical difference between all the treatments in terms of germination in both symtopmatic and symptomless pods. In symptomatic pod, Ife brown had highest germination but was statistically not similar to other treatments and the control had 100 % germination. In symptomless pod, however, SAMPEA-7 had 80 % germination and was statistically similar to Ife brown, followed by Ife brown (65 %) in symptomless pod and was similar to SAMPEA-7 statistically. In seedling establishment, seed inoculation had the lowest (35.50, 57.00 and 47.00 % in vegetative inoculation. Similar trend was also observed in symptomless pod. The rate of transmission was high (52.00 %) in symptomatic pods compared to 22.00 % in symptomless pods in seed inoculation. The rate of transmission was lower 30.00 % and 11.00 % in symptomatic and symptomless pod in vegetative inoculation. Similar trend of result was observed in population of *Xav* isolated from both symptomatic and symptomless pods in seed and vegetative inoculation.

Table 12: Transmission of *Xav* by Symptomatic and Symptomless Pod in 2011

Variety	Treatment	Symptomatic pods				Symptomless pods			
		Germination (%)	Seedling establishment (%)	Infection (%)	Population (%)	Germination (%)	Seedling establishment (%)	Infection (%)	Population (%)
Ife brown	Seed inoculation	67.00b	35.50e	50.00b	$2.3x10^5$a	65.00c	57.00d	20.00b	$2.0x10^3$a
	Vegetative inoculation	65.00c	57.00b	30.00d	$1.4x10^5$b	80.00b	60.10c	11.50d	$0.7x10^3$c
SAMPEA-7	Seed inoculation	65.00c	37.50d	52.00a	$2.5x10^5$b	64.60d	55.60e	22.00a	$1.2x10^3$b
	Vegetative inoculation	63.00d	47.00c	32.00c	$1.3x10^5$b	80.00b	62.40b	13.00c	$0.5x10^3$d
Control		100a	100a	0.00e	0.00c	100a	100a	0.00d	0.00e
SE		1.91	3.00	0.42	1.91	3.50	3.20	0.52	0.40

Means in a column followed by the same letter are not significantly different at 5 % level of significance using SNK test.

Table 13 shows the infection of CoBB after flower inoculation. After 24 h of inoculation *Xav* population vary from 0.5 x 10^5 in stalk to 2.3 x 10^8 in corolla in Ife brown while in SAMPEA-7, the population varies from 0.8 x 10^5 in stalk to 1.3 x 10^8 in corolla which was the highest population. There was no *Xav* detected in carpel and ovary. After 48 h of inoculation, the highest population (10^9) was recorded in receptacle and corolla of both cowpea varieties, followed by calyx (10^8). Within 48 h of inoculation the bacteria was able to infect the carpel and ovary. Similar trend of population growth was observed after 72 h of inoculation. Floral inoculation resulted to high flower and pod arbortion. In Table (14), germination ranges from 50 % in symptomatic pod to 58 % in symptomless pod in Ife brown but was statistically similar to SAMPEA-7 (47-55 %). Similar result was also obtained in seedling establishment.

The percentage infection was higher in symptomic pods (75.00 % and 85.00 %) respectively in Ife brown and SAMPEA-7 while in symptomless pods the rate of transmission was 65.00 % in Ife brown and 55.00 % in SAMPEA-7. Similarly, the population of *Xav* was lower (10^3) in symptomless pods compared to (10^7) in symptomatic pods. Table 15 shows the population of *Xav* within parts of the pod after 24 h of inoculation. The population of *Xav* was higher (2.6x 10^8) in pod stalk of SAMPEA-7 and was statistically similar to pod stalk of Ife brown (2.4 x 10^8) inoculated at flat pod stage and were significantly higher than the mature pod inoculation (1.0 x 10^8 – 1.1x10^8) in Ife brown and SAMPEA-7 respectively. Similar trend of result were observed in fruit coat and dorsal suture. There was no *Xav* detected in funicle, testa and cotyledons at neither stages of inoculation. At 48 h of inoculation, there was statistical difference in

population of *Xav* in pod stalk, coat and dorsal suture. *Xav* was detected in funicle. There was statistical difference between the two varieties at both stages of inoculation. Again, no *Xav* was detected in seed testa and cotyledon. There was pod arbortion in flat pod inoculation and there was no pod abortion in mature pod inoculation.

Table 13: Population of *Xav* on the floral parts after 24, 48, and 72 hours after Inoculation in 2011

		Floral Parts					
Treatment/ Variety	Hours after inoculation	Stalk	Calyx	Receptacle	Corolla	Carpel	Ovary
Ife brown	24	$0.5x10^5$	1.2×10^5	1.4×10^6	2.3×10^5	0.00	0.00
	48	$1.5x10^6$	1.6×10^8	2.5×10^9	3.0×10^9	$1.0x10^6$	$1.3x10^6$
	72	$1.6x10^6$	1.7×10^8	2.7×10^9	3.1×10^9	$1.0x10^6$	$1.5x10^6$
SAMPEA-7	24	$0.8 x10^5$	1.5×10^5	1.3×10^6	1.3×10^8	0.00	0.00
	48	1.4×10^6	1.4×10^8	2.7×10^9	2.9×10^9	$1.2x10^6$	$1.2x10^6$
	72	1.5×10^6	1.6×10^8	3.0×10^9	3.3×10^9	$1.0x10^6$	$1.4x10^6$
Untreated SAMPEA-7		0.00	0.00	0.00	0.00	0.00	0.00

Table 14: Transmission of *Xav* to Seed from Symptomless and Symptomatic Pods following Inoculation at flowering stage in 2011 Combine Analysis of Two Trials

	Symptomless pod				Symptomatic pod			
Treatment	Germination	Seedling establishment	Rate	Population	Germination	Seedling establishment	Rate	Population
Ife brown	58b	46b	65a	$3.6x10^3$b	50b	29c	0.75b	$2.7x10^7$a
SAMPEA-7	55c	35c	0.55b	$3.7x10^3$a	47c	35b	0.85a	$2.6x10^7$b
Control	100a	100a	0.00c	0.00c	100a	100a	0.00c	0.00c
S.E	1.50	2.32	0.25	1.23	3.2	2.44	0.21	1.23

Means in a column followed by the same letter are not significantly different at 5 % level of significance using LSD test.

Table 15: Population of *Xav* (cfu/g) on Pod after 24, 48, 72, 96 hours after Inoculation

Variety	Treatment	Hours after inoculation	Floral parts					
			Stalk	Fruit coat	Dorsal suture	Funicle	Testa	Cotyledon
Ife brown	Flat pod inoculation	24	$2.4x10^8$b	$2.7x10^8$a	$2.8x10^8$a	0.00	0.00	0.00
		48	$1.2x10^8$a	$2.8x10^8$a	$3.4x10^9$a	$2.2x10^9$a	0.00	0.00
		72	$13x10^8$b	$2.9x10^8$a	$3.4x10^9$a	$2.3x10^9$a	0.00	0.00
		96	$1.3x10^5$a	$2.9x10^6$a	$3.5x10^7$a	$2.4x10^6$a	0.00	0.00
	Matured pod inoculation	24	$1.0x10^8$d	$1.1x10^8$c	$2.2x10^8$c	0.00	0.00	0.00
		48	$1.1x10^8$b	$2.6x10^8$b	$2.4x10^9$c	$2.0x10^9$ac	0.00	0.00
		72	$1.4x10^8$a	$1.5x10^8$c	$1.3x10^9$d	$1.0x10^8$d	0.00	0.00
		96	$1.1x10^3$b	$1.2x10^5$c	$1.5x10^5$c	$1.4x10^5$c	0.00	0.00
SAMPEA-7	Flat pod inoculation	24	$2.6x10^8$a	$2.6x10^8$b	$2.8x10^8$a	0.00	0.00	0.00
		48	$1.0x10^8$c	$2.5x10^8$c	$3.0x10^9$b	$2.1x10^9$b	0.00	0.00
		72	$1.3x10^8$b	$2.8x10^8$b	$3.2x10^9$b	$2.2x10^9$b	0.00	0.00
		96	$1.3x10^5$a	$2.7x10^6$b	$3.4x10^6$b	$2.3x10^6$b	0.00	0.00
	Matured pod inoculation	24	$1.1x10^8$c	$1.0x10^8$d	$2.4x10^8$b	0.00	0.00	0.00
		48	$1.0x10^8$c	$2.4x10^8$d	$2.4x10^9$c	$2.1x10^9$b	0.00	0.00
		72	$1.2x10^8$c	$1.3x10^8$d	$1.4x10^9$c	$1.2x10^9$c	0.00	0.00
		96	$1.0x10^3$c	$1.3x10^5$d	$1.4x10^5$d	$1.2x10^5$d	0.00	0.00
Control			0.00e	0.00e	0.00e	0.00e		
SE $\pm$			1.20	1.30	1.32	1.25		

Meansin a column followed by the same letter is not significantly different at 5 % level of significance using LSD test

The flat pod inoculation leads high pod infection in both varieties SAMPEA-7 and Ife brown (Table 16). There were no obvious or conspicuous symptoms on pods following mature pod inoculation. The epiphytic population of *Xav* was higher in mature pod inoculation than than the flat pod inoculation. The percentage of infection was higher in flat pod inoculation (75 % and 80 %) compared to (10 %) in matured pod inoculation. Similar result was obtained in population of *Xav*.

Unshed flower lead to canker development on pod (Plate XI). After flower inoculation canker symptom was observed on pods (Plate XII), flower cusion (Plate XIII) and pod suture (Plate XIV). Pod shattering symptoms were observed after flat pod inoculation (Plate XV). Heavy seed infection from symptomatic pods resulted to early CoBB symptoms on the emerging cowpea seedling (Plate XV).

Table 16: Seeds Infection and Population growth following Inoculation at Flat and Matured Pod in 2011 Two Trials Combine Analysis

Treatment		% infection	Population(cfu)
Flat pod inoculation	Ife brown	75	$2.6x10^7$a
	SAMPEA-7	80a	$2.5x10^7$b
Mature pod inoculation	Ife brown	10c	$0.2x10^2$c
	SAMPEA-7	10c	$0.3x10^2$d
	Control	0.00d	0.00e
S.E		3.45	0.32

Means in a column followed by the same letter are not significantly different at 5 % level of significance using SNK test.



