EVALUATION OF PLANT EXTRACTS FOR THE CONTROL OF SEEDLING BACTERIAL BLIGHT OF COTTON INDUCED BY *Xanthomonas campestris* pv. *malvacearum* (Smith) Dye

By

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A thesis submitted to the Post-Graduate School, Ahmadu Bello University, in partial fulfillment of the requirements for the Degree of Master of Science in Crop Protection.

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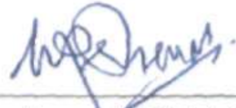
APRIL, 2002

DECLARATION

I hereby declare that this thesis has been written by me, and that it is a record of my own research work. It has not been presented before in any previous application for a higher degree.



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CERTIFICATION

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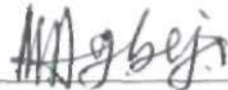

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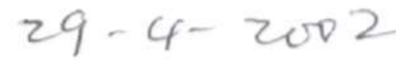

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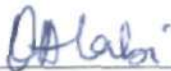

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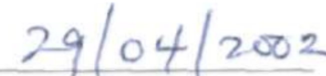

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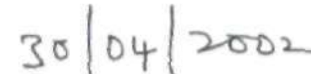

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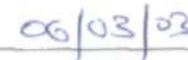

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DEDICATION

To my mother, Mrs. Josephine H. Shenge (who painstakingly laid the foundation of my life), my wife, Mrs. Mercy N. Shenge (whose presence in my life has brought many blessings), and my daughter, Miss Deborah M. Shenge (who has given me a new understanding of life)

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My profound gratitude goes to my major supervisor, Dr A.D. Akpa for suggesting the topic. I am very grateful for his attention, suggestions, constructive criticisms and corrections which have guided me whenever I found myself at cross-roads, and for his critical review of the manuscript.

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I am indebted to Mrs. J.A. Uwakwe, A. Maaji, Mrs. M.O. Akpa, and Mal. Yahaya Abdullahi for their enormous technical assistance.

ABSTRACT

Aqueous extracts of six plants, namely Garlic {*Allium sativum* L), Custard apple *Annona senegalensis* L), *Borreria verticillata* L., *Jatropha curcas* L, the Sweet basil (*Ocimum basilicum* L), and Neem (Azadirachta indica (A.), Juss) were evaluated for the control of bacterial blight of cotton. Streptomycin and three preprietory chemicals; bronocot (12% broropol), Apron plus (10% metalaxyl, 6% carboxin, and 34% Furathiocarb) and Apron star (20% Thiamethoxam, 20% Metalyaxyl-M, and 2% Difenoconazole) served as checks alongside an untreated control. Assessments of the extracts was done *in vitro,* in the glasshouse, and in the field to determine the efficacy of the extracts against the disease.

Two methods were used to evaluate the plant extracts *in vitro,* namely, dilution plate count and agar cup methods. Garlic *(A. sativum)* was found to be most effective, and significantly reduced bacterial population and growth over the untreated control. *A. senegalensis, B. verticillata,* and *J. curcas* were moderately effective, while the performance of 0. *basilicum* and *A. indica* relative to untreated control was very marginal. In the glasshouse experiment, the extracts were evaluated for their effect on seed-seedling parameters. Germination, stand establishment, shoot length root length, stem diameter and vigour of seedlings arising from seeds treated with the various plant extracts were assessed. Treatments were found to have no effect on germination stand establishment and root length. Extracts of *A. sativum* were found to be phytotoxic at high concentrations, as evidenced by the significantly reduced shoot length and plant vigour. However specific experiments to investigate this observed phytotoxicity did not confirm this observation. Plant extracts were also evaluated under field conditions for their effect on germination, stand establishment and seedling bacterial blight. In these experiments, extracts of garlic produced rates of germination which were marginally higher than those of all the other plant extracts. All the plant extracts significantly reduced the incidence of seedling

bacterial blight although they had no effect on severity of the disease. However, extracts of garlic were significantly more effective in reducing the incidence seedling bacterial blight over the untreated control; and was indeed on the same level of effectiveness with bronocot.

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1.0 INTRODUCTION

1.1 General

Cotton is an attractive host to a number of fungal, bacterial and viral diseases. Emechebe *et al.* (1980) listed diseases associated with cotton in Nigeria. Amongst the fungal diseases are *Alternaria* leaf spot (*Alternaria macrospora* Zimm. and other *Alternaria* spp), Areolate mildew (*Ramularia areola* Ath.,), Ascochyta leaf spot induced by *Ascochyta* sp., Anthracnose (*Colletotrichum gossypii* South), Stem blight (*Nematospora gossypii* Ashby and Nowell), Boll rot *Rhizopus stolonifer*) collar rot and wilt (*Sclerotium rolfsii* Sac.), and a host of other fungi isolated from bolls, stems and leaves. The only nematode disease is root knot induced by *Meloidogyne incognita* (Kufoid and White), Chitwood and *M. javanica* (Treub). Chitwood; while the viral diseases are leaf curl Germinivirus and cotton mosaic virus.

Bacterial blight of cotton induced by *Xanthomonas campestris* pv. *malvacearum (Xcm)* (Smith) Dye is the most important bacterial disease (Tarr, 1959; Brinkerhoff, 1970; Verma, 1986). Bacterial blight, *Alternaria* leaf spot,, and *Ramularia* leaf spot are considered the most important diseases of cotton in Nigeria (Emechebe et *al.*, 1980). Bacterial blight is, however, the most serious economic disease of cotton, causing an estimated yield loss of 10 – 20% in affected plants (King and Lawes, 1957; Dransfield, 1968; Erinle, 1981).

1.2 Purpose of the Study

Several attempts to check the spread of bacterial blight of cotton and bring it under control concentrated on the development of resistant cotton varieties (Knight, 1948; Brinferhoff, 1963, 1970; Hunter and Brinkerhoff, 1964) and the

use of synthetic pesticides (Dransfield, 1971; Pulido, 1973; Pulido and Bolton, 1974; Attang, 1988).

While the use of resistant varieties often offers a cost-effective and reliable solution, especially in the long run, it has been shown by various workers that sooner or later, a race or other sub-specific variant of the pathogen acquires morphological, genetic or physiological modifications which enable it to compromise the genetic basis of host resistance (Chew *et al*, 1969; Brinkerhoff, 1963; Verma *et al.*, 1979). Indeed, with particular reference to cotton, highly virulent isolates of *Xcm* (HV_1, HV_3, HV_7, CH_1, CH_2, CH_3, SU_1, SU_2 and SU_3) that evolved in Burkina Faso, Chad and Sudan are now known to have rendered all cotton cultivars with the B_2, B_3, B_4, B_6 and B_7 major genes for resistance and their combinations susceptible to the disease (Follin, 1983; Bird *et al.*, 1984; Poswal *et al.*, 1985). In such situation, one has to resort to the use of synthetic pesticides. While pesticides provide immediate seeming solution, they have neither solved the purely agricultural problem of the small scale farmer, nor have they improved his financial situation. On the contrary, their use has, in the long run, resulted in a series of consequences which politically, economically, ecologically and socially, are self-defeating (Stoll, 1998). Some of these attendant problems include toxicity to man and his animals as a result of direct contact, phytotoxicity, environmental contamination, resistance to the chemical by target organisms, high cost of purchase/application and residual toxicity.

The aim of this research, therefore, is to explore the use of natural substances, plants extracts, for the control of cotton bacterial blight. Nature herself has offered

us a profusion of plants for use in crop protection; a potential which deserves our interest.

1.3 Objectives

The objectives of this research are:

- To evaluate the efficacy of some plant extracts for the control of *Xcm* in laboratory and glasshouse studies.
- To evaluate the efficacy of some plant extracts for the control of seedling bacterial blight.

2.0 REVIEW OF LITERATURE

2.1 Origin and Distribution of Cotton

Cotton belongs to the family Malvaceae. The genus *Gossypium*, with about thirty species of annual sub-shrubs, perennial shrubs or small trees is distributed in the tropical and sub-tropical regions of Africa, Asia and America (Purseglove, 1974).