Plate XI. Unshed flower lead Canker on Pod after flower Inoculation



Plate XII. CoBB Canker on Pod after flower Inoculation

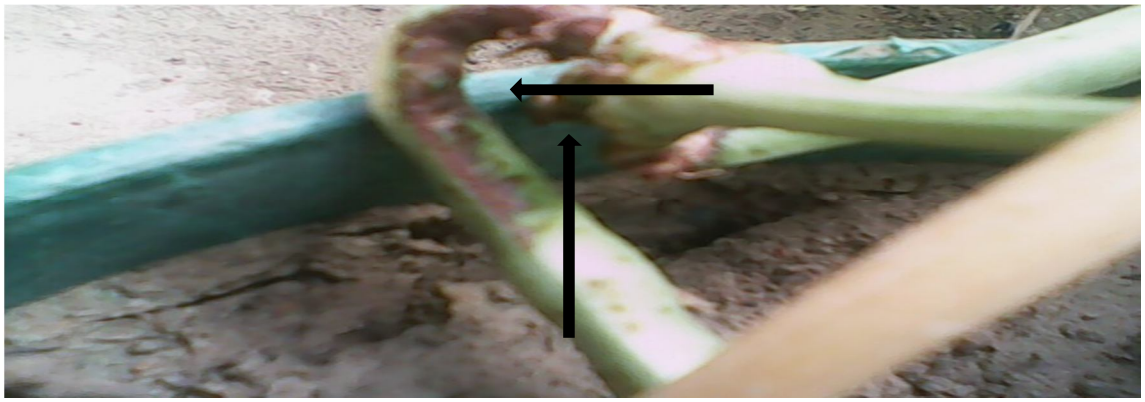


Plate XIII. Canker on Cushion/Pod after flower Inoculation

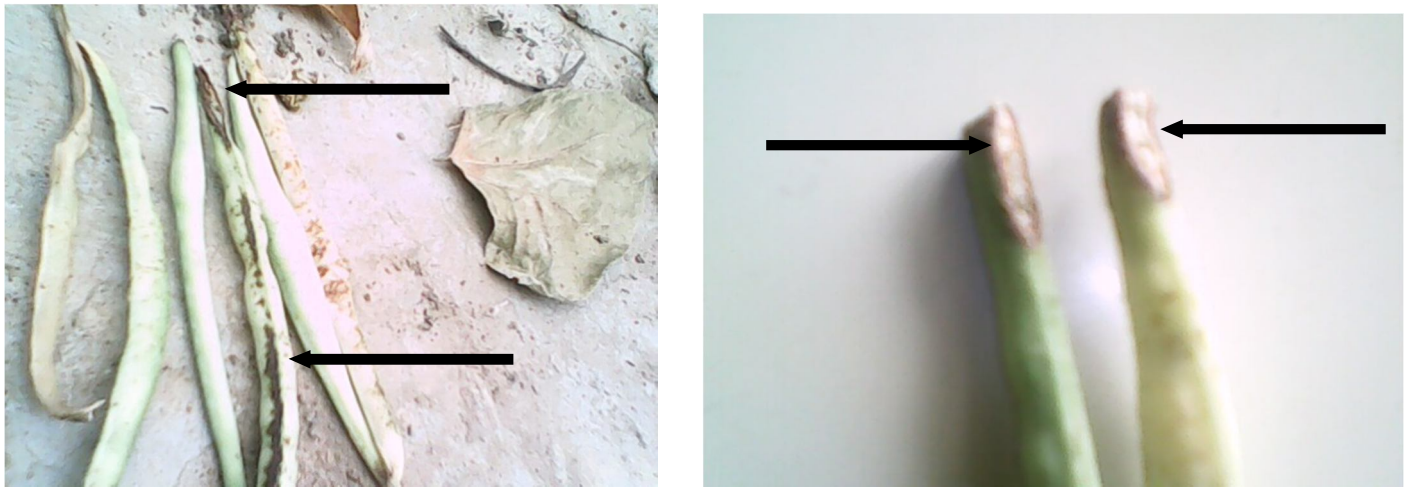
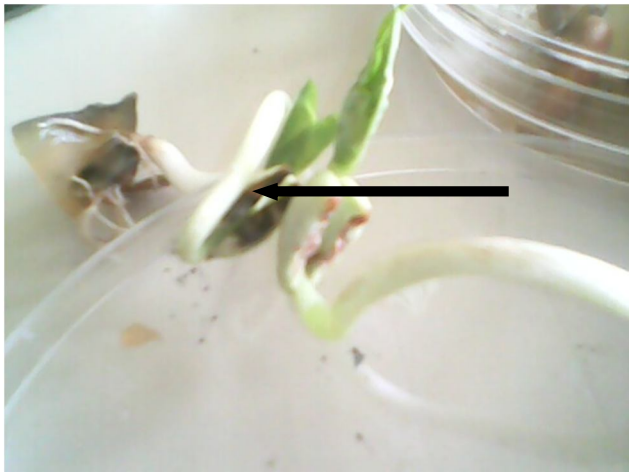


Plate XIV. Symptoms on Pod suture after flat pod Inoculation



Plate XV. Pod Shattering Symptoms after flat pod Inoculation



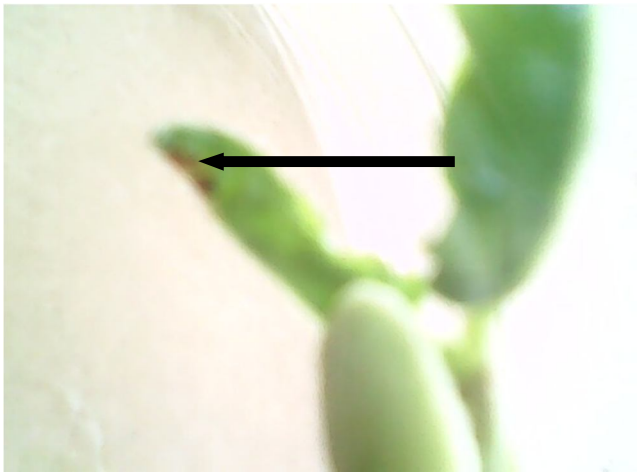




Plate XVI. Heavy seed Infection from Symptomatic pods leads to Eearly Symptom Development on the Emerging Seedling

4.5 Transmission of *Xav* by Non- host Cereal Crops.

Table 17 shows the result of detection of *Xav* on non-host cereal crops using seed washing assay, seed plating and seed destruction assay. *Xav* was not detected on millet samples from Borno, Adamawa, Sokoto and Kebbi state using washing assay. The population of *Xav* on millet samples ranges from $1.0x10^2$-$1.4x10^2$ cfu/ml. *Xav* was consistently detected on millet samples collected from all the states except kebbi state using seed destruction assay. In seed destruction assay however, the population of *Xav* detected on millet samples ranges from $1.0x10^2$ - $3.3x10^2$ cfu/ml. There was no *Xav* detected on maize and sorghum samples. Biochemical/morphological tests show the characteristics of the nine (9) isolates and were identified as *Xav*. All isolates produced colonies of mucoid texture on YDCA (Table 18).

All the isolates were gram negative (-) and produced copious amount of EPS, hydrolised starch, but not all developed typical yellow pigmentation on YDCA. Also variation occurs in the number of days various colonies took to developed yellow colony and others were creamy yellow. It took some three (3) day to develop typical yellow, while some seven (7) days. Also observed under the microscope is the possession of one polar flagellum, produce no fluorescence under UV light, oxidase reaction nagetive, catalyse reaction positive and oxidative metabolism. Table 19 shows the result of the result of pathogenicity test of the nine (9) isolates of *Xav* on the three (3) varieties of cowpea and tobacco plant. All the nine (9) isolates were pathogenic on the 3 cowpea varieties and also induce hypersensitivity reaction on tobacco plant. Variations among the isolates were observed in the number of days taken to induce reaction on the plants.

Table 17: Detection of *Xav* on Millet in 2010

Source	Seed washing a assay	Seed plating	Seed destruction assay
Borno (Bama)	-	+	$0.2x10^2$
Gombe (Akor)	$1.0x10^2$		$1.3x10^2$
Adamawa (Mubi)	-	-	$1.0x10^2$
Sokoto (Duchin Kura)	-		$1.4x10^2$
Kaduna (Saminaka)	$1.2x10^2$	-	$3.3x10^2$
Kebbi (Jega)	-	-	-
Nasarawa (Keffi)	$1.1x10^2$	+	$1.2x10^2$
Kogi (Idah)	$1.0x10^2$	+	$2.4x10^2$
Niger (Mokwa)	$1.4x10^2$	+	$1.0x10^2$

Key

+ = *Xav* dectected, - =*Xav* not detected

Table 18: Biochemical/Morphological Test on Isolate from Millet in 2010/2011

Isolate/Strain	Gram reaction	Pigmentation	EPS[c]	Starch hydrolysis	Inoculation period (days)
Borno (Bama)	-	T	+	+	3
Gombe (Akor)	-	T	+	+	3
Adamawa (Mubi)	-	T	+	+	3
Sokoto (Duchin kura)	-	T	+	+	4
Kaduna (Saminaka)	-	CY	+	+	4
Kebbi (Jega)	-	T	+	+	3
Kogi (Idah)	-	T	+	+	3
Nassarawa (Keffi)	-	LY	+	+	4
Niger (Mokwa)	-	CY	+	+	4

Key: T= Typical yellow, Ly = Light Yellow, CY = Creamy yellow. c = Extracellular polysaccharide

Table 19: Pathogenicity of Twelve *Xav* Isolates from Millet on Three Varieties of Cowpea and Tobacoo in 2010/2011

Isolate/strain	IT86D-721	SAMPEA-7	Ife brown	Tobacco	Period (Day)
Borno (Bama)	+	+	+	HR	15-21
Gombe (Akor)	+	+	+	HR	15-21
Adamawa (Mubi)	+	+	+	HR	12-21
Sokoto (Duchin kura)	+	+	+	HR	10-21
Kaduna (Saminaka)	+	+	+	HR	10-21
Kebbi (Jega)	+	+	+	HR	8-21
Kogi (Idah)	+	+	+	HR	7-21
Nassarawa (Keffi)	+	+	+	HR	7-21
Niger (Mokwa)	+	+	+	HR	9-21

HR= Hypersensitive reaction
+ = compatible (susceptible) reaction

Table 20 shows the population of *Xav* in *vivo* 7, 14 and 21 DAS. At 7 DAS, the rhizosphere population of *Xav* was significantly higher than the control ($P \leq 0.05$). There was however no statistical difference between the treatments.

Table 20: Population of *Xav* (cfu) in Vivo 7, 14 and 21 DAS in 2011 Combine Analysis of Two Trials

	Days after sowing (DAS)					
	7		14		21	
Treatment	Root	Shoot	Root	Shoot	Root	Shoot
Ife brown	$3.20x10^{7}$a	$1.6x10^{7}$a	$3.4x10^{8}$a	$2.7x10^{8}$a	$2.4x10^{8}$a	$3.1x10^{8}$a
Samsorg-12	$2.5x10^{7}$b	$1.0x10^{7}$c	$2.2x10^{7}$ab	$1.2x10^{7}$b	$1.9x10^{6}$b	$1.0x10^{6}$c
Millet	$3.3x10^{7}$a	$1.4x10^{7}$ab	$3.2x10^{7}$b	$2.4x10^{7}$b	$2.2x10^{6}$ab	$2.1x10^{6}$b
Maize	$2.4x10^{7}$b	$1.3x10^{7}$b	$2.1x10^{7}$b	$1.1x10^{7}$b	$1.4x10^{6}$c	$0.3x10^{7d}$
Ife brown	0.00c	0.00d	0.00c	0.00c	0.00d	0.00e
Millet control	0.00c	0.00d	0.00c	0.00c	0.00d	0.00e

Means in a column followed by the same letter are not significantly different at 5 % level of significance using SNK test.

At the shoot level, there was statistical difference between the treatments ($P \leq 0.05$). The phylosophere population of *Xav* was higher in Ife brown ($1.6x10^8$), followed by millet ($6.4x10^7$) and Samsorg-12 ($1.0x10^7$). At 24 DAS, the rhizosphere population of *Xav* increases and there was significance difference between the treatments ($P \leq 0.05$). The rhizosphere population of *Xav* was higher in Ife brown ($3.4x10^8$), millet ($3.2x10^7$) and Samsung12 ($2.2x\ 10^7$) which is statistically similar to maize ($2.1x10^7$). At the shoot region, again, Ife brown had the highest phyllosphere of *Xav* ($2.7x10^8$) followed by millet $2.4x10^7$), followed by Samsorg-12 ($1.2x10^7$) which was statistically similar to maize ($1.1x10^7$) . Similar results were obtained at 21 DAS but with decline population of *Xav* both in rhizosphere and phyllosphere.

Table 21 shows the result of *Xav* population in vivo at 35 and 49 DAS. On the leaves, and steam Ife brown had higher *Xav* population ($2.3x10^8$, $1.5x10^8$ followed by millet $1.5x10^4$ $5x10^4$ cfu/cm^2) with *Xav* not detected on other treatments. Similar results were obtained at 49 DAS. The *Xav* population in Ife brown and millet leaves were higher than that of the stem.

Table 22 shows the result of transmission of *Xav* through direct vascular system and indirect floral system. In direct vascular transmission, *Xav* was only detected on millet by seed destructive assay and seed plating while in the indirect floral system, *Xav* was detected on millet and sorghum in all the isolating methods. No *Xav* was detected on maize both direct vascular transmission and indirect floral system. Colonies of *Xav* observed on millet samples are outlined in Plate XVII and subcultured colonies shown in Plate XVIII.

Table 21: Population of *Xav* (cfu) *in Vivo* 35 and 49 DAS in 2011 Combine Analysis of Two Trials

	Days after sowing (DAS)			
	35		49	
Treatment	Leaves	Stem	Leaves	Stem
Ife brown	$2.3x10^8$a	$1.5x10^8$a	$2.4x10^8$a	$1.5x10^8$a
Samsorg- 12	0.00c	0.00c	0.00c	0.00c
Millet	$1.5x10^4$a	$0.5x10^4$b	$1.2x10^4$b	$0.6x10^4$b
Maize	0.00c	0.00c	0.00c	0.00c
Ife brown	0.00c	0.00c	0.00c	0.00c
Millet control	0.00c	0.00c	0.00c	0.00c

Means in a column followed by the same letter are not significantly different at 5 % level of significance using SNK test.

Table 22: Transmission of *Xav* through Direct vascular and indirect floral system in 2011

Treatment	Direct vascular transmission			Indirect floral system transmission		
	Seed destruction	Seed washing	Seed plating	Seed destruction	Seed washing	Seed plating
Maize	-	-	-	$2.0x10^2$(cfu)	-	-
Millet	$2.3x10^4$(cfu)	-	+	$1.0x10^3$(cfu)	$1.4x10^3$ (cfu)	+

Keys

- **= not detected**

\+ **= detected**

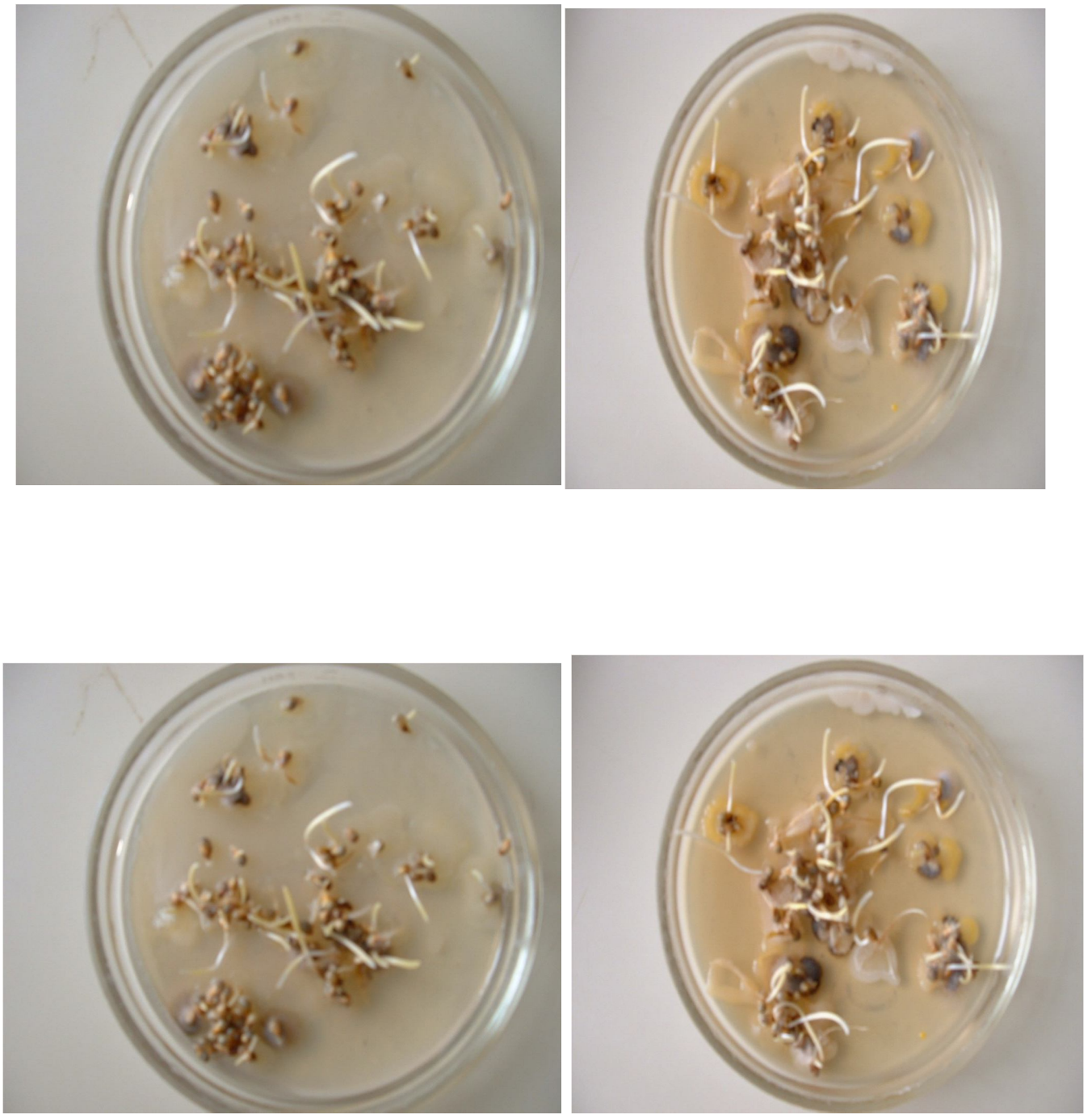


Plate XVII. Colonies of *Xav* on Millet samples

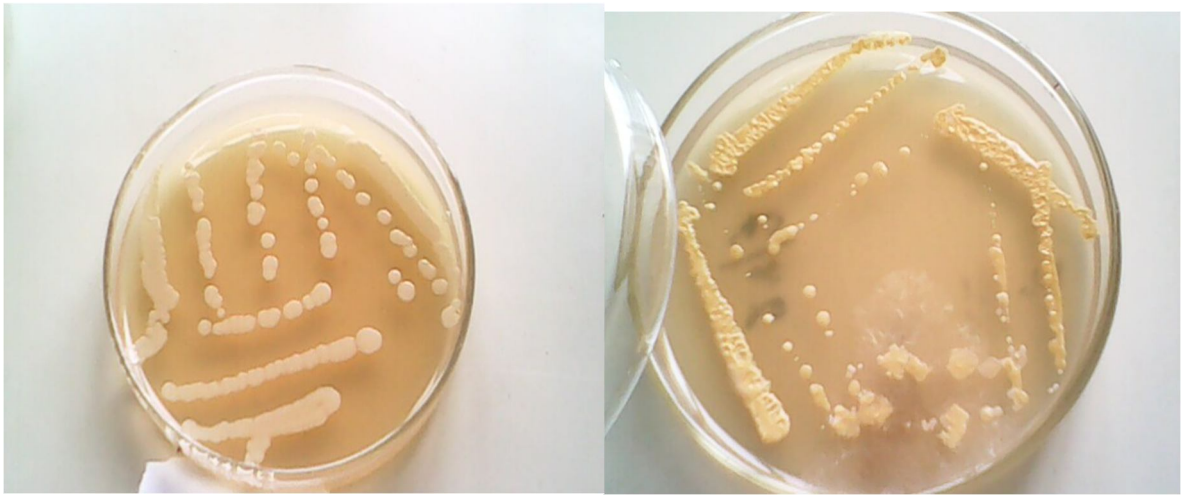


Plate XVIII. Sub cultured colony of *Xav* on YDCA Media after 48 h

CHAPTER 5

5.0 DISCUSSION

The study demonstrated that the cowpea seeds used by farmers in Nigeria were infected by *Xav* both locally produced seeds and seeds produced by the seed companies. The lowest level of seed infection was 1.0 % and the bacterial population on seed lots ranges between 10^2-10^3 cfu/ml (Table 1). The minimum bacteria population necessary to incite infection in the field was found to be (10^2) cfu/kg of seeds (Opio *et al*., 1993). A level of seed infection of 0.02 % in 1000 seeds was found to initiate and cause severe infection in the field (Schaad, 1988). The low incidence of *Xav* on seeds collected from, Maiduguri, Gombe, Adamawa, Kebbi and Sokoto and that of seed companies could be as a result of physical stress and chemical injury inflicted on the pathogen from the origin of hosts seeds. Liao and Shollenberger (2004) reported that bacteria cells that had been exposed to high temperature (physical stress) and some form of chemical treatment have difficulty in growing on selective and semi- selective media like YDCA.

However, the injury or stress did not affect their pathogenic ability on the host. The physical stress could be as a result of unfavourable climatic conditions in the far north where bulks of cowpea are produced in Nigeria. This environmental/climatic condition could be an advantage in seed production programmes. This result confirm the report of Maude (1996), Agrawal and Sinclair (1996) that because of extremely low tolerance for pathogens in seed crops has resulted in specialized areas of seed production in regions where pathogens are unable to establish or usually remain below threshold levels during seed development in United States. In United States seed production occurs primarily in

the western states where pressure from fungal and bacterial pathogens is reduced by low rainfall and relative humidity (du Toit 2004). Higher rainfall and prolonged leave wetness has been cited to be the singular reason for high population of pathogens in the high rainfall areas (Maina *et al.,* 2006). Maina *et al*. (2006) observed that disease spread has been observed to differ between low altitude and high altitude agroecological regions where outbreaks have been reported. Extended precipitation and mostly cloudy weather in high altitude increase the relative humidity and reduce evaporative demand on plants, which keeps the foliage wet for longer periods.

The characteristics of the 12 bacteria strains/ isolates from cowpea varieties across the cowpea producing regions were determined, all of which had been identified as *Xav*, as shown in (Table 4). Most of them produced typical yellow pigment (Xanthomonadin) with few showing variable pigmentation colour. The typical yellow colour of the pigment was as a result of the presence of a particular carotinoid pigment which is generally indicative of genus *Xanthomonas* and the concentration of the carotinoid vary from strain to strain (Masterson, 2007). This result indicates the existence of morphologicaly, pathologically and geographically distinct *Xav* isolates (Alexander *et al.,* 2004). These results also corroborate the finding of Todar (2010) that many pathogenic bacteria exist in nature as multiple antigenic (meaning that they are variant strains of the same pathogenic species). The washing assay recovers low population of *Xav* attached to the surface of seeds. The presence of *Xav* on the seed surface is mainly through infestation/contamination during harvesting, threshing and processing. Bacteria are carried on seeds by contamination/infestation. In this case the pathogen may be

independent of seeds but accompany them. Walcott (2005) reported that seed contamination or infestation during threshing provide a possible explanation for the production of contaminated seed lots from virtually inspected fields and also may be the reason why seeds collected from the seeds companies were infected/infested with *Xav* in this study. *External contamination of seeds occurs via fruit as a consequence of contact of the seed with fruit tissue showing symptoms, or at harvest and threshing with residues carrying large bacterial populations (Maude, 1996). It is also important to note that saprophytic and antagonistic bacteria associated with bean seeds often interfere with the isolation of Xav on plating media such as YDCA. The number of colonies of Xav recoverable from various seed lots often depends upon the number of saprophytic and antagonistic bacteria present on the seeds, and this may partly account for the differential incidence and population of Xav in various seed lots. Up to 50 % fewer colonies of Xanthomonas campestris were recovered when antagonistic bacteria were present in the seed washing (Randhawa and Schaud, 1984). Wettability and hydrophobicity also plays a key role in bacterial adhesion (Morais et al., 2008). Hadas et al. (2001) paralleled this by reporting that population of Erwinia carotovora sub sp carotovora decline rapidly within six months in storage at 23 ^{o}C and after 8 months at 6 ^{o}C.* Direct planting of infested seed results in transmission to seedling under ideal environmental condition (Walcotte, 2005). But Murty and Davadath (1984) reported that infested seeds did not give rise to infected seedlings but did introduce the pathogens into the soil. Kauffman and Reddy (1975) reported that viable bacteria could not be detected on seeds stored for two (2) months. This was corroborated by Gottwald *et al*. (2002) who reported that exposed bacterium on contaminated surfaces perishes when the surface dry

out. This may account for the low *Xav* population on seed lots collected from Borno, Gombe, Adamawa, Sokoto, Kaduna, Kebbi and seed lots collected from seed companies. But bacteria in biofilm are resistant to desiccation and washing (Fett and Cooke, 2003) and could also be the reason for differences in *Xav* incidence and population among the states. Bacterial pathogens that stick to seeds during harvesting or post harvest do so through biofilm formation. This could be by firmbrial (flagella, pilli) and non fimbrial (production of extracellular polymeric substance) (Wassenaar, 2009).