Commercially cultivated cotton comprises the Old World linted, and the New World linted cottons (Purseglove, 1974). The Old World linted cottons consist of two species; *Gossypium herbaceum* L. with five cultivated races in Asia and Africa, and *G. arboretum* L. with six cultivated races. The New World cottons contain three species; namely *G. barbadense* L. (syn. *G. peruvianum* Cav. to which the famous sea inland cotton belongs), *G. hirsutum* L. with seven races; four of which occur wild in Central America (Hutchinson, 1962) and three with varietal status (Purseglove, 1974); with *G. tomentosum* Nutt. ex Seem being endemic in the Hawaii Islands.

The Old World linted hybrid species *G. herbaceum* is said to be more primitive, cytogenetically, than *G. arboreum, G. herbaceum* race *africanum* occurs wild in the dry, bushy regions of Southern Africa from Angola and Namibia to Mozambique (Purseglove, 1974). Hutchinson (1959) considered race *acerifolium* to have originated from race *africanum*, which later found its way to Southern Arabia where cotton was first domesticated; and is found often as a perennial in Ethiopia, Southern Arabia, and Southern Baluchistan. The annual races of *G. herbaceum; persicum* (in Persia), and *Kuljianum* (in Turkistan) are said to have been taken to India through the Arabian coast and the Persian coast along the trade routes

about 1780, and gave rise to the race *wightianum* (Purseglove, 1974; Verma, 1986).

Gossypium arboreum race *indicum* is a primitive perennial in Western India, embracing the oldest forms, and relating more closely to *G. herbaceum race acerifolium* (Purseglove, 1974). The oldest known specimens of Asiatic cotton dated about 3000 B.C. found in excavations at Mohanjo-Daro in West Pakistan belong to *G. arboreum* (Purseglove, 1974). It is believed to have spread eastwards towards Burma and Assam, giving rise to race *burmanicum*, and westwards across Southern Arabia to Northern Sudan, the southern borders of the Sahara to West Africa, giving rise to race *Soudenensis*. The northern perennial *arboreums* gave rise to annual cottons including race *bengalense* (of Northern India) and *sinense* (of China).

The origin of New World cottons has been attributed to allopolyploidy resulting from the cross of the 13-chromosome American wild species and the Old World cultivated types bearing "A" genome. The American wild species *G. raimondi* is probably the nearest relative of the "D" genome that has entered into the ancestry of the present day New World 26-chromosome cultivated cottons (Verma, 1986). Harland (1932) made similar conclusions, suggesting that *G. barbadense* must have arisen from a cross between an Old World linted species and a New World species like *G. ramondi*; while *G. hirsutum* occurring wild in N.E. Brazil is speculated to be the result of a cross between an old world linted cotton and a species resembling *G. thurberi*. Seeds of *G. barbadense* from West Indies gave rise to Sea Inland cotton in South Carolina in 1786, while perennial forms of *G. barbadense* spread in post Columbian times from Eastern South America to West

Africa; giving rise to the Ishan cotton of Western Nigeria. From this, the commercial cotton crop in Egypt was established in 1820 by Jummel (Purseglove, 1974). It spread to the U.S.A. from Mexico about 1700, establishing the annual habit in the upper country and hence known as "Uplands", distinguishing them from the coastal "Sea Inland" cotton.

Upland cotton was distributed worldwide as a result of the rise of the British cotton industry. It is now the basis of all commercial cotton crops of Africa outside the Nile Valley, all South America except Peru and Northern Brazil, the Russian crop and much of Northern India and Pakistan (Purseglove, 1974).

Cotton cultivation in Nigeria pre-dates the geographical constitution of the country as it is presently known, with cultivation largely restricted to providing the local needs of the hand weaving and spinning industry. According to French (1926), cotton was exported from South Western Nigeria as early as 1865. Cultivation was based on the low-yielding perennial Asiatic types or Old World species in which commercialization was very limited (Johnsrud, 1960). This subsistent level of production persisted until 1903 when commercial cotton-growing was commenced by the British Cotton Growers Association (BCGA) in the now Lagos State with the aim of producing a commercial crop suitable for mechanical textile factories in Europe (Derrick, 1976). The introduction of the American Allen gave rise to a boost in cotton commercialization and production in Northern Nigeria; which has since replaced the Southern provinces as a major area of production (Faulkner, 1974). From 1916, farmers were encouraged to grow "Allen" due to its obvious advantages over the Asiatic types. Gradually, a step-by-step "gazetting" of "Allen" cotton areas started (French, 1926); a process which eventually resulted to the present three cotton growing zones.

2.2 Economic Importance of Cotton

Cotton has aptly been described as "the queen of fibre crops" (Faulkner, 1974) due to its status as the most important vegetable fibre in the world. Its economic importance is such that its products and by-products play a vital role in the socio-economic and political life of a country (Verma, 1986; Prasad, 1998; Personal communication). Cotton lint is woven into fabrics alone or combined with other fibres. The invention of the textile mills led to a rapid expansion in the utilization of cotton. By 1890, cotton provided 78.6% of the world's textiles, increasing to about 84.2% in the period 1924-1928 (Purseglove, 1974). Since then, production has been declining; due probably to competition from man-made synthetic fibres. Cotton lint also provides yarn, cordage, twine and tyre cord. The fuzz provides linters for use in felts, upholstery, carpets, mattresses, wicks, surgical cotton and in chemical industries. It is used to produce rayons, plastics, lacquers, papers, photographic films, cellulose, explosives and sausage skins (Purseglove, 1974).

Commercial cotton seed is approximately 92% dry matter, yielding about 20% semi-dry edible oil, which on extraction leaves a 42% protein cotton-cake or meal. Cotton seed oil is yellow and is used in lard substitutes as salad and cooking oil, and in margarine manufacture. Low grade oil is used in the manufacture of soaps, lubricants, sulphonated oils and protective coatings. In the animal industry, the residual seed cake is a valuable protein supplement for livestock (Oyenuga, 1968; Verma, 1986). Low grade cake is also used as manure. The whole seed may also be fed to livestock. Cotton seed hulls are used as roughage for livestock, as a source of stuffing for bedding, as fertilizer and fuel.

2.3 Bacterial Blight of Cotton

2.3.1 General

Bacterial blight of cotton (hereafter referred as CBB) has apparently been associated with cotton in the U.S.A. from the beginning of cotton culture (Atkinson, 1891). However, Erwin F. Smith was the first to reproduce the disease and describe the inducing organism in 1901 and 1920 (Verma, 1986; Poswal, 1981).

CBB is a collective term for the disease representing numerous manifestations descriptively named according to the part of the plant attacked. The "angular leaf spot" phase is manifested as water-soaked spots on the cotyledons and leaves, turning later into angular lesions delimited by veins. Another phase of the disease occurs as initially water-soaked lesions which later become blackish, on the mid-rib and veinlets. This phase is referred to as "vein blight". "Black arm disease" occurs as blackish lesions on the young stems, while the presence of blackish spots which are sunken and rounded in outline on the mature bolls is a symptom of a phase of the disease usually referred to as 'boll rot" (Stoughton, 1928; Wickens, 1953; Verma, 1986). The black arm phase of bacterial blight rarely occurs in Nigeria (Erinle, 1981; Dranfield, 1965) while it is common in Egypt (Wickens, 1953).

CBB is represented in almost all the cotton-growing regions of the world in one, two or more of its manifestations (Hayward and Waterson, 1964). Host range studies have so far revealed that bacterial blight is found on almost all the *Gossypium* spp., *Thurberia thespesiodes*, *Eriodendron anfractuosum*, *Jatropha*

curcas, *Hibiscus litifolus* (Wickens, 1953; Davis and Sandige, 1977) and a cotton field weed (*Lochnera pussila*) (Sivaprakasan et *al.*, 1965).

2.3.2 Economic Importance

Of the diseases responsible for heavy crop losses in cotton, bacterial blight is the principal one in all cotton-growing areas of the world (Tarr, 1959; Wickens, 1953; Brinkerhoff, 1970).

Seed dressing trials have revealed a wide range of economic losses due to CBB (Wickens, 1961). There appears to be some variability in yield loss due to CBB from region to region. The disease is said to be most severe in the sub-humid and semi-arid areas of the cotton belt where the rainfall varies from 10 - 30 mm (Verman, 1986). This variability is probably due to different phases of the disease, differences in levels of host resistance, cultural practices, edaphic and climatic factors, etc. (Poswal, 1981). Although higher magnitudes of losses are rare, losses of 10 - 30% are of common occurrence (Innes, 1970; Ramapandu *et al.*, 1979), and complete crop failure is also known (Last, 1960). Hansford and Hosking (1938) showed a 63% yield loss to bacterial blight. Wickens (1961) cited Jameson and Thomas (1952) who showed that seed-dressing increased yields up to between 7 - 19%. King and Lawes (1957) in Nigeria showed a yield increase of 15.6% as a result of seed-dressing. Peat *et al.* (1955) showed statistically significant yield increases from seed treatment against bacterial blight to the tune of 25 - 29%.

2.3.3 Symptomatology

Symptoms and their development can be grouped as those of the primary infection phase and those of the secondary infection phase.

The primary infection phase: This usually occurs at the seedling stage and is characterized by the development of round, water-soaked lesions often dark-green in reflected light and translucent by transmitted light (Stoughton, 1928; Massey, 1934; Wickens, 1953, 1956) on cotyledons. On the true leaves, large initially water-soaked angular leaf spots develop. Intensive and severe seedling attack would normally lead to death of the seedlings (Stoughton, 1928; Wickens, 1956).

The secondary infection phases: The initially water-soaked lesions at the true leaf phase later turn dark-brown with occasional intensive coalescing, giving rise to large necrotic areas. Vein blighting normally associated with petioles and stem rotting does not necessarily arise from the progression of parenchymatous infection of the leaf, but may be due to internal upward movement of the pathogen in the vascular element (Wickens, 1956). The black arm phase of the disease results from the gradual, but progressive movement of the pathogen in the vascular element, and not externally as was earlier supposed (Wickens, 1956). Wickens advanced evidence from the progression of symptoms, and isolation of the pathogen from various parts of the plant at different stages to show that stem lesions or black arm is primarily vascular, progressing from the edges of the cotyledons into the hypocotyls and upwards into the vascular tissues from where it may break into the parenchymatous tissues, leading to more characteristic forms

of the disease. Vascular infection has been reported to be greater in hot dry weather (Rolfs, 1935).

Bacterial boll rot by *Xcm* may occur indirectly by extension of infection of calyx, bracts or receptacle or through the vascular system from infection occurring lower down on the fruiting branches (Hansford, 1934; Wickens and Logan, 1957), or it may occur directly, after corolla drop, through the boll-wall epidermis (Logan, 1958).

2.3.4 Some epidemiological aspects of the disease

2.3.4.1 Disease transmission

The primary source of disease infection is the seed (Hibbard, 1910; Jenkins, 1919; Poswal, 1981; Verma, 1986). Since the bacterium is both externally and internally seed-borne (Massey, 1931; Laycock, 1936; Knight and Hutchinson, 1950; Brinkerhoff and Hunter, 1963), disease dissemination to areas formally uninfected is achieved through seed transmission. World wide distribution of the pathogen has been affected by long distance transportation of contaminated seeds (Wickens, 1953).

Secondary spread of the disease amongst plants and from field to field is achieved by wind-driven rain, causing the dispersal of water droplets with the disease from field to field (Faulwetter, 1917). Where long dry periods, strong, convectional rains, and high winds abound, widespread outbreak of the disease as a consequence of widespread dissemination by wind is likely to occur (Wickens, 1953).

2.3.4.2 Favourable weather for disease development

High relative humidity and high air temperature have been found to be conducive to the development of bacterial blight with maximum infection occurring at 35 - 36°C and relative humidity exceeding 85% (Stoughton, 1931). Infection decreases at progressively lower temperatures (Stoughton, 1932). A rainfall of 25–28mm a week has been shown to be optimum for disease transmission.

2.3.4.3 Seed infection, contamination and their relationship to bacterial blight development

Since the cotton seed is the primary source of inoculum, the sowing of naturally infected seeds, especially if undressed, leads to a high percentage of seedlings with primary infection, and is, in many cases, an important factor in disease epiphytotics.

Patel and Kulkami (1950) collected seeds from infected bolls, delinted them and dropped them into potato dextrose broth. A cloudy growth was obtained at 31°C in 3 days in 70% of inoculated tubes, and when sprayed on susceptible plants, gave positive infection on leaves. They demonstrated a 10% internal seed-borne infection. Seeds were finally shown to be carrying both internal and external primary inoculum (Tarr, 1953; Wickens, 1953; Ark, 1958; Schnathorst *et al.*, 1960; Nayudu, 1969; Verma and Singh, 1974; Verma *et al.*, 1979), and healthy seeds were considered to be contaminated during ginning (Schnathrost, 1964). Brinkerhoff and Hunter (1963) further showed that internal infection was 6.4% in discoloured seeds from infected bolls as compared to 0 – 4% in commercial seeds. They found that *Xcm* survived in seeds (both internally and externally) for 56 months; thus showing the long survival ability of the pathogen. It was concluded that a single infected plant per 6000 plants (0.017%) was sufficient to

cause epiphytotic under favourable conditions (Tarr, 1961), thus emphasizing the importance of internally seed-borne inoculum. Hunter and Brinkerhoff (1964) assessed the level of bacterial survival and seedling infection from naturally infected seeds from infected bolls of upland cotton fields near Perkins, Oklahoma. They examined the occurrence of lesions on cotyledons 21 days after sowing and found that the percentage of infected seedlings declined from 60.3 after six months storage of fuzzy seed to 1.0 after 56 months storage. Acid-delinted seeds gave 11.64% of infected seedlings at 6 months storage and 0.7% at 56 months storage. Infected bolls however gave 41.0% and 4.0% of infected seedlings at 6 and 56 months respectively. Acid-delinted seeds gave 3.6% and 0.3% after 6 and 56 months of storage respectively.

Schnathorst (1969) showed that the percentage of infection of cotton seedlings was related directly to the concentration of *Xcm* in suspensions in which the seeds were soaked before sowing. According to Jameson and Thomas (1952), the areas where seed infection is heaviest does not necessarily mean that these areas have heaviest loss resulting from blackarm damage. Conditions may be right for seed infection and wrong for spread in the growing crop. Conversely, areas with moderate seed infection may be the start of a severe epiphytotics (Jameson and Thomas, 1952).

With respect to seed contamination, seeds from commercial gins have been shown to have high levels of bacterial contamination, leading to high levels of the disease, and introduction of the disease into areas previously not affected, and constituting a setback to control programmes (Poswal, 1981; Poswal and Erinle, 1983). Mechanical delinting of seeds has been found to increase the proportion of

infected plants (Jameson and Thomas, 1952). Over 90% losses at germinanation was associated with bacterial infection when one lot of mechanically delinted seed was used. Schnathorst (1964) demonstrated that ginning or deliniting of blight-free cotton in gins contaminated with the pathogen could result in surface contamination of previously disease-free seed.

2.3.4.4 Bacterial blight zonal/regional surveys in Nigeria

Soon after CBB was first noted in Nigeria by Farquaherson in 1912, various workers elsewhere in the country also established the presence of the disease in their areas. Thornton (1922) reported what he considered to be *Phytomonas malvacearum* from the Ilorin district and later found it to be generally distributed in Nigeria, being common in Oyo province. He also reported the collection of diseased specimens at Kano, Zaria and Ilorin in the North, and Umuahia in the South East.

In a comprehensive disease survey of Nigeria, Laycock (1936) found that bacterial blight was absent in the extreme southern parts of Nigeria. This he attributed to high temperatures of both soil and air. In the middle cotton belt, the disease was considered to be economically important. He cited Golding who in 1928-29 seasons stated that CBB was the principal disease of cotton at Ilorin. At Badeggi, he found that boll shedding was due to the disease. Limited surveys were conducted in the northern belt. He cited Jones (1926) who gave a rough estimate of 15 – 20% loss of leaf area due to the disease at Samaru. He thus considered the disease to be of major importance in this region, and that, were it absent, the yield of cotton would probably be considerably increased.

Little subsequent mention of the disease in Nigeria appeared in literature until King (1955) at Samaru summarized the status of bacterial blight in the country between 1949 and 1952. Quoting Wickens to express the level of disease in 1953, King (1955) stated that the general level of leaf infection was high enough to cause a considerable reduction in yield, particularly if the branch infection was causing direct loss of fruiting.