The high percentage of *Xav* on the hilum indicated the suitability of the site for the pathogen. Contamination outside the seed's testa can produce healthy seedling, but the inoculums infects the soil and from there it can cause infections at more developed stages of the plant (Nome *et al.,* 2011). This is because hilum is a natural opening, through which the pathogen can hide (Nome *et al*. 2011). Hilum is also an intermediate between the internal pants (Microphyle) and the external parts (funiculus). The pathogen that could not enter the microphyl may remain in the hilum and feeds on the nutrient provided by the funiculus from the pod placenta (Cafati and Saettler, 1980). The invasion of hilum by pathogenic bacteria has been reported and constituted the main area of seed for the bacteria habitation (Darsonval *et al.,* 2009). Low population of *Xav* was detected on the seed embryo. The blight organisms may also enter the hilum via the vascular tissue of the pod suture (Cafati and Saettler, 1980). Embrayo can be infected only in the early stage of seed development after which it remain closed (inaccessible) until harvest (Wang and Maule, 1992). There is only a limited 'window' in the seeds developmental process during which bacteria can enter. Such a 'window' might easily differ with the

physiological status of the host plant and could account in part for the commonly observed variation in incidence and population of *Xav* on the seeds samples (Wang and Maule, 1992). When the seed germinate, if the embryo is infected, the pathogen initiates its growth together with the plant. Symptoms can show up during different stages of development (Nome *et al.,* 2011). The testa which is the seed cover consists of stony, leathery membranous layer. The nature of the surface influence the attachment of the pathogen to the surface while rough and fleshy surface attract adhesion, the stony and leathery surface was not suitable for attachment (Morais *et al*., 2008). Testas are formed by one or more layers of cells originated from the ovule integuments and sometimes from the pericarp made from the walls of the ovary (Wang and Maule, 1992). The pathogen was not detected in the seed cotyledons (Table 3). Phytopathogenic bacteria can only enter the cotyledons through breakage of the testa, establishing itself in the endopleura or the endosperm. This is because bacteria lack the capacity to penetrate the testa directly unlike the fungal pathogens which can penetrate through the testa as a result of the germination of the Teliospores or mycelium (Nome *et al*., 2011), and this may account for the non detection of *Xav* on the cotyledons.

The percentage germination of control treatments was lower (60 %) than the other treatments. This was as a result of soaking control seeds in ethanol for 20 mins. Peter *et al*. (1999) reported that ethanol was very effective in killing naturally occurring microorganisms in/on seeds but it inhibites seed germination. The low seedling establishment ranging from 43 %-64 % in the naturally and artificially infected seeds shows the compatible reaction of *Xav* on all the seedlings. The variable effect of *Xav* on

the seedling establishment may be partly due to variation in pathogenicity and the location of the pathogen on the seeds (Shoaga, 1996). The internally located pathogen (especially on the embryo can cause seedling mortality while the pathogen located on the surface of the seed will contaminate the soil (Murty and Devadath, 1984). Similarly the variability in disease incidence, severity and population of *Xav in vivo* (Table 5) could be attributed to the variability in pathogenicity and the differential susceptibility of the different varieties. Varietal susceptibility or other wise of common bean to bacterial blight have been reported (Singh and Monoz, 1999), while Shoaga (1996) reported the variability in pathogenicity of different isolates of *Xav* and Okechukwu *et al*. (2010) reported the variability in pathogenicity of different isolates of *Xav* due to different agro ecological differences.

There was high CoBB incidence on root and leaf of all the treatments inrespective of the variety. This shows the nutritional differences in the various parts of the seedling. This result is in agreement with Green *et al*. (2007) who reported that rhizospher support high growth of pathogens. Also Yaryura *et al*. (2008) paralleled this observation by reporting that extract produced when bacteria and roots are simultaneously present, allow colonization to occur and that bacterial growth are facilitated. The high population of pathogen usually associated with leaves (Phyllosophere colonization) could be a survival strategy (Darsonval *et al*., 2009). The genus Xanthamonas is particularly well adapted to epiphytic survival in the phylosphere because it is able to aggregate in biofilm that protect it against environmental stresses (Jacques *et al*., 2005). Symptoms appear when environmental conditions are favourable (warm temperature and high relative humidity)

and when its population sizes are sufficient (10^6 cfu/g fresh weight). Colonization of bean by *Xav* in the absence of symptoms depends on a functional T3SS (type III secretion system). Functional T3SS is essential for inplanta colonization but not the epiphytic survivl. The mutant bacteria do not colonise endophysically the host plant and do not contaminate seeds by vascular pathway. However, they are still able to transmit to seeds by the floral route (Daresse *et al*., 2007; Dansoval *et al*., 2009). The low incidence of CoBB on cotyledons and stem observed could be as a result of the differential location of pathogen on the seeds at the time of germination (Buyer *et al*., 1999). Bacteria carried on the hilum can move to any parts of the developing seedling and cause disease symptoms during favourable condition (Dath and Devadath, 1983), while bacteria carried on the embryo could lead to seedling mortality (Nome *et al*., 2011). There was general reflection of susceptibility of SAMPEA-7 to *Xav* as observed in the various parts of all the seedlings (Root, cotyledon, stem and leaf). Also the fungicides used in the seed treatment did not have significant effect on the pathogen attached to the seeds.

These results confirm the report of McMullen and Lamey (2000), Shenge (2007) that most fungicides do not control bacterial pathogens and most will not control all types of fungal diseases. Most seed treatment products are either fungicides or insecticides were usually applied to seed before planting (McMullen and Lamey, 2000). There was slight increase in population of *Xav* after inoculation by artificial inoculated treatments. Transfer of bacteria from one medium to another, where there exist chemical differences between the two media; typically results in slow cell division. Time will be taken by the pathogen for physiological adaptation to the new environment, prior to their resumption

of cell division (this explain why there was slight increase in population after 14 DAS for the artificially inoculated treatment. The inherited attached bacteria cells are better adapted to the seeds than the inoculated cells. This result parallel the report by Feil *et al*. (2007) that bacteria in biofilm was more fit to cause disease than the planktonic cell (free moving cells). These account for statistical difference between all the treatments in term of seed germination and establishment. The statistical difference in disease incidence and disease severeity was as a result of the pathogenecity variation among the isolates (Okechukwu *et al.,* 2010). The statistical difference in the disease incidence (Table 6) on the different seedling parts would be as a result of differential distribution of nutrients in plant parts, host susceptibility/resistant and variability in virulence among the isolates (Upper *et al.,* 2003; Feil *et al.,* 2007; Okechukwu *et al*., 2010).

The relatively low *Xav* population on the surface (phyllosophere population) might be due to time taken to adapt to the new environment and also outside the host (Gottwald *et al*. 2002). There was general dramatic increase in population of *Xav* both in/on the surface of the plant from 42-56 DAI. This was as a result of the pathogen getting full adaption to the new environment (Upper *et al*., 2003; Feil *et al*., 2007) however; the phyllosphere population was higher than the inplanta population. Canopy provides condusive environment for the pathogen to grow and form biofilms. This result is in agreement with the report of Ramey *et al*. (2004) that aerial plant surface support large population of bacterial epiphytics, including plant pathogen that multiply on the leaf surface before initiating disease. The leaf surface is partitioned into prefered microhabitats along veins, near trichomes and stomata (Beattie and Lindow, 1999;

Monier and Lindow, 2004). Spread of the pathogen often occur independent of lesion occurrence (Upper *et al*., 2003). Lesions on the other hand, occur when extensive multiplications require the bacterial to rely on expression of T3SS (Dersonval *et al*., 2003). Foliage and stems of bean are known to habor relatively high population of plant pathogenic bacteria without exhibiting descernable symptoms (Mabagala, 1997; Barak *et al.,* 2002). The differences in population of *Xav* observed indicated the different level of virulence factor within the pathogens and differences in varietal susceptibility to the pathogens (Okechukwu *et al*., 2010). The initial high population of *Xav* in plants from ground assay (Fig. IV) shows the high nutrient status of young succulent plants. The gradual decrease in population of *Xav* in the ground assay was as a result of plant growing more cellulose and lignifications of tissues, thereby creating challenge for pathogen growth and development (Ramey *et al.,* 2004). Fig. V shows the result of disease incidence and severity. The disease severity was generally higher in all the treatments 14 DAI. This was as a result of population pressure on the plant resulting from the cumulative inoculation on the plant after 14 days after sowing. The cumulative population of *Xav* resulting from the inoculation and the inherent population account for the high incidence and severity observed.

This result corroborate the report of Rouse *et al*. (1985) that as population sizes increase, so does the probability of disease development. While the disease incidence remain fairly constant, disease severity steadily declined at 28 and 42 DAI. This result paralleled the report of Upper *et al*. (2003) who reported that bacterial growth becomes limited as the population intensity increases *in vivo*. Why no overt symptom development at this stage

may be due to bacteria through quorum sensing may avoid provoking damage or induce minimal or no inflammation required for eliciting defence response of the host (Todar, 2010). Many phytopathogenic bacteria multiply quite successfully in association with susceptible tissues without causing lesion (Upper *et al.,* 2003). Table 7 shows the result of severity of blight on the leaf. The percentage defoliation was higher in the susceptible variety (SAMPEA-7) than other varieties. There was a general steady increase in the percentage defoliation from 21-91 DAS in all the varieties. This was as a result of corresponding increase in the population of *Xav in vivo*. This defoliation was usually accompanied with symptoms at the base of the petiole or extensive blight on the leaves stalk plate (8). Defoliation was mainly due to large population of *Xav* colonizing the xylem vessels (Gartemann *et al*., 2003). Bacterial pathogen can reduce phosphorylation by causing a loss of chloroplast structure and function (Kosuge and Kimpel, 1982). Plant pathogenic bacteria produce Extracellular Polymeric Substances (EPS) that cause water soaking of intercellular spaces of leaves. Water soaking is a result of altered plant membrane functions, which will also cause a loss of compartmentation and possibly a disruption of chloroplast function (Kosuge and Kimpel, 1982). Also, the pathogens secrete effectors into the apoplast and/ or cytoplasm of the host plants to suppress host defense. These effectors may include the T3SS or enzyme whose products act as elicitor (Hogenhout *et al*., 2009). Disruption of membrane permeability is an important cause of defoliation (Chlaupowicz *et al*., 2010) and this might possibly account for the high percentage defoliation of all the treatments inrespective of variety. The pathogens that infect green aerial tissues (photosynthetic tissues) are more likely to affect plants productivity. The pathogen, once established in host tissue, redirect the host nutrients for

their own use. In most diseases the water flow through the xylem is reduced to a mere 2-4 % of that flowing through stems of healthy plants (Goodwin, 1992; Chaube and Pundhir, 2005).

There was increase in population of *Xav* from the root having the lowest population to flower and flower cusion having the highest population. This was as a result of the available nutrient in plant moveing towards the reproductive parts such as peduncle, flower cusion and flower. The pathogens also move in that direction. The low population of *Xav* may be due to poor nature of root extract as the plant matures (Yaryura *et al*., 2008; Green *et al*., 2007). The low population of *Xav* in the mature stem could be due to morphological and biochemical factors that regulate the pathogen population in plant at this stage. The morphological factor is as a result of maturing stem producing more waterproof substance called suberin. The suberin is deposited in the cell walls in the form of b and s called suberia strips (Taylor, 1997). This will control and regulate the movement of solutes and pathogens through to the xylem vessels, such control is necessary as a protective measure against the entry of toxic substances, and harmful disease causing bacteria and fungi (Taylor, 1997). Plants are known to produce varieties of biochemical substance that acts as defense against the invading pathogen. The production of salicylic substance acts as defense that are active against pathogens with biotrophic lifestyle, while the production of Jasmonate and ethylene substances, acts as defense that are active against the necrotrophic pathogens like bacteria (Koornneet and Piesterse, 2008), also peptide are known to be generated by Pat-1 from a specific plant protein. These signaling peptides are known to play major roles in animal and plants

defense system (Chalupowicz *et al*., 2010). The high population of *Xav* in leaf, petiole, Peduncle, flower cusion and flower could be due to high nutrients available in these parts of the plant at maturity. This result was in line with Goodwin (1992) who reported that photosynthetic rate in cowpea increase at flowering to a maximum rate at pod set, and the amount of this increase is positively correlated with yield. The high population of *Xav* in both the flower cusion and the flower is consistent with the fact that flowers are considered excellent microbial habitats, being well supplied with nutrients (Ngugi and Schem 2006) and offering protecting habitats against abiotic stresses (Dawsonval *et al*., 2009). Furthermore, flowers provide short lived but non hostile environment to microbes (Stockwell, 2005). Most importantly flowers do not induce defense responses (Ngugi and Scherm, 2006) and this account for the high population of *Xav* isolated in this environment. Table 8 shows the result of CoBB severity on the floral parts. There was general increase in flower abortion from 70-91 DAS and there was no apparent varietal difference in CoBB effect on flower. Similar results were also observed in pod abortion. Most yield losses, however, presumably result from high flower and pod abortion (Goodwin, 1992). This heavy flower and pod abortion could be due to the high population of *Xav* in the floral parts (Fig. VI) and flower does not induce defense response making the pathogen population often grow explosively (Ngugi and Scherm, 2006). Disruption of membrane permeability is an important cause of flower and pod abortion (Van Alfen, 1989). Similarly, there was varietal difference in the severity of blight on pods. Some losses may be attributed to pod lesions which can expand to the seed if infection occurs during pod development and can result in rotting and shriveling of the seed plate 14 and 15 (Yoshii, 1980; Goodwin, 1992).