Prior to the introduction of seed-dressing in Nigeria, Dransfield (1965) using undressed seed samples from various locations in 1955-1956, 1956-1957 and 1957-1958 seasons was able to establish the wide distribution of the disease in Northern, Eastern and Southern cotton zones. Seed samples in the Northern and Eastern zones gave high infection of seedling blight of between 30-60%. In the Southern zone, however, consistently low disease incidence was recorded, mostly below 30%. Dransfield (1970) found most seed market samples from the Northern zone developing more or higher seedling blight than the Eastern zone seed market samples. These trends were, many years later, confirmed by Poswal and Erinle (1983) who conducted similar surveys to determine the extent of the disease in the Northern states of Nigeria. They obtained seed samples from 32 hand-ginned markets and commercial gins which they grew in the 1979 and 1980 seasons at Samaru, and scored the incidence and severity of CBB on seedlings grown from the samples. Results showed that the Northern and Southern zones where Samaru 71 was the commercial cultivar gave higher levels of CBB than the Eastern zone where Samaru 72 was the commercial cultivar. They also reported variability in blight levels from samples obtained from the Northern and Eastern zones. They found commercial gin samples to have a higher susceptibility to CBB than hand-ginned market samples.

Poswal (1988) confirmed the long-held suggestion as to the probable occurrence of strains of the bacterium, that may vary in virulence, in the cotton-growing zones of Nigeria (Hayward, 1964; Dransfield, 1971; Poswal and Erinle, 1983). Using bacterial isolates from diseased leaf samples obtained from 12 locations covering the three cotton zones of Nigeria, he identified races 6, 7 and 10 from the 3 zones in trials based on the grouping of Hunter *et al.* (1968). These were found to correspond to race biotypes 20A, 30A and 32A respectively (Verma and Singh, 1974, 1975).

2.3.5 Breeding Varieties for Resistance to CBB

The breeding and screening of bacterial blight resistant cotton cultivars and strains is the ultimate and most economic approach to controlling the disease (Tarr, 1959; Presley and Bird, 1968; Brinkerhoff, 1963, 1970; Verma and Singh, 1971). Brinkerhoff and Hunter (1963) concluded that the generation of new races of *Xcm* will be a serious problem in the maintenance of bacterial blight resistant varieties of cotton.

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Over the years, various methods have been employed by different workers in many parts of the world to assess cultivars, lines and strains of cotton for their levels of resistance. Knight (1954), Knight *et al.* (1941, 1950, 1956) screened over 1000 types of New World and Old World cottons in the Sudan. Their critical experiments on inheritance, etc, laid the basic foundation for breeding CBB disease-resistance. They reported 9 bacterial blight resistant genes which were termed BAR (black arm resistant) genes and later renamed "BBRG" (bacterial blight resistant genes). Of these, the genes B_2 and B_3 (*Gossypium hirsutum* race *punctatum*) and B6m (an

intensifying gene from *Gossypium arboreum*) were found most useful when transferred to Sudan sakels (*G. barbadense*). B2 alone gave fair resistance, B_2 + B_3 gave high resistance, B_2 + B_{6m} imparted field immunity and B_2 + B_6 + B_{6m} gave immunity (Knight, 1954, 1956). Since this work, the search for resistant varieties has become a constant endeavour.

2.4 Control of CBB

2.4.1 Seed treatment

Since the bacterium is externally and internally seed-borne (Massey, 1931; Laycock, 1936; Knight and Hutchinson, 1950; Brinkerhoff and Hunter, 1963), seed treatment has been found to be effective in reducing primary infection and enhancing seedling germination and vigour (Attang, 1988). In many cases, seed treatment has also been found to reduce secondary spread of the disease.

Various chemicals have been tried over the years as dusts, slurries, soaking, etc. These include inorganic mercurials (e.g. $HgCl_2$), organic mercurials (ceresin, agrosan, etc.), copper compounds (e.g. copper trichlorophenolate (20%)), formalin, hydrogen sulphide, etc. Some of these chemicals have been replaced by less toxic ones such as Bronocot (12% bronopol), Busan 72 and Busan 30 (Dransfield, 1971; Pulido, 1973; Pulido and Bolton, 1974). Antibiotics such as streptomycin have also been applied as disinfectant, soil drench in combination with a fungicide (8-quinolinol), seed dips, etc. with favourable results. Other methods such as hot water treatment, exposure to high temperatures of between 60 - 100°C and varying periods of time, exposure to ionizing radiation, acid and flame delining, etc. have also been applied with satisfactory results (Verma, 1986).

2.4.2 Crop sanitation

Sanitary measures such as the use of acid-delinted seeds, crop rotation, deep ploughing followed by irrigation, change from sprinkler to furrow irrigation (if disease recurred), use of uncontaminated gin seeds, non-usage of susceptible varieties, destruction of crop residues, the cotton close season, etc. have also proved effective when used in combination (Schnathorst, 1966).

2.4.3 Resistant varieties

The development of resistant varieties appears to be the best way of fighting the disease. Until this is done seed treatment and crop sanitation would remain only palliative measures to minimize loss (Tarr, 1959, Verma, 1986). Cotton varieties which have shown a high level of resistance to the disease include RASA (76)101, REBA B-50, RSA (68)27, and BJA-592.

2.5 The Use of Plant Extracts and Local Materials for the Control of Plant Diseases

The adoption of practices and the use of materials that are environment-friendly constitutes a vital component of sustainable agriculture. All over the world, current agricultural practices are moving away from the use of synthetic chemicals due to their long term effects on the environment. The fact that nature itself has provided us with a profusion of plants and other materials which may be employed in fighting the purely natural problem of plant disease is no longer in doubt, although, the use of these materials has only recently attracted scientific attention in Nigeria.

Tokin (1957) isolated a substance from wheat and tested it against *Xcm* on a cotton seed at 1 = 500 and 1 = 700, which he reported to be 100% effective and did not adversely affect germination. In another experiment, bacterial and bacteriostatic effects of phytoncides in volatile and non-volatile fractions of extracts from garlic were demonstrated against *Xanthomonas campestris* pv *malvacearum* by Abdullaeva (1962).

Various parts of neem have been used over the years in experiments to demonstrate its pesticidal properties (Emechebe, 1996; Obagwu, 1997; Emechebe and Alabi, 1997; Stoll, 1998; Aliyu, 1999). Although with respect to its efficacy in disease control, parts of neem have been reported to be highly effective in controlling a wide range of fungal diseases, Emechebe (1996) found foliar applications of aqueous neem seed extracts to be ineffective in controlling bacterial blight of cowpea. The active principle of neem is azadirachtin, which is said to be more concentrated in the seed. Almost every part of the tree is bitter, and has found application in natural pest control. The bitter principles of neem are said to be nimbidin (m.p. 90 - 100°C; 1.2 – 1.6%) which contains sulphur (Bhatnagar *et al.*, 1948). On hydrolysis, it yields nimbidinic acid which is equally bitter and retains sulphur. Besides nimbidin, two bitter compounds free from sulphur (m.p. 192°C and 205°C) have also been obtained in very small quantities.

The anti-fungal ability of garlic extracts against several rhizoshere and rhizoplane fungi (*in vitro*) has been demonstrated (Murthy and Amonkar, 1974; Ahmed and Agnihotri, 1977; Agrawal, 1978; Shashikanth *et al.*, 1981; Obagwu, 1997). In the Philippines, Pordesimo and Ilag (1976) reported that crude juice extract from fresh garlic bulb was toxic (*in vitro*) in varying degrees to *Colletotrichum gloesporoides*.

The conidial germination of *C. gloesporoides* and *C. capsici* was also inhibited by garlic extract (Gupta *et al.*, 1981). The bactericidal activity of garlic is attributed to the presence of an essential oil (0.06 – 0.1% of fresh weight) which contains allyl-propyl disulphide ($C_6H_{12}S_2$; 6%), diallyl disulphide ($C_6H_{10}S_2$; 6%) and two or more sulphur-containing compounds. The fresh garlic bulb consists of 62.8% moisture, 6.3% protein, 0.1% fat, 29.0% carbohydrate, 0.03% calcium, 0.31% phosphorus, 1.3 mg iron and 13 mg/100g vit C (Bhatnagar *et al.*, 1948).

The sweet Basil (*Ocimum basilicum*) is a plant better known as a vegetable and as a source of perfume; the oil of Basil, and as a flavouring agent. However, apart from its insect repellant properties, it has also been reported to possess bactericidal properties as demonstrated against *Salmonella typhosa* and *Staphylococcus aureus* (Chopra *et al.*, 1956). The seeds of the plant are odourless with an oily, slightly pungent taste. When steeped in water, they liberate a mucilage which is semi-transparent and nearly tasteless. The mucilage (9.3%) on hydrolysis, yields uronic acid, glucose, xylose and rhamose. The seeds contain a drying oil with the following fatty acid composition: palmitic acid 7.0%, stearic acid = 0.2%; Oleic acid = 11.0%, linoleic acid = 60.0% and linolenic acid = 21%. The unsaponifiable fraction is reported to contain β sitosterol, oleonolic acid and ursolic acid. Aqueous extracts of the seeds are active against Gram-positive bacteria and mycobacteria (Zaheer *et al.*, 1966). The plant is considered stomachic, anthelmintic, antipyretic, expectant and stimulant.

Jatropha curcas Linn is wildly known in traditional medicine in both Africa and Asia, although its efficacy against plant pathogenic organisms has not been evaluated. The seeds possess poisonous and purgative properties, although they are rarely used as a purgative. The seeds also yield a valuable oil known as "Curcas oil" which burns without emitting smoke, and is used as external application for skin disease and rheumatism (Ram *et al.*, 1959). A decoction of leaves and roots is given for diarrhoea, and the root bark is used in external application for sores. Roots are also steeped in water and used for the treatment of various diseases, including some sexually transmitted diseases (Omo-Eboh, 1999; Personal communication).

Although very little documentation has been done about *Borreria verticillata* beyond its botanical description and taxonomy, its external application for the control of skin diseases dates back to ancient times. It has also been used for the treatment of typhoid fever (Ihom, 1999; Personal communication).

Emechebe and Alabi (1997) evaluated wild custard apple also known as "wild pawpaw" (*Anonna senegalensis* L.) for its efficacy against a wide range of cowpea diseases at Samaru, and found it to be effective in controlling Septoria leaf spot, foliar, peduncle, pod and stem scab, but not bacterial blight. Other experiments conducted by Aliyu (1999) showed it to be effective in enhancing germination of sorghum seeds as well as other seedling parameters (except germination) above those of control. The plant is known for its broad-spectrum of anti-fungal activity (Burkhill, 1985).

In view of the almost ubiquitous nature of these plants, their efficacy against plant pathogens and the immense advantages in utilizing them in disease control, it is hoped that efforts will continue in evaluating them and others to determine their efficacy and range of anti-microbial ability.

3.0 EFFECT OF PLANT EXTRACTS ON THE POPULATION OF *Xcm IN VITRO* USING DILUTION PLATE COUNT AND AGAR CUP METHODS

3.1 Materials and Methods

3.1.1 Collection of materials for the study

The plant materials collected at Samaru and its environs were; bulb of garlic onion (*Allium sativum* Linn.), leaves of the sweet Basil (*Ocimum basilicum* Linn), root bark of *Jatropha* curcas Linn, stem bark of *Annona senegalensis* Linn, seeds of the neem tree (*Azadirachta indica* (A) Juss) and leaves of *Borreria verticillata* Linn.

Agrochemicals which were also used are bronocot (12% bronopol), Streptomycin, Apron plus (10% metalaxyl, 6% carboxin, 34% furathiocarb) and Apron star (20% thiamethoxam, 20% metalaxyl, 2% difenoconazole). These were purchased from agrochemical stores in Zaria.

Infected plant materials were collected from the IAR research farm at Samaru in August, 1999.

3.1.2 Plant extracts

The plant materials used were those listed in 3.0 above. Bronocot (12% bronopol) was used as a check alongside Apron plus (10% metalaxyl, 6% carboxin, 34% furathiocarb) Streptomycin, Apron star (20% thiamethoxam, 20% metalaxyl-M, 2% difenoconazole) and an untreated control. Five grams of each plant material were pounded separately in a porcelain mortar to form a paste. This was then mixed with 100 ml of water and allowed to stand for 6h after which they were filtered through whatman filter papers before filter-sterilizing them by means of Millipore filters (Millipore Filter Corporation, Bedford, Massachussetts, U.S.A.). Two hundred

milligrams of bronocot, Streptomycin, Apron plus and Apron star were dissolved in 100 ml of sterile distilled water (SDW).

3.1.3 Preparation of bacterial inoculum

Leaf samples were obtained from advancing margins of bacterial blight lesions. These were placed in a few drops of SDW and teased apart with a flame-sterilized needle and scarpel and allowed to stand for 10 - 15 mins, after which a loopful was removed with the aid of a sterilized wire loop and streaked unto sucrose peptone agar (SPA) plates. The petri-dishes were then incubated at 25 - 28C and examined daily for 4-5 days. Pure colonies were obtained by the single colony method (Bradbury, 1970). A loopful of bacterial growth was taken from a 24 - 48h old culture and suspended in 10 ml of SDW from which a serial dilution of up to 10^{-5} was prepared.

3.1.4 *Testing Sensitivity of the Organism to the Extracts*

Two methods were employed in testing the sensitivity of the organism to the test materials as follows.

3.1.4.1 Dilution plate count method

SPA was prepared in a flask and allowed to cool to about 45C. Prior to pouring the medium, 0.2 ml of the aqueous plant extracts was introduced unto the bottom of the petri-dishes with the aid of a blue-tipped micro-pipette, after which 20 ml of the molten SPA were poured into the dishes. When this had been done, 10 µl of the bacterial suspension adjusted to ca 4.7×10^7 cfu/ml was then introduced unto the medium with the aid of a yellow-tipped micro-pipette. The contents of the petri-

dishes were mixed thoroughly by swirling five times each in clock-wise, anti-clock-wise, and north-south directions. The plates were allowed to solidify, after which they were incubated accordingly. All the treatments were screened in this way. The experiment was laid out using a Completely Randomised Design (CRD) with three repetitions. Colony counts were made after 48h. Data collected were analysed statistically using analysis of variance (ANOVA), and means separated by the least significant difference (LSD) test.

3.1.4.2 Agar cup method

Slightly cooled (*ca* 45C) SPA was poured directly into sterile petri-dishes unto which 10 µl of bacterial suspension adjusted to *ca* 4.7 x 10^7 cfu/ml was introduced with the aid of a yellow-tipped micro-pipette and mixed thoroughly as outlined above. The medium was allowed to solidify, after which eleven cups (one cup for each treatment) were made in the solid medium by means of a sterile 5 mm diameter cork borer at the rate of 4 cups/plate. 0.1 ml of samples to be evaluated were then introduced into the cups with the aid of a blue-tipped micro-pipette, after which the plates were incubated for observation. The experiment was based on a CRD with three repetitions. The extent of the zone of inhibition around each cup was measured as an indication of sensitivity of the organism to the material being tested. Data collected was analysed statistically using ANOVA, and treatment means separated by the LSD. The experiment was repeated once.

3.2 Results and Discussion

The effect of plant extracts on the population of *Xcm in vitro* using the dilution plate count method is outlined in Table 4.1. The trend of the results was similar for runs I and II. All the plant extracts significantly reduced the population of the bacterium compared with the untreated control. Population reduction was, however, greater with garlic extract than all other plant extracts. *A. indica* had the least inhibitory effect on bacterial population. In order of effectiveness, *A. senegalensis* and *J. curcas* compared fairly with *A. sativum*. In comparison with the untreated control and all the plant extracts, bronocot had the greatest inhibitory effect. However, its effect was not significantly different from that of *A. sativum*, *A. senegalensis* and *J. curcas*. The effect of these three plant extracts did not differ significantly, but were superior to *O. basilicum*.

Table 3.1. Effect of plant extracts on the population of *Xcm in vitro* using dilution plate count method.