Table 9 shows the result of population of *Xav* in aborted flowers/pods. There was high population of *Xav* in flowers and pod even on the normally shed flowers. The inffected shed flowers can lead to contaminated pod which may lead to pod infection plate 11 and 12 (Goodwin, 1992). This high population of *Xav* in the aborted flowers and pod could lead to serious soil contamination (Murty and Devadath, 1984). The result of this work is completely at variance with the report of Leith and Reynolds (1988) that the effect of a phytophagous pest on plant growth was considered to be equivalent to the amount of defoliation it caused. However, this will not be appropriate for common bacterial blight of bean, because photosynthesis is reduced by more than merely a loss of radiation interception (RI) due to blight lesions (visibly destroying green leaf tissue) (Goodwin, 1982). To reflect accurately the effect of common bacterial blight on plant growth the disease severity alone should not be used to rate the effect of CoBB but population build up in-vivo and leaf lesion should be incorporated into plant disease assessment since population growth does not correlate to symptom development.

Bacterial adhesion is the initial step in colonization and biofilm formation and it begins with the attachment of free floating bacteria to a surface through weak, reversible vander waal forces (Horia and Matsumotob, 2010). The 100 % of infection on cowpea seeds at 2 h after inoculation could be partly due to high inbibition of cowpea seeds that draw the bacteria to the seeds and tissue tropism (Mah amd O. Toole, 2001; Todar, 2010). Though bacteria attachment to surfaces appear to be nonspecific (Darsunval *et al*., 2009), particular bacteria pathogens are known to have an apparent preference for certain tissues over other. For example *Salmonella mutan* is abundant in dental plaque but does not

occur on epithelial surfaces of the tongue; the reverse is true for *Salmonella salivariu*s which is attached in high numbers to epithelial cells of the tongue but is absent in dental plague (Todar, 2010). The nonspecific adherence involves nonspecific attractive forces which allow approach of the bacterium to the eukaryotic cell surfaces. Possible interactions are (a) hydrophobic interactions (b) electrostatic attractions (c) Brownian movement and (d) recruitment and trapping by biofilm polymers interacting from fluctuating dipoles of similar frequencies (Todar, 2010). When the bacteria are not properly adhered or docked to the surface, it can easily slough, this explain why some seeds have little to no *Xav* at 2 h inoculation (Ramey *et al*., 2004; Todar, 2010). The non-reversible (dock) attachment of bacteria cells to surfaces depend on surface properties of plant tissue, time, nutrient and water availability (Ramey *et al*., 2004). The nature of the surface influence the attachment of the pathogen to the surface while rough and fleshy surface attract adhesion, the stony and leathery surface was not suitable for attachment (Morais *et al*., 2008). The surface properties of seeds used in this work vary considerably. After 4 h of inoculation, all the seeds had *Xav* attached to it.

Individual bacterium coalesces by linking extracellar polysaccharides on their cell walls. Polysaccharide chains exhibit chemical properties that make them polar and thus very "sticky". This polarity is what leads to surface adhesions and cell cohesion and is one of the properties that make biofilms tough to remove (Ramey *et al.,* 2004). This result is in agreement with the report of Huber *et al*. (2003) that many bacteria utilize sophiscated regulatory system to ensure that some functions are only expressed when a particular

population density has been reached. The term "quorum sensing" has been coined to describe this form of density dependant gene.

Fig. VIII shows the result of biofilm formation induced by seed extracts. Ife brown extract + 0.5 g glucose induced the highest biofilm formation followed by millet extract, followed by sorghum extract, and maize extract. Biofilm formation induced by nutrient agar and two cow pea varieties were not statistically different ($P \leq 0.05$).

The biofilm formation did not significantly increase with time except Ife brown extract + 0.5 g glucose where biofilm formed at 96 h was higher than that formed 72 h but did not increase at 120 h. Non reversible attachment and biofilm formation are quorum sensing regulated (Huber *et al*., 2003). Adhesion is a widely conserved virulence mechanism among gram-negative bacteria, no matter whether they are human, animal or plant pathogens, since attachment to the host tissue is one of the key early steps of the bacterial infections process (Mhedbi-Hajri *et al*., 2011).

In Fig. IX the populations of *Xav* consistently increase with time. This shows that the bacteria population increases as the nutrient availability increases. These results indicate that bacterial colonization of seedlings relies mostly on surface attachment and population continues to grow until carrying capacity is reached (Darrasse *et al*., 2010). Under this raised nutrient status of the germinating seedlings, bacteria multiplication did not require a functional T3SS, and indeed, T3SS genes are not induced in nutrient rich medium (Valls *et al.*, 2006). The induction of type III secretion system (T3SS) by the

bacteria that leads to symptom development might be due to competion for nutrients as plant grows, nutrient availability decreases (Yaryura *et al*., 2008).

The non - presence of *Xav* on seed coat 5, 7 and 8 DAI could be as a result of bacteria moving along with nutrient gradients (Taormina *et al*., 1999). As in many pathogenic bacteria the production of virulence factors is quorum-sensing regulated, it has been suggested that this form of gene regulation allows the bacteria to remain invisible to the defence systems of the host until the population is sufficiently large to successfully establish the infection (Huber *et al*., 2003). The high population of *Xav* (10^7) observed in radicle and side roots support the report of Bais *et al*. (2006) that plant root axis excrete an enormous variety of chemotoxis inducing compounds to the rhizosphere, which encompass some of the most complex chemical, physical, and biologic interactions involving the roots of the plants and microorganisms present therein (Hiroyuki *et al*., 1998; Barak *et al*., 2005). The low population of *Xav* (10^6) in hypocotyle and (10^3) in the emerging leaves could be due to concentration of the pathogen moving in the direction of shoot systems at the germinating time (Barak *et al*., 2002; Danhorn and Fuqua, 2007). *Escherichia Coli* and *Salmonella enterica* on the seed coats grow epiphytically and firmly adhere to alfalfa sprouts (felt and Cooke, 2005; Singh *et al*., 2005). Bacteria adhere to the hilum could move in either direction (shoot ward or root -ward) (Dalt and Devadah, 1983), but most often, bacteria colonize the developing rhizoshpere via chemotaxis and motility (Tumbull *et al*., 2001). Bacteria on the embryo will mostly colonize the shoot axis of the developing seedling (Fraedrich, 1996; Maude, 1996).

The higher percentage of non-infection in the vegetative stage of inoculation was due to the inability of the bacteria to move into the systematic parts of the plant and spread systemically in the vascular system (Upper *et al*., 2003). This was due mainly to the fact that movement of bacteria into internal parts of the plant were influenced by many factors such as wetness of the leaves caused by rain, duration of wetness, and injury caused by insects or other animals (Chaube and Pundhir, 2005). In the screen house however, these conditions were not readily available. The higher percentage of pod infection in seed inoculation was due mainly to the fact that bacteria pathogen can invade the vascular tissue of the host and spread systemically in the xylem, reaching the pods and seeds through the funiculus (Goodwin, 1992). The percentage infection 50 % in symptomatic pods compared to 0.20 in symptomless pods (Table 13) was the cause of low seedling establishment (35.50 and 37.50 %) and high population of *Xav* observed. High pod infection can leads to high seed infection (Yoshii *et al*., 1980). Heavily infected seed can lead to high seedling manifesting symptoms (plate 16). Though the percentage infection of *Xav* (20 %)and population (10^3) was low in symptomless pods, the significant reduction of seedling establishment (55.60 %) compared to 100% in the control treatments was an indication that bacteria can cause serious epidemics even at low population level (Opio *et al*., 1999). The general lower rate of transmission and population of *Xav* observed in vegetative inoculation was due to the age of the host plants. These results corroborate the report of Singh and Munoz (1999) that stages of crop maturity at infection affect the spread of the disease.

The population of *Xav* within the floral parts (Table 13) indicated that flower receptacle and corolla had the highest population (10^6). This couled be due to the elevated nutrients in these parts of the plants. The results agree with the report of Ngugi and Schem (2006) that flowers are considered excellent microbial habitants, being well supplied with nutrients. The bacteria were not detected in carpel and ovary because they had not yet colonised the floral parts down to these area being the inner parts of flower. At 48 h after inoculation all the floral parts were infected and the population ranges from (10^6) in flower stalk, carpel and embryo sac to (10^8) in calyx and (10^9) in receptacle and corolla. Similar results were also obtained 72 h after inoculation. There was high percentage of flower aborted at 7 and 14 DAI. This development might be due to the accumulation of the pathogen in the vessels thereby clogging and prevent free flow of water and nutrients to the flower (Yoshii, 1980). Similarly Darsonval *et al.* (2009) reported that the ability of bacteria to form biofilm in planta leads to the congestion of some rare vessels that culminated to leaf shedding, flower and fruit abortion and the general wilting of plants. The high rate of transmission (75 % and 85 %), high population (10^7 and low seedling establishment 29 and 35 %) was due to high seed infection resulting from symptomatic pods (Table 14). This result corroborate the finding of Cafati and Sealtler (1980) who reported that in symptomatic pods, the blight organisms may enter the pod cavity via the stomata of the pod or by breaking through the vascular tissues of the pod suture; the bacteria then pass into the funiculus and the raphe or the microphyle leading into the seed. Plate 7, 12 and 13 shows the canker symptoms on stems and pods. Canker develop when bacteria pathogen invade the xylem vessels, impairing water transport and lead to tissues death (Xu *et al*., 2010). Though very low (10^3) population of bacteria titre was

obtained in the symptomless pods, indicating that bacteria transmission did not entirely depends on the pod symptoms. This result agree with the earlier work of Weller (1978) who reported the production of symptompless navy bean seed containing low population levels of blight bacteria in symptomless pods. Also corroborated the result was the report of Mabagala (1997) that CoBB has the ability to enter pods through vascular system and infect the seed without causing lesions on the surface of the pods.

The low germination percentage 58 and 55 % compared to the control 100 % low seedling establishment 46 and 35 % compared to the control 100 % and high rate of transmission 65 % and 55 % in symptomless pod indicated that bacteria pathogenicity on seeds had little to do with population but more to do with virulence strain of the bacteria, and the location of bacteria in/on the seeds (Adipala *et al.,* 1995; Okechukwu *et al*., 2010). The significant difference ($P \leq 0.05$) between the flat pod inculation and mature pod inoculation indicated the susceptibility of the pod area to *Xav* at different stages of development (Upper *et al.,* 2003).

The absence of *Xav* in the testa and cotyledons was due mainly to the fact that bacteria pathogen lack the ability to directly penetrate through the testa and the cotyledons unlike the fungal pathogens (Nome *et al*., 2011). However, the abscence of bacteria in testa and embryo might suggest that there is only a limited 'window' in the developmental process during which bacteria can enter. Such a 'window' might easily differ with the physiological status of the host plant and could account in part for the observed differences in these results (Wang and Maule, 1992; Maude, 1996). The high population

of *Xav* in dorsal suture and funicle was due to these sites acts as an umbilical cord to the seeds making the place rich in nutrients. This finding suggests that seed infection was primarily a sequel of pod colonization.

The lower population of *Xav* observed in mature pod inoculation might be due to the pod being lignified at this stage (Nome *et al*., 2011; Sigee, 1993). This was in line with the report of Singh and Munoz (1999) who reported that severity of yield loss varies according to levels of infection, environment and stage of crop growth and maturity at infection. There was no varietal resistance observed in this result. Agour *et al*. (1989) reported that cowpea genotypes may show resistance in leaves, but susceptible in pods, or vice versa. Bacteria tend to move and spread faster through vascular of succulent tissues of susceptible hosts (Singh and Munoz, 1999). These results corroborate the report of Goodwin (1992) that infection of pod at early state of pod development can result in pod abortion, rotting, shattering and shriveling of the seed (plate 14 and 15). Pod abortion will be the end product of bacterial pathogen in vascular system of pod because the bacteria will block the movement of water and nutrients to and within the pods by depositing extracellular polysaccharide (EPS), secretion of toxin and the formation of biofilm in the vessels (Sigee, 1993; Darsunral *et al.,* 2009). The gradual reduction in pod abortion 14 DAI and general absence of pod abortion in mature pod inoculation may be due to corresponding liginfication of pod tissues which hinder the free movement of the pathogen (Sigee, 1993; Nome *et al*.,2011).