Treatment	Mean bacterial count (cfu)/plate*		
	Run I	Run II	Mean
A. sativum	7.6^{e}	15.6^{g}	11.6^{g}
J. curcas	29.0^{de}	189.0^{de}	109.0^{de}
B.. verticillarta	56.6^{de}	189.3^{de}	122.9^{d}
A. senegalensis	28.6^{de}	106.6^{f}	67.6^{f}
O. basilicum	78.3^{d}	260.3^{c}	169.3^{c}
A. indica	347.0^{b}	360.6^{b}	354.8^{b}
Untreated check	477.6^{a}	483.6^{a}	480.6^{a}
Bronocot	3.0^{e}	0.0^{g}	1.5^{g}
Streptomycin	3.0^{e}	170.6^{e}	86.8^{ef}
Apron plus	4.6^{e}	179.0^{de}	91.8^{ef}
Apron star	172.0^{c}	211.0^{d}	191.5^{c}
LSD	52.51	39.52	30.71

**Means followed by the same superscript(s) are not significantly different at P=0.05.*

Plate 1: Effect of *Allium sativum* on *Xcm in vitro*

Plate 2: Effect of *Azadirackta indica* on *Xcm in vitro*

Plate 3: Effect of *Borrerta verticilata* and Jatropha *curcas* on *Xcm in vitro*

Plate 4: Effect of *Ocimum basilicum* on *Xcm in vitro*

The effect of the plant extracts on the growth of *Xcm in vitro* using the agar cup method is presented in Table 4.2. Again, the trend of the results were similar in both runs. Among the plant extracts, *A. sativum* exhibited the greatest inhibitory effect, followed by *J. curcas* and *B. verticullata*. Extracts of *O. basilicum* and *A. indica* had no inhibitory effect on *Xcm*, and in fact, were similar to the untreated control. Compared with the treatments, bronocot had the greatest inhibitory effect. Both methods of evaluation have demonstrated the superiority of extracts of *A. sativum* over all the other plant extracts. In addition, similar patterns of results were obtained with the two methods used to evaluate the sensitivity of *Xcm* to plant extracts.

Table 3.2. Effect of plant extracts on *Xcm in vitro* using the agar method.

Treatment	Diameter of clear zone (mm)*		
	Run I	Run II	Mean
A. sativum	21.0^{b}	26.6^{b}	23.8^{b}
J. curcas	6.6^{d}	6.3^{c}	$6.4c^{de}$
. verticillarta	6.8^{d}	8.5^{c}	7.6^{cd}
A. senegalensis	8.5^{c}	8.5^{c}	8.5^{c}
O. basilicum	0.0^{f}	0.0^{e}	0.0^{f}
A. indica	0.0^{f}	0.0^{a}	0.0^{f}
Untreated check	0.0^{f}	0.0^{c}	0.0^{f}
Bronocot	44.6^{a}	43.3^{a}	44.0^{a}
Streptomycin	0.0^{f}	1.6^{d}	0.8^{f}
Apron plus	5.1^{e}	5.0^{de}	5.0^{e}
Apron star	5.5^{e}	5.0^{de}	5.2^{de}
LSD	1.18	4.24	2.30

**Means followed by the same superscript(s) are not significantly different at P=0.05.*

This work has shown the effectiveness of garlic (*A. sativum*) in significantly reducing both the population and growth of *Xcm in vitro*. The anti-microbial activity of garlic against a broad spectrum of fungal and bacterial organisms has been

reported previously (Murthy and Amonkar, 1974; Ahmed and Agnihotri, 1977; Agrawal, 1978; Shashikanth *et al.*, 1981; Gondwe *et al.*, 1996). The anti-microbial properties of garlic have been attributed to the presence of an essential oil (0.06 - 0.1% of fresh weight) which contains allyl propyl disulphide ($C_6H_{12}S_2$), diallyl disulphide ($C_6H_{10}S_2$) and two or more sulphur-containing compounds (Bhatnagar et *al.*, 1948). The liberal amounts of these various forms of sulphur present in garlic are believed to be the basis of its antimicrobial properties, as other forms of organic sulphur compounds have been demonstrated to be efficacious against *Xcm in vitro* (Verma et *al.*, 1975).

Although the activity of *B. verticillata* against micro-organisms has not been documented, its local use as herbal treatment for eczema among the Tivs and other Nigerian tribes is widespread. Its mode of action, when applied to infected skin tissue is by necrosis of infected skin cells, and subsequent exfoliation of the dead skin layer following the formation of a new skin layer (usually 6 - 10 days after tratment). It is likely that the biochemistry and physiology of this activity may be related to the action of this plant material against the bacterial blight pathogen, although this view needs to be investigated.

It is interesting to note that in all the experiments, neem seed extract was not as effective as other materials in reducing the population of *Xcm*, given that its antifungal activity has been widely acknowledged (Emechebe, 1996; Emechebe and Alabi, 1997; Obagwu, 1997, Stoll, 1998).

Jatropha curcas Linn. is widely known in traditional medicine in both Africa and Asia, although its efficacy against plant pathogenic organisms has not been

evaluated. This work has shown the effectiveness of this plant material in significantly reducing the population and growth of the cotton bacterial blight pathogen *in vitro*. These results also confirm the report by Omo-Eboh (1999) that roots of *J. curcas*, when steeped in water are active against sexually transmitted diseases; many of which are induced by bacteria. Apparently, this activity is through bactericidal and bacteriastatic properties present in the plant material as has been demonstrated in this investigation

Although Chopra *et al*. (1956) found that *O. basilicum* demonstrated bactericidal properties against *Salmonella typhosa* and *Staphylococcus aureus*, they did not indicate the part of the plant assayed. The findings of Zaheer *et al.* (1966) that aqueous extracts of the seeds were active against Gram-positive bacteria and mycobacteria tended to confirm the earlier report by Chopra *et al*. (1958). However, extracts of the leaves of *O. basilicum* evaluated in this study were not found to possess the same antibacterial properties against *Xcm in vitro*. The reason for this difference could be due to the fact that while the ingredient that is antibiotic is highly concentrated in the seed, its presence in the leaves (and perhaps other vegetative parts) may be so scanty that it fails to manifest. Specific investigations may be required to confirm this view.

4.0 EFFECT OF PLANT EXTRACTS ON SEED-SEEDLING PARAMETERS UNDER GLASSHOUSE CONDITION

4.1 Materials and Methods

4.1.1 Collection of materials for the study: As in 3.1.1.

4.1.2 Preparation of plant extracts

Fifty grams of each of the plant materials were pounded in a porcelain mortar to form a paste which was then mixed in one litre of water and allowed to stand for six hours before being filtered through a muslin cloth to obtain a crude extract which was used to dress the seeds before sowing.

4.1.3 Seed inoculation, dressing and sowing

The pot experiment was conducted in a glasshouse. A Completely Randomised Design (CRD) was used to layout the treatments. Thirty three plastic pots of 18 cm diameter were filled with heat-sterilized topsoil obtained from the IAR. These were arranged randomly on a bench.

Three hundred grams (300g) of cotton seed were soaked in a litre of bacterial suspension adjusted to 4.7×10^7 cfu/ml for 24 hours before it was drained, and the seeds dried in the shade for 72 hours. After they had dried thoroughly, they were divided into eleven equal portions of 27.27 gms. Six of these seedlots were treated with the extracts obtained in 5.1.1 by soaking them in the extracts and allowing to stand for three hours before they were dried in the shade. Four were treated with Bronocot, Streptomycin, Apron plus and Apron star at the rates of 160 mg/27.27g of seed, 160 mg/27.27g of seed, 270 mg/27.27g of seed and 68

mg/27.27g of seed respectively. the remaining seedlot was not treated and served as untreated check.

Six seeds from each treatment were sown per pot and labeled accordingly. Treatments were arranged randomly, and the pots watered as, and when appropriate.

4.1.4 Data collection

Data was collected on the following parameters; germination, stand establishment, seedling vigour, shoot length and stem diameter.

Records of seedling emergence were taken at 14 days after sowing (DAS) by counting the number of seeds that have germinated in each treatment, and expressing this as a percentage of the total number of seeds sown for that treatment. Records of stand establishment were taken at 21 DAS. Measurement of shoot length, root length, stem diameter and seedling vigour were done at 35 DAS. Plants to be used as samples were removed from the soil by pulling gently to ensure that the roots were not broken in the process. Measurements of shoot and root length were done with a meter rule, while those of stem diameter were done with a vernier caliper. Seedling vigour was assessed on a scale of 1-6, being a modified form of that originally developed by Kim (1988), as follows:

1. Completely killed
2. Only few green tissues present
3. Definite growth reduction, severe toxicity symptoms and less likely to survive.
4. Distinguishable inhibition of growth and other injury symptoms.