Flat pod inoculation leads high seed infection while there was no infection in mature pod inoculation. Severity of pod infection varies with stage of pod maturity at infection

(Singh and Munoz, 1999). The high population of *Xav* (10^4) in symptomatic pod might be due to biofilm formation on the pods by *Xav* pathogen (Lewis, 2001). This result parallel the report of Barak *et al*. (2002) that population of bacteria recover from the surface of the plant tissue was higher than the population recoverd from the inside of the plant tissue. Aggregation followed by biofilm formation is a strategy used by *Xav* during colonization of bean plylosphere for its protection from stresses and maintenance of inoculation reservoirs (Jacques *et al*., 2005). If *Xav* is present, the epidemic of bacterial blight disease should be driven almost completely by the suitability of conditions for multiplication of the pathogen in association with leaves or pods in the field (Upper *et al.,* 2003). The bacteria penetrate the pods via pods stomata or wounds and remain in the apoplast (intercellular spaces) of infected tissues or remain deposited on the pods. The epiphytic populations serve as reservoirs and contribute to either secondary or primary infection (Walcott, 2005).

The higher transmission rate and *Xav* population in flat pod inoculation may be due to the succulent nature of pod tissue at that stage and also the stomata openings are large enough for active penetration and passive entrance of bacterial cells (Chuabe and Pundhir, 2005). Conversely, the low rate and low population of *Xav* in mature pod might be attributed to mechanical barriers to infection as a result of lignifications. The toughness of epidermis was due to the polymer of cellulose, hemicelluloses, lignin, and polymerized organic compounds (Chuabe and Pundhir, 2005). Bacteria can adhere to the pod surface and multiply epiphytically through bacteria surface constituent such as lipopolysaccharides which are the principal components of the outer membrane of gram-

negative bacteria (Attensach and Robatzek, 2007). This result is in line with the report of Mabagala (1997) that foliage and pod of beans are known to harbor relatively high population of plant pathogenic bacteria. These phyllosphere bacteria can lead to seed contamination and infection. The pathogen itself can stick or get mixed with seeds during any of the processes during seed harvesting, extraction, threshing and packaging (Nome *et al*., 2011)

The non-detection of *Xav* on maize and sorghum (Table 17), from all samples collected across the cowpea producing states indicated that these crops did not transmit *Xav* and therefore could not contribute to introduction of primary inoculums in non-infested field. The non-consistency in detecting *Xav* on millet samples in some states could be due to low incidence of the disease in the field of origin or stress imposed on the pathogen by seed treatment or more importantly may be due to biofilm formation on the seeds by *Xav* (Liao and Shollenger, 20004; Darsonval *et al.,* 2009). It has been reported that following exposure of seed to a variety of physical (heating, drying, freezing) or chemical stresses, bacteria will be killed or injured (Ligo and Shollenbenger, 2004). Seed plating and seed destruction assay consistently recover *Xav* in the millet samples collected across the states. This shows that the pathogen was firmly attached to the seeds, that washing assay could not recover (Darsonval *et al.,* 2009). Felt and Cooke (2003) reported that bacteria in biofilms are notoriously resistant to washing and other antibacterial treatments.

The population of *Xav* was significantly high (10^7) (Table 18) in the roots of both cowpea and the cereal crops. At 7 DAS the population of *Xav* was higher in the root system

($2.4x10^7$-$3.3x10^7$) than the shoot system ($1.0x10^7$-$1.6x10^7$). This result corroborated the report of Gilbertson and Maxwell (1992) that seed borne bacteria especially *Xanthomonads* colonise the surface of seedlings and have no early endophytic development. One observation made previously by Hirano *et al*. (1999) that *Pseudomonas syringe*a pv *syringea* did not behave as a pathogen during bean germination, as its type III secretion system (T3SS) does not seem to play a major role during seed germination and seedling colonization, suggesting that nutrient are not a limiting factors in the spermosphere, indeed, T3SS gene are not induced in nutrient rich medium (Vall *et al.,* 2006; Darrasse *et al*., 2010). At 14 DAS the populations of *Xav* was significantly higher (10^8) in both root and shoot of Ife brown followed by millet $3.2x10^7$ and $2.4x10^7$ but statistically similar to other treatments. The steady decline in the population of *Xav* from 10^7-10^6 in all the cereal crops indicated the corresponding decline in nutrient available for the pathogen since the cereal plants at this stage resume exponential growth. Inside non host plants is hostile to invading pathogen (Chaube and Pundhir, 2005). At 35 and 49 DAS, the population of *Xav* was significant ($P \leq 0.05$) only on Ife brown followed by millet.

The factors that influence the in planta or endophytic population of pathogen had been advanced by many workers. According to Wilson *et al*. (2003), all successful pathogens must firmly attach themselves to the wall of the host, must counter the active and passive defense systems of the plants and must be capable of causing minimal damage to plant cell so as to grow in or maintain population. Chaube and Pundhir (2005) reported that in planta environment is hostile to non pathogenic organisms to survive and multiply. Also

as lignification, suberization and deposition of carbohydrate increases with age, so also, it become increasingly difficult for pathogen to survive and multiply (Taylor *et al*., 1997; Chaube and Pundhir, 2005). However, bacteria pathogens are known to survive and multiply in a volunteer host and this may account for the presence and population of *Xav* in millet host (Walcott, 2005). For pathogen to accomplish systematic transmission, the pathogen must initiate at least minimal infection during the vegetative growth of the host (Wang and Maule, 1992) or through molecular mimicry where the bacteria cells are so closely related chemically to host "self" components that the immunological cell can not distinguish between the two and immune response can not be raised (Todar, 2010).

Bacterial population growth was observed in Ife brown and millet plant at 35 and 49 DAS Table 19 show *Xav* transmission through direct vascular and indirect floral system. The non-detection of *Xav* on seeds of maize and sorghum at harvest indicated that the pathogen fails to systemically transmit. The results corroborate the report of Wang and Muale (1992) that for a pathogen to systemically transmit to seeds, the pathogen must initiate infection during the vegetative growth of the host. Although the transmission of bacteria pathogen by volunteer host had been reported previously Walcott, 2005), there has been no report of *Xav* being transmitted by millet. Also, it has been reported that some human pathogens establish themselves endophytically in plants and may be inherited from generation to generation (Taylor and Triplett, 2008). The diversity of bacteria-host interactions is remarkable. These interactions range from mutualistic symbiotic relationships that benefit both parties to explosive infections in which the pathogen rapidly killed the host (Parsek and Singh, 2003).

Others argued that biofilm formation increases the resistance of bacteria to killing and may make organisms less conspicuous to the immune system, as antigens may be hidden and key ligand repressed (Mah and O' Toole, 2001) making it possible for non host plant to transmit bacterial pathogens. This finding could further be explained by three theories: According to evolutionary theory, optimal virulence of a pathogen increased with horizontal transmission (between non relative hosts) and may decrease with vertical transmission (within the same host).

Trade-off hypotheses: At one time, some biologist argued that pathogen would tend to evolve towards ever decreasing virulence because the death of host (or even serious disability) is ultimately harmful to the pathogen living inside for example, if the host dies, the pathogen population inside may die out entirely.

The coincidental evolution hypothesis: some forms of pathogenic virulence or pathogenesis did not co-evolve with the host for example; tetanus is caused by soil bacterium *Clostridium tetani*. After *C. tetani* enter a human wound, the bacteria may grow and divide rapidly, even though human body is not their normal habitat (Bruce and Carl, 2000; Scolt and Jon, 2007; Encyclopedia Britanica, 2009; Horia and Matsumotob, 2010).

Xanthomonas axonopodis pv. *vignicola* was detected on both seeds of sorghum and millet and was not detected on maize after harvest. Why *Xav* was not detected on maize seeds may be due to fact that maize seed are beard in the husk which was to penetrate by the pathogen and the pathogen could not transmit to the seed with pollen grains. Seed

infection is sequel to fruit, pod and husk infection (Yoshii *et al*., 1980). The external contact of seed and the pathogen transmission of incompatible bacteria to seedlings from contaminated seeds suggest a probable role of seeds of non host plant as an inoculums source for seed-borne pathogenic bacteria. Associations developing on germinating seeds mark the first point of contact between plants, pathogens, and micro organisms, with beneficial or harmful results for plant health, growth and development. These results indicate that bacteria colonization of seedlings relies mostly on surface colonization and not on parasitic or pathogenic relationships (Darrasse *et al*., 2010).

The attachment of bacteria to seeds of incompatible hosts suggests a probable role of seeds of nonhost as an inoculums source for seed-borne pathogenic bacteria. Interaction occurring in the developing seeds and bacteria allowing bacteria to multiply and having no negative impact on seeds defined a commensal interaction (Darrasse *et al*., 2010). Attachment to seeds represents a key step for the introduction of bacteria into the rhizosphere and would represent an advantage for seed-borne pathogens (Danhorn and Fuqua, 2007).

A plant pathogenic bacterium can efficiently transmit from seeds to non host seedlings as a consequence of saprophytic multiplication, resulting in the establishment of a primary inocula focus. It was previously demonstrated that plant pathogenic bacteria can survive in the phyllosphere of non host (Sikirou and Wydra, 2004; Darsonval, *et al*., 2008). These results are coherent with the idea that *Xanthomonads* host a large repertoire of bacteria surface structures necessary to exploit the various environments they have to face in their

lives (Darsonval *et al*., 2009). Consequently, if seeds become contaminated with a pathogen, the sprouting or germinating process provides excellent condition for its growth and distribution to primary inoculums focus. This seed contamination or infestation is refers to as passive relationship of a pathogen and seeds (Nome *et al*., 2011). The pathogen can stick or adhere to the seeds. Adhesion to host tissues is essential for successful infection by many bacteria pathogens. The bacterial surface structures important for adhesion cover a broad group of fimbrial and non timbrial structures commonly known as adhesions (Latasa *et al*., 2006). This unspecific attachment of pathogens to seeds and seedlings make it possible for some human pathogens (*salmonella* and *Escherichia coli*) to be transmitted by alfafa and Mung bean sprouts (Taormina *et al*., 1999). It is now understood that *Xav* can be carried on nonhost seeds (millet and sorghum) but how this inoculum focus can leads to field infection remain to be investigated. However, it must be noted that Singh and Ajeigbe (2002) reported the reduction of shoot, as well as root growth of cowpea growing in crop mixtures resulting in very low grain and fodder yields. A quantitative assessment of the traditional cropping systems revealed the existence of up to 43 crop mixtures in the Sudan savanna of Nigeria with a millet-cowpea mixture being predominant, representing 22 % of the fields (Henriet *et al*., 1997).

CHAPTER 6

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

Seed borne inoculum is important in the survival and dissemination of plant pathogenic bacteria. Most seed born bacteria survive as long as the seed remains viable. The study demonstrated that cowpea seeds used by farmers in cowpea producing areas in Nigeria were heavily infected by *Xav* both locally produced seeds and the seeds produced by the seed companies. There was low *Xav* incidence from seeds collected from Borno, Adamawa, Kebbi, Gombe and Sokoto. The highest *Xav* population was isolated from the hilum while no *Xav* was detected on the cotyledons. Putting together, the result of this work shows the external parts of seeds were the most infected by *Xav*. Both externally and internally infected seed are important source of primary inoculum of *Xav*. All the fungicides used for the treatment of seeds by the seed companies did not have significant effect on the pathogen both *in-vitro* growth and pathogenicity. This study indicates the existence of morphologicaly, pathogenetically and geographically distinct *Xav* isolates.

The result also demonstrate the importance of identifying the type or types of cowpea bacteria blight prevalent in a region in order to select appropriate strains for breeding for resistance. In order to study the correlation between population build up *in vivo* and symptom manifestation, seeds collected from different sources reflecting the different source farmers obtained their seeds were used. All the seed lots were treated with fungicides that were used to treat seeds produced by the seed companies.

There was no significance difference in pathogenicity between the high population (10^7) in artificially inoculated seeds and low population (10^3) in natural infected seeds. This was because bacteria adhere to seeds often form biofilms on seeds. Bacteria in biofilm or in association with seeds were better adapted to the environment than the artificially inoculated bacteria. Population build up did not correlate to folial symptoms development, indicating the latent debilitating effect on the plant by the pathogen. Although there were varietal differences in terms of disease severity, there was no varietal difference in terms of population build up *in vivo* and symptoms manifestation and this was due mainly to the toxin produced by pathogen binding to sensitive sites in the host plant. Only the plants that have such sensitive sites become diseased or developed symptoms. The symptoms of *Xav* did not only manifest as leave lesions but also cause leaves defoliation, aborted flowers and aborted pods. Measurement of disease severity base on leave lesions alone could be misleading as most of the defoliated leaves did not have extensive lesions, and most importantly flower and pod aborted were not showing obvious symptoms.

Adhesion to host tissue is essential for successful infection by many microbial pathogens. The study of adhesion of bacterial to seed surfaces demonstrated that the different seeds types influences adhesion by the phytopathogenic bacteria. While it took 2 h for all seeds of cowpea to be attached by *Xav*, it took some seeds like maize and millet 4 h to have about 40-60 % adhesion by the *Xav*. All the seeds extract induce biofilm formation and the level of biofilm formation varies with time and the nutrient status of the media or medium. While NGA and cowpea extracts favour bacterial growth, other extract maize,

millet and sorghum favor biofilm formation. Colonization pattern from seed to seedling of *Xav* reveals that at germination, seedlings were colonized by *Xav* at the root sides than the shoot sides.

The study of route of seed infection showed that seed inoculation, vegetative inoculation, flower inoculation, flat pod inoculation and mature pod inoculation gave rise to infected pods and seeds, indicating the capability of *Xav* to move from plant surface to the internal tissue and systemically transmit to seeds. Flower and flat pod inoculation gave rise to heavy flower and pod abortion. Severely infected pods produce high infected seeds but pod lesions is not an index to measure the level of seed infection since symptomless pods also gave rise to infected seeds. Seeds are the passive carriers of a diversified microbial cohort in commensal or parasitic relationship. The study of role of non-host cereal crops in the transmission of *Xav* showed that *Xav* was systemically transmitted from inoculated millet seeds to harvested seeds, although *Xav* population increases at the seedling stage of all the cereal crops but fail to transmit to all harvested seeds. Inoculation of cereal crops at flowering stage lead to infected seeds in millet and sorghum except maize.

6.2 Conclusion

The result of this work shows the external parts of seeds (hilum and seed coat) were the most infected/infested by *Xav*. Fungicides used for the treatment of seeds has no significant effect on the pathogen both *in-vitro* or *in vivo*. Population build up did not correlate to foliar symptom development. The study demonstrated the adhesion capacity of *Xav* to seed and formation of biofilm by *Xav* was demonstrated for the first time. The

study showed the capability of *Xav* to systemically or epiphytically transmit to seeds and the transmission of *Xav* by non-host cereal crops was reported for the first time. It was demonstrated that seed destruction assay was better in recovering pathogen from infected seeds.

6.3 Recommendations

From this work it is recommended that:

i) Antibiotics should be included in seed treatments either in isolation or in combination with other fungicides, bearing in mind the problem of biofilm formation.

ii) Seed assay should be performed on every seed lot since visibly symptomless pod/seeds is not a guarantee to getting bacterial-free seeds.

iii) Pathogen population assessment be undertaken in conjunction with disease incidence not disease severity.

iv) More studies on transmission of diseases from non-host cereal crops to cowpea plants should be conducted since cereal and cowpea crops are usually grown in crop mixtures in Nigeria.

CHAPTER SEVEN

7.0 REFERENCES

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APPENDIX

Appendix 1. ANOVA Tables for incidence and population of *Xav* on seed samples.

A.1.1. ANOVA For incidence

Source	Df	SS	MS	F	Pr > F
Treatment	11	16.080	1.462	458.55	< 0.0001
Error	12	0.40	0.00		
Total	23	16.85			

A.1.2. ANOVA for population

Source	Df	SS	MS	F	Pr > F
Treatment	11	116,14	10.56	infinity	< 0.0001
Error	12	0.16	0.01		
Total	23	116.30			

Appendix 2. ANOVA Tables for relative distribution of *Xav* on seed surface using plating of seed and washing assay.

A.2.1. ANOVA for washing assay

Source	Df	SS	MS	F	Pr > F
Treatment	11	0.18	0.02	1.95	< 0.0001
Error	12	0.10	0.01		
Total	23	0.23			

A.2.2. ANOVA for Hilum up

Source	Df	SS	MS	F	Pr > F
Treatment	11	0.04	0.30	infinity	<0.0001
Error	12	0.00	0.00		
Total	23	0.05			

A.2.3. ANOVA for Hilum down

Source	Df	SS	MS	F	Pr > F
Treatment	11	0.15	1.27	2.38	0.0763
Error	12	0.00	0.00		
Total	23	0.15			

Appendix 3. ANOVA Tables for relative distribution of *Xav* in internal parts of seeds.

A.3.1. ANOVA for Testa

Source	Df	SS	MS	F	Pr > F
Treatment	11	8.53	0.04	0.07	0.999
Error	12	8.00	5.78		
Total	23	8.53			

A.3.2. ANOVA for Hilum

Source	Df	SS	MS	F	Pr > F
Treatment	11	2.47	0.24	0.34	0.9679
Error	12	12.58	0.67		
Total	23	11.23			

A.3.3. ANOVA for Embryo

Source	Df	SS	MS	F	Pr > F
Treatment	11	0.17	0.06	1.95	0.9982
Error	12	0.10	1.04		
Total	23	0.28			

Appendix 4. ANOVA Tables for incidence, severity and population build-up 14 DAS

A.4.1 ANOVA for germination

Source	Df	SS	MS	F	Pr > F
Treatment	13	360.71	27.75	infinity	< 0.0001
Error	14	0.00	3.25		
Total	27	360.71			

A.4.2. ANOVA for establishment

Source	Df	SS	MS	F	Pr > F
Treatment	13	4270	328.53	1.01	0.0001
Error	14	0.00	5.21		
Total	27	4270.85			

A.4.3. ANOVA for incidence

Source	Df	SS	MS	F	Pr > F
Treatment	13	4270.85	915.06	12.66	< 0.0001
Error	14	0.00	0.00		
Total	27	4270.85			

A.4.4. ANOVA for severity

Source	Df	SS	MS	F	Pr > F
Treatment	13	8004.46	615.75	1.18	< 0.0001
Error	14	0.00	5.08		
Total	27	8004.73			

A.4.5. ANOVA for population

Source	Df	SS	MS	F	Pr > F
Treatment	13	49.33	3.79	1062.43	< 0.0001
Total	14	0.00	0.00		
Total	27	49.33			

Appendix 5. ANOVA Tables for incidence 0f CoBB 0n seedling parts 14 DAS

A.5.1. ANOVA for root

Source	Df	SS	MS	F	Pr > F
Treatment	13	31.63	3.43	4.79	< 0.0001
Error	14	0.00	0.00		
Total	27	31.63			

A.5.2. ANOVA for cotyledon

Source	Df	SS	MS	F	Pr > F
Treatment	13	227.48	17.48	infinity	< 0.0001
Error	14	0.00	5.08		
Total	27	227.26			

A.5.3. ANOVA for stem

Source	Df	SS	MS	F	Pr > F
Treatment	13	25.00	1.92	infinity	< 0.0001
Error	14	0.00	0.00		
Total	27	25.00			

A.5.4. ANOVA for leaf

Source	Df	SS	MS	F	Pr > F
Treatment	13	82.44	6.34	infinity	< 0.0001
Error	14	0.00	9.91		
Total	27	83.44			

Appendix 6. ANOVA Tables for severity of blight on the leaf part at 21, 35, 49, 63, 77 and 91 DAS (% Defoliation)

A.6.1 ANOVA for leaf part 21 DAS

Source	Df	SS	MS	F	Pr > F
Treatment	13	499.46	38.42	infinity	< 0.0001
Error	14	0.00	1.02		
Total	27	499.46			

A.6.2 ANOVA for leaf part 35 DAS

Source	Df	SS	MS	F	Pr > F
Treatment	13	19.12	5.11	1.49	< 0.0001
Error	14	0.00	0.00		
Total	27	19.17			

A.6.3 ANOVA for leaf part 49 DAS

Source	Df	SS	MS	F	Pr > F
Treatment	13	390.00	30.00	2.96	< 0.0001
Error	14	0.00	0.00		
Total	27	390.00			

A.6.4 ANOVA for leaf part 63 DAS

Source	Df	SS	MS	F	Pr > F
Treatment	13	447.57	34.43	infinity	< 0.0001
Error	14	0.00	0.00		
Total	27	447.58			

A.6.5 ANOVA for leaf part 77 DAS

Source	Df	SS	MS	F	Pr > F
Treatment	13	499.57	38.43	infinity	< 0.0001
Error	14	0.00	0.00		
Total	27	499.57			

A.6.6 ANOVA for leaf part 91 DAS

Source	Df	SS	MS	F	Pr > F
Treatment	13	2697.26	207.48	1.45	< 0.0001
Error	14	0.00	0.00		
Total	27	2697.26			

Appendix 7. ANOVA Tables for severity of *Xav* on floral parts.

A.7.1 ANOVA for % aborted flower 70 DAS

Source	Df	SS	MS	F	Pr > F
Treatment	13	3864.11	297.23	infinity	< 0.0001
Error	14	0.00	3.05		
Total	27	3864.11			

A.7.2 ANOVA for % aborted flower 77 DAS

Source	Df	SS	MS	F	Pr > F
Treatment	13	1082.50	83.27	infinity	< 0.0001
Error	14	0.00	2.54		
Total	27	1082.50			

A.7.3 ANOVA for % aborted pod 84 DAS

Source	Df	SS	MS	F	Pr > F
Treatment	13	7253.70	518.12	infinity	< 0.0001
Error	14	0.00	0.00		
Total	27	7373.45			

A.7.4 ANOVA for % aborted pod 91DAS

Source	Df	SS	MS	F	Pr > F
Treatment	13	3168.49	243.73	815	< 0.0001
Error	14	0.00	8.92		
Total	27	3168.49			

A.7.5 ANOVA for severity on pod 98 DAS

Source	Df	SS	MS	F	Pr > F
Treatment	13	21.76	167.38	infinity	< 0.0001
Error	14	0.00	0.00		
Total	27	65.32			

Appendix 8. ANOVA Tables for population **of *Xav* in shed, aborted flowers and aborted pods**

A.8.1 ANOVA for cfu/g of shed flower 70 DAS

Source	Df	SS	MS	F	Pr > F
Treatment	13	2176.17	167.40	infinity	< 0.0001
Error	14	0.00	9.91		
Total	27	2176.17			

A.8.2 ANOVA for cfu/g of aborted flower 77 DAS

Source	Df	SS	MS	F	Pr > F
Treatment	13	3490.14	268.47	3.01	< 0.0001
Error	14	0.00	0.00		
Total	27	3490.14			

A.8.3 ANOVA for cfu/g of aborted pod 84 DAS

Source	Df	SS	MS	F	Pr > F
Treatment	13	7.93	0.57	10.55	< 0.0001
Error	14	0.00	0.00		
Total	27	8.73			

Appendix 9. ANOVA Tables for adhesion of *Xav* to seeds.