5. Slight discolouration and low vigour/discolouration and necrotic spots.
6. Most healthy in the experiment.

All data collected were analysed statistically using ANOVA, and mean values separated by LSD.

4.2 Results and Discussion

The effect of plant extracts on seed and seedling parameters under glasshouse condition is outlined in Tables 4.1.

The effect of plant extracts on germination, stand establishment, root length and stem diameter did not differ from the untreated control. In comparison with all the plant extracts and the untreated control, bronocot and Apron plus produced the highest germination percentage. This did not, however, differ significantly from those of the other treatments.

The results showed that extracts of garlic (*A. sativum*) marginally lowered stand establishment and significantly reduced the shoot length and vigour of seedlings arising from that treatment. The phytotoxic effects observed with garlic indicate that this plant material is harmful to seed/seedlings at higher levels of concentration. Aliyu (1999) assessed extracts of garlic as a seed dressing material for sorghum at the rate of 25 gms/lL of water and found that it enhanced all the parameters assessed except germination; thus showing that the phytotoxic effect observed in this work could most likely be due to the relatively higher concentration of the plant material. This view necessitated the assessment of garlic at different concentrations (10g/L of water, 30g/L of water and 50g/L of water) to determine their relative effects on seed germination and other seedling parameters in cotton.

The results of this investigation, however did not show the same phytotoxic effect (Table 4.2). More investigations may be required to determine those conditions under which garlic products are toxic to seedlines or otherwise, and the reason(s) for this.

Table 4.1. Effect of plant extracts on seed-seedlings parameters under glasshouse.

Treatment	Germination (%)	Stand establishment (%)	Shoot* length (cm)	Root length (cm)	Stem diameter (cm)	Plant vigour score*
A. sativum	52.7	63.8	9.8^{b}	2.8	1.8	4.0^{b}
J. curcas	55.5	88.3	13.6^{b}	4.8	2.1	6.0^{a}
B. verticillarta	61.0	76.3	16.0^{b}	3.6	2.1	6.0^{a}
A. senegalensis	61.1	87.4	15.0^{b}	3.7	2.0	6.0^{a}
O. basilicum	61.0	85.8	13.0^{ab}	3.4	2.0	6.0^{a}
A. indica	55.5	81.6	13.6^{b}	3.5	1.9	6.0^{a}
Untreated check	61.0	85.8	15.1^{b}	4.7	2.1	6.0^{a}
Bronocot	69.4	85.8	16.2^{b}	3.8	2.1	6.0^{a}
Streptomycin	55.5	91.6	17.2^{b}	4.7	2.1	6.0^{a}
Apron plus	69.4	96.6	15.0^{b}	4.1	2.1	6.0^{a}
Apron star	66.6	80.5	15.8^{b}	3.2	2.0	6.0^{a}
LSD	NS	NS	3.37	NS	NS	0.25

**Means followed by the same superscript are not significantly different at P=0.05*

Table 4.2. Effect of different levels of garlic extracts on cotton seed-seedling parameters under glasshouse condition.

Garlic treatment (g/l)	Shoot length (cm)	Root length (cm)	Seedling vigour score
10	22.60	8.20	6.0
30	19.20	10.66	6.0
50	19.43	9.33	6.0
LSD	NS	NS	NS

Although the effects of the plant extracts were not significantly different from those of untreated control in the assessed parameters, this work has shown that apart from garlic, all the other plant extracts can be used safely in crop protection.

5.0 EFFECT OF PLANT EXTRACTS ON GERMINATION, STAND ESTABLISHMENT AND SEEDLING BACTERIAL BLIGHT OF COTTON

5.1 Materials and Methods

Collection of materials, preparation of plant extracts, seed inoculation and dressing was done in the same way and proportions as in sections 4.1.1 and 4.1.2 above. The trial was based on a Completely Randomised Block Design (CRBD) with three replications. The cotton variety recommended for the Northern Cotton Zone, SAMCOT 9 was used in the experiment. A total of 120 seeds were sown per plot at the rate of 6 seeds/stand, with a spacing of 40 cm apart within the row. Each plot was a standard ridge with inter-row spacing of 0.9m, and was 10m long. Each ridge had a total of 20 plant stands. All the treatments were allocated randomly. A total of 360 seeds (6 seeds x 20 stands x 3 reps) of each treatment were sown.

5.1.2 Data collection

Data collection on germination and stand establishment was done as in section 5.1.3 above. Assessment of seedling blight was done at 35 DAS using a modified scale of 1-6 originally developed by Poswal (1981) as follows:

1. No infection on cotyledons
2. Water-soaked lesions numbering from 1-5 and less than 0.5 mm in diameter.

3. Water-soaked lesions ranging from 0.5 – 2 mm, no coalescing.
4. Profuse 2 mm water-soaked lesions with occasional coalescing, no browning of lesions or necrotic areas.
5. Profuse 3 – 4 mm lesions, extensive coalescing, browning of lesions or necrotic areas.
6. Large, brown necrotic lesions above 4mm, extensive coalescing, frequent yellowing or browning of cotyledons and/or dead cotyledons.

On this scale,

1	=	highly resistant (no infection)
2 - 3	=	resistant (minimum infection)
4	=	moderately resistant (moderate infection)
5 - 6	=	susceptible (severe infection).

Mean values of disease grade were calculated for each treatment sample.

Disease incidence was expressed as a percentage of the proportion of diseased plant stands relative to the total number of stands using the formula:

$$\text{D.I.} = \frac{\text{Total number of stands infected}}{\text{Total number of stands observed}} \times 100$$

All data collected was analysed statistically using ANOVA, and mean values separated by LSD.

5.2 Results and Discussion

The effect of plant extracts on germination stand establishment and seedling bacterial blight under field condition is summarized in Tables 5.1 and 5.2 (for the 1999/2000 and 2000/2001 rainy season respectively). The results show that for

both seasons, the plant extracts had no effect germination and stand establishment. All the plant extracts, however significantly reduced the incidence of bacterial blight more than untreated control. In comparison with all the plant extracts, and the other treatments, bronocot produced the highest germination rates, although these were not significantly different from other treatments. It was also marginally more effective in reducing the incidence of seedling bacterial blight than all the other treatments, although the performance of *B. verticillata* was marginally better than bronocot in enhancing seedling stand establishment. Treatments had no effect on the severity of seedling bacterial blight.

Table 5.1. Effect of plant extracts on germination, stand establishment and seedling bacterial blight (1999/2000 season)

Treatment	Germination (%)	Stand establishment (%)	Disease incidence* (%)	Disease severity score
A. sativum	70.8	80.0	18.3[b]	2.0
J. curcas	71.1	80.0	18.3[b]	2.0
B. verticillarta	68.8	81.6	18.3[b]	2.0
A. senegalensis	70.0	71.6	16.5[b]	1.6
O. basilicum	66.9	78.3	20.0[b]	1.6
A. indica	58.0	70.0	20.0[b]	2.0
Untreated check	69.1	73.3	31.6[a]	2.3
Bronocot	74.1	80.0	15.0[b]	1.6
Streptomycin	68.0	66.6	15.0[b]	2.3
Apron plus	69.1	76.6	18.3[b]	2.0
Apron star	68.8	75.0	16.5[b]	2.0
LSD	NS	NS	2.15	N.S

Means followed by the same superscript are not significantly different at P=0.05

For the 2000/2001 season, a similar trend was observed. Extracts of garlic (*A. sativum*) were found to have produced the highest percentage of seedling emergence (germination) over those of the other treatments except bronocot. These differences were, however, not statistically significant. With respect to stand establishment, all the plant extracts performed better than the proprietory pesticides, including bronocot. Among the plant extracts, *O. basilicum* and *B. verticilla* performed better than the other plant extracts. These differences were, however, not significant.