A.9.1 ANOVA for % infection 2 h after inculation

Source	Df	SS	MS	F	Pr > F
Treatment	13	33.77	2.60	infinity	< 0.0001
Error	4	0.00	0.00		
Total	7	33.77			

A.9.2 ANOVA for population

Source	Df	SS	MS	F	Pr > F
Treatment	3	3.86	0.30	infinity	0.0001
Error	4	0.00	0.00		
Total	7	3.86			

A.9.3 ANOVA for % infection 4 h after inoculation

Source	Df	SS	MS	F	Pr > F
Treatment	3	2.93	0.23	infinity	< 0.0001
Error	4	0.00	1.78		
Total	7	2.93			

A.9.4 ANOVA for population

Source	Df	SS	MS	F	Pr > F
Treatment	3	2.70	0.21	5.21	<0.0001
Error	4	0.130	0.00		
Total	7	2.70			

Appendix 10 ANOVA Tables for colonization pattern of *Xav* on seedling

A.10.1 ANOVA for radicle

Source	Df	SS	MS	F	Pr > F
Treatment	2	0.160	0.08	4.32	<0.0001
Error	3	0.00	0.00		
Total	5	0.160			

A.10.2 ANOVA for hypocotyle

Source	Df	SS	MS	F	Pr > F
Treatment	2	0.280	0.14	infinity	<0.0001
Error	3	0.00	0.00		
Total	5	0.280			

A.10.3 ANOVA for side roots

Source	Df	SS	MS	F	Pr > F
Treatment	2	0.250	0.125	infinity	<0.0001
Error	3	0.00	0.00		
Total	5	0.355			

A.10.4 ANOVA for primary roots

Source	Df	SS	MS	F	Pr > F
Treatment	2	0.253	0.13	infinity	0.0001
Error	3	0.00	0.00		
Total	5	0.253			

A.10.5 ANOVA for emerging leaves

Source	Df	SS	MS	F	Pr > F
Treatment	2	0.273	0.19	infinity	0.0001
Error	3	0.00	0.00		
Total	5	0.273			

Appendix 11. ANOVA Tables for transmission of *Xav* to seed by symptomatic and symptomless pods

A.11.1 ANOVA for germination

Source	Df	SS	MS	F	Pr > F
Treatment	4	0.06	0.05	infinity	<0.0001
Error	5	0.00	0.00		
Total	9	0.06			

A.11.2 ANOVA for seedling establishment

Source	Df	SS	MS	F	Pr > F
Treatment	4	4.62	1.15	infinity	0.0001
Error	5	0.00	9.09		
Total	9	4.62			

A.11.3 ANOVA for % infection

Source	Df	SS	MS	F	Pr > F
Treatment	4	0.34	0.08	infinity	< 0.0001
Error	5	0.00	0.00		
Total	9	0.34			

A.11.4 ANOVA for population

Source	Df	SS	MS	F	Pr > F
Treatment	4	7.57	1.892	126.10	< 0.0001
Error	5	0.08	0.00		
Total	9	7.64			

A.11.5 ANOVA for germination

Source	Df	SS	MS	F	Pr > F
Treatment	4	1660.00	415.00	20.75	0.0026
Error	5	100.00	0.00		
Total	9	1760.00			

A.11.6 ANOVA for seedling establishment

Source	Df	SS	MS	F	Pr > F
Treatment	4	2773.60	693.40	infinity	< 0.0001
Error	5	0.00	0.00		
Total	9	2773.60			

A.11.7 ANOVA for population

Source	Df	SS	MS	F	Pr > F
Treatment	4	4.896	1.224	42.21	0.0005
Error	5	0.145	0.029		
Total	9	5.041			

Appendix12. ANOVA Tables for transmission of *Xav* to seed from symptomless and symptomatic pods following inoculation at flowering stage.

A.12.1 ANOVA for germination

Source	Df	SS	MS	F	Pr > F
Treatment	2	2532.00	1266.00	infinity	<0.0001
Error	3	0.00	7.58		
Total	5	2532.00			

A.12.2 ANOVA for seedling establishment

Source	Df	SS	MS	F	Pr > F
Treatment	2	4762.33	2381.17	3.19	<0.0001
Error	3	0.00	0.00		
Total	5	4887.33			

A.12.3 ANOVA for rate of transmission

Source	Df	SS	MS	F	Pr > F
Treatment	2	0.49	0.25	infinity	0.0028
Error	3	0.00	0.00		
Total	5	0.50			

A.12.4 ANOVA for population

Source	Df	SS	MS	F	Pr > F
Treatment	2	27.52	8.762	infinity	< 0.0001
Error	3	0.00	0.00		
Total	5	17.55			

A.12.5 ANOVA for germination

Source	Df	SS	MS	F	Pr > F
Treatment	2	3545.33	1772.67	infinity	< 0.0001
Error	3	0.00	0.00		
Total	5	3545.33			

A.12.6 ANOVA for seedling establishment

Source	Df	SS	MS	F	Pr > F
Treatment	2	6201.00	3100.00	infinity	< 0.0001
Error	3	0.00	0.00		
Total	5	6201.00			

A.12.7 ANOVA for rate of transmission

Source	Df	SS	MS	F	Pr > F
Treatment	2	0.84	0.042	6316.00	< 0.0001
Error	3	0.00	0.00		
Total	5	0.84			

A.12.8 ANOVA for population

Source	Df	SS	MS	F	Pr > F
Treatment	2	9.37	4.69	5.28	<0.0001
Error	3	0.00	1.89		
Total	5	9.3			

Appendix 13. ANOVA Tables for movement of *Xav* within the pod after 24 h after inoculation

A.13.1 ANOVA for stalk

Source	Df	SS	MS	F	Pr > F
Treatment	4	9.286	2.322	infinity	< 0.0001
Error	5	0.00	7.11		
Total	9	9.336			

A.13.2 ANOVA for fruit coat

Source	Df	SS	MS	F	Pr > F
Treatment	4	10.524	2.631	infinity	< 0.0001
Error	5	0.00	0.00		
Total	9	10.589			
Source	Df	SS	MS	F	Pr > F

A.13.3 ANOVA for dorsal suture

Treatment	4	10.926	2.732	infinity	< 0.0001
Error	5	0.00	0.00		
Total	9	11.001			

Appendix 14. ANOVA Tables for movement of *Xav* within parts of the pod after 48 h of inoculation

A.14.1 ANOVA for stalk

Source	Df	SS	MS	F	Pr > F
Treatment	4	1.930	0.484	infinity	0.0003
Error	5	0.00	0.00		
Total	9	1.981			

A.14.2 ANOVA for fruitcoat

Source	Df	SS	MS	F	Pr > F
Treatment	4	10.856	2.714	infinity	<0.0001
Error	5	0.00	0.00		
Total	9	11.091			

A.14.3 ANOVA for dorsal suture

Source	Df	SS	MS	F	Pr > F
Treatment	4	13.886	3.472	infinity	< 0.0001
Error	5	0.00	0.00		
Total	9	13.901			

A.14.4 ANOVA for funicle

Source	Df	SS	MS	F	Pr > F
Treatment	4	7.324	1.831	infinity	< 0.0001
Error	5	0.00	2.22		
Total	9	7.369			

Appendix 15. ANOVA Tables for movement of *Xav* within parts of the pod after 72 h of inoculation

A.15.1 ANOVA for stalk

Source	Df	SS	MS	F	Pr > F
Treatment	4	7.10	1.77	infinity	<0.0001
Error	5	0.00	0.00		
Total	9	7.1			

A.15.2 ANOVA for fruit coat

Source	Df	SS	MS	F	Pr > F
Treatment	4	2.74	2.79	infinity	< 0.0001
Error	5	0.020	0.004		
Total	9	2.74			

A.15.3 ANOVA for dorsal suture

Source	Df	SS	MS	F	Pr > F
Treatment	4	11.48	4.069	infinity	< 0.0001
Error	5	0.00	0.00		
Total	9	1148			

A.15.4 ANOVA for funicle

Source	Df	SS	MS	F	Pr > F
Treatment	4	16.30	4.08	infinity	< 0.0001
Error	5	0.00	0.00		
Total	9	16.30			

Appendix 16. ANOVA Tables for movement of *Xav* within parts of the pod after 96 h of inoculation

A.16.1 ANOVA for stalk

Source	Df	SS	MS	F	Pr > F
Treatment	4	2.30	0.57	infinity	< 0.0001
Error	5	0.00	0.00		
Total	9	2.32			

A.16.2 ANOVA for fruit coat

Source	Df	SS	MS	F	Pr > F
Treatment	4	11.38	2.84	infinity	< 0.0001
Error	5	0.00	0.00		
Total	9	11.30			

A.16.3 ANOVA for dorsal suture

Source	Df	SS	MS	F	Pr > F
Treatment	4	17.92	4.48	infinity	< 0.0001
Error	5	0.00	0.000		
Total	9	17.96			

A.16.4 ANOVA for funicle

Source	Df	SS	MS	F	Pr > F
Treatment	4	7.58	1.80	infinity	< 0.0001
Error	5	0.00	0.00		
Total	9	7.58			

Appendix 17. ANOVA Tables for transmission rate of *Xav* to seeds following inoculation at flat and matured pod

A.17.1 ANOVA for % infection

Source	Df	SS	MS	F	Pr > F
Treatment	4	1.220	0.31	infinity	<0.0001
Error	5	0.025	0.005		
Total	9	1.245			

A.17.2 ANOVA for population

Source	Df	SS	MS	F	Pr > F
Treatment	4	13.74	3.43	2.42	< 0.0001
Error	5	0.00	0.00		
Total	9	13.74			

Appendix 18. ANOVA Tables for population of *Xav* in-vivo 7, 14 and 21 DAS

A.18.1 ANOVA for root 7 DAS

Source	Df	SS	MS	F	Pr > F
Treatment	5	22.747	4.543	545.92	< 0.0001
Error	6	0.050	0.006		
Total	11	22.797			

A.18.2 ANOVA for shoot 7 DAS

Source	Df	SS	MS	F	Pr > F
Treatment	5	5.057	1.011	101.13	< 0.0001
Error	6	0.060	0.010		
Total	11	5.117			

A.18.3 ANOVA for root 14 DAS

Source	Df	SS	MS	F	Pr > F
Treatment	5	22.317	4.464	255.06	< 0.0001
Error	6	0.105	0.175		
Total	11	22.423			

A.18.4 ANOVA for shoot 14 DAS

Source	Df	SS	MS	F	Pr > F
Treatment	5	12.918	2.584	83.79	< 0.0001
Error	6	0.185	0.0308		
Total	11	13.103			

A.18.5 ANOVA for root 21 DAS

Source	Df	SS	MS	F	Pr > F
Treatment	5	11.587	2.317	99.33	< 0.0001
Error	6	0.140	0.023		
Total	11	11.727			

A.18.6 ANOVA for shoot 21 DAS

Source	Df	SS	MS	F	Pr > F
Treatment	5	16.097	3.219	643.87	< 0.0001
Error	6	0.030	0.005		
Total	11	16.127			

Appendix 19. ANOVA Tables for population of *Xav* in-vivo 35 and 49 DAS

A.19.1 ANOVA for leaves 35 DAS

Source	Df	SS	MS	F	Pr > F
Treatment	5	10.267	2.053	infinity	< 0.0001
Error	6	0.00	0.00		
Total	11	10.267			

A.19.2 ANOVA for stem 35 DAS

Source	Df	SS	MS	F	Pr > F
Treatment	5	3.667	0.733	infinity	< 0.0001
Error	6	0.00	0.00		
Total	11	3.667			

A.19.3 ANOVA for leaves 49 DAS

Source	Df	SS	MS	F	Pr > F
Treatment	5	9.724	1.945	2333.80	< 0.0001
Error	6	0.005	0.0008		
Total	11	9.729			

A.19.4 ANOVA for stem 49 DAS

Source	Df	SS	MS	F	Pr > F
Treatment	5	3.750	0.750	infinity	< 0.0001
Error	6	0.00	0.00		
Total	11	3.750			

Apendix 20. Layout of treatments used in objective II

S/N	Treatment	Abbreviation
1	Alheri seed treated with Apron star	Alheri A
2	Premier seed treated with Dress force	Premier D.F
3	Masalaha seed treated with Team	Masalaha T
4	IT86D-721 treated with Apron star	IT86D-721A
5	IT98K-503-1 treated with Team	IT98K-503-IT
6	Local Variety purchased from wusasa	L.Ifb.W
7	Local Variety purchased from sabon-gari	L.Ifb.SG
8	Local variety purchased from Samaru	L.Ifb.S.
9	SAMPEA-7 treated with Apron star	SAMPEA-7 A
10	SAMPEA-7 treated with Team	SAMPEA-7 T
11	SAMPEA-7 treated with Dress force	SAMPEA-7D.F
12	SAMPEA-7 inoculated	SAMPEA-7I
13	Ife brown inoculated	Ifeb I
14	SAMPEA-7 control	SAMPEA-7 C

Appendix 21. WEATHER DATA FOR 2010-2011

Month	2010		2011	
	Av. Temp ^{0}c	Av. R/H (%)	Av. Temp ^{0}c	Av. R/H (%)
January	27.50	44.50	29.97	50.34
February	34.50	44.36	36.86	49.21
March	30.50	46.13	36.99	34.68
April	33.50	58.80	36.47	58.30
May	32.65	81.94	35.05	88.71
June	30.67	90.96	31.06	91.23
July	29.52	93.68	28.77	94.93
August	27.48	94.65	29.29	95.23
September	29.10	93.80	30.48	95.00
October	28.00	93.97	32.40	91.77
November	32.23	70.83		
December	29.16	59.52		