Table 5.2. Effect of plant extracts on germination, stand establishment and seedling bacterial blight (2000/2001 season)

Treatment	Germination (%)	Stand establishment* (%)	Disease incidence* (%)	Disease severity score
A. sativum	71.1	72.6^{bc}	5.5^{d}	2.0
J. curcas	69.9	77.8^{bc}	33.3^{ab}	1.6
B. verticillarta	70.5	80.4^{b}	27.7^{abc}	1.6
A. senegalensis	69.9	80.2^{b}	27.7^{abc}	2.0
O. basilicum	67.2	81.7^{b}	38.8^{ab}	2.3
A. indica	68.8	79.0^{bc}	38.8^{ab}	2.0
Untreated check	68.6	76.6^{bc}	44.4^{a}	2.0
Bronocot	74.4	100.0^{a}	5.5^{d}	1.6
Streptomycin	69.4	82.4^{b}	11.1^{cd}	2.0
Apron plus	70.5	100.0^{a}	22.2^{bcd}	2.0
Apron star	70.2	100.0^{a}	22.2^{bcd}	1.6
LSD	NS	7.31	21.41	N.S

Means followed by the same superscript(s) are not significantly different at P=0.05

In respect of their effects on seedling bacterial blight, extracts of garlic (*A. sativum*) showed the highest level of efficacy. Its effect was the same as that of bronocot,

and was significantly higher than those of all the other treatments, including streptomycin. It was followed by streptomycin, *B. verticillata*, and *A. senegalensis*. These were similar to each other, but, although they were marginally more efficacious than *O. basilicum* and *A. indica*, these differences were not statistically significant. All the treatments significantly reduced levels of seedling bacterial blight more than untreated control.

This work has again confirmed the effectiveness of garlic (*A. sativum)* in significantly reducing bacterial blight. These results are in agreement with those of the *in vitro* evaluation of garlic for its efficacy against the cotton bacterial blight pathogen. A review of available literature has shown that garlic has hitherto not been assessed as a seed-dressing for cotton under field conditions, but Emechebe (1996) reported that foliar applications of 1% aqueous extracts of the plant material applied as a foliar spray were moderately effective in reducing cowpea bacterial blight induced by *X.c.* pv *vignicola* (Burkholder) Dye at Samaru. The efficacy of garlic against a wide variety of pathogens has been attributed to the liberal amounts of sulphur compounds present in the bulb. These results have shown that the active principle responsible for this antibiotic effect remains effective even under field condition. It is interesting to note that the phytotoxic effects observed with this plant material in the glasshouse were not noticed in the field. The reason(s) for this disparity is/are not clear. The positive results obtained with *A senegalensis* are in agreement with those of Emechebe and Alabi (1997) who found it to be effective in controlling a variety of fungal diseases associated with cowpea at Zaria, when applied as a foliar spray. The failure of *A. indica* and *O. basilicum* to significantly affect seedling bacterial blight in the field, is, again

consistent with results of the *in vitro* experiments; and in agreement with the findings of Emechebe and Alabi (1997) who reported that foliar application of neem seed extract was ineffective in reducing cowpea bacterial blight.

Borreria verticillata has not been documented as possessing antibiotic properties, although its use as a herbal treatment for eczema is widespread among many Nigerian tribes. It is suggested that the basis of its action against this notorious skin infection in humans may be related to its ability to also act against the bacterial blight pathogen.

6.0 SUMMARY AND CONCLUSION

Extracts of six plant materials viz; garlic (*Allium sativum* Linn.), *Jatropha curcas* L.; *Borreria verticillata* L., cussstard apple, (*Annonia senegalensis* L.); the sweet basil (*Ocimum basilicum* L.); and neem (*Azadirachta indica* (A) Juss) were evaluated for the control of cotton seedling bacterial blight induced by *Xanthomonas campestris* PV *malvacearum* (Smith) Dye under laboratory, glasshouse and field conditions at Samaru.

In vitro studies were conducted to assess the effect of plant extracts on the cotton bacterial blight pathogen. Two methods were employed in carrying out the assessments, namely the dilution plate count and agar cup methods. Extracts were evaluated at the rate of 5 gms/100 mls of water. Streptomycin, and three proprietary pesticides; bronocot, Apron plus, and Apron star were used as checks alongside the untreated control. These were assessed at the rate of 20mg/ml of water. Garlic extract was found to be the most effective, and significantly reduced

bacterial population and growth over the untreated control. *A senegalensis*, *B. verticillata* and *J. curcas* were moderately effective, while the performance of *O. basilicum* and *A. indica* relative to untreated control was very marginal. In comparison to the plant extracts, and all other treatments, bronocot had the greatest inhibitory effect, however, this was not significantly different from that of *A. sativum* in the dilution plate count method.

In order to determine their effect on seed-seedling parameters, aqueous extracts of the plant materials were assessed under glasshouse condition. Cotton seeds pre-inoculated with the pathogen by soaking them for 24 hours in a bacterial suspension of the pathogen adjusted to *ca* 4.7×10^7 cfu/ml and then air drying them before treatment with the plant extracts were done, and the seeds sown in plastic pots at the rate of 6 seeds/pot. Data on germination, stand establishment, shoot length, root length, stem diameter, and plant vigour were collected. The experiment was based on a CRD with three replications, and was repeated once. Results showed that extracts had no significant effect on germination, stand establishment, root length and stem diameter. Scores of seedling vigour and measurements of root length, however, revealed that extracts of garlic significantly reduced shoot length and the vigour of seedlings arising from that treatment. In both parameters, extracts of garlic significantly differed negatively from the other treatments. Another experiment was conducted to determine the concentration of garlic extract which could be considered safe for seedlings, while at the same time retaining its potency. It was found that concentrations up to 50g/L of water were safe enough.

A field experiment was also conducted in order to evaluate the effect of extracts on germination, stand establishment, and seedling bacterial blight of cotton. Seed inoculation and sowing was as in the glasshouse experiment. Treatments were done on plots 10m long. Six seeds were sown per hole at a spacing of 40 cm within the row. Data on germination, stand establishment and seedling bacterial blight were collected and analysed. Results showed that the extracts did not significantly affect germination, although extracts of garlic performed marginally better than all the other extracts over the untreated control. Plant stand establishment was also not affected by the treatments, although bacterial blight incidenced was reduced significantly by all the treatments over untreated control.

This work has demonstrated the effectiveness of garlic (*Allium sativum*) in significantly reducing cotton seedling bacterial blight. It is worthy of mention that efforts at controlling this disease in Nigeria have hitherto concentrated on seed treatment with bronocot. However, apart from the hazards associated with the indiscriminate use pesticides, erratic supply and rising costs have placed many of them beyond the reach of many resource-poor farmers on whom the production of cotton in Nigeria largely depends. Bulbs of garlic are relatively cheap and available all year round. The methodology adopted for this work is also simple and straight-forward. It is, therefore, hoped that the results of this study will be relevant to improved cotton production in Nigeria.

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Appendix 1. ANOVA procedure for dilution plate count method (Experiment I).

Source	DF	SS	MSS	F
Treatment	10	77039.5152	77003.9515	80.07**
Error	22	21158.0000	961.7273	
	32	98197.5152	77965.6788	

LSD = 52.51.

Appendix 2. ANOVA procedure for dilution plate count method (Experiment II).

Source	DF	SS	MSS	F
Treatment	10	582488.0606	58248.8060	106.96**
Error	22	11980.6667	544.5758	
	32	594468.7273	58793.3819	

LSD = 39.52.

Appendix 3. ANOVA procedure for agar cup method (Experiment I).

Source	DF	SS	MSS	F
Treatment	10	5332.2121	533.2212	1099.8787**
Error	22	10.6667	0.4848	
	32	5342.8788	533.7060	

LSD = 1.179.

Appendix 4. ANOVA procedure for agar cup method (Experiment II).

Source	DF	SS	MSS	F
Treatment	10	5338.0757	533.8075	85.0000**
Error	22	138.1666	6.2803	
	32	5476.2424	540.0878	

LSD = 4.244.

Appendix 5. ANOVA procedure for germination in the glasshouse (Experiment I).

Source	DF	SS	MSS	F
Treatment	10	1851.5926	185.1592	0.4489^{NS}
Error	22	9073.9262	412.4511	
	32	10925.5188	597.6103	

Appendix 6. ANOVA procedure for seedling stand establishment in the glasshouse (Experiment I)

Source	DF	SS	MSS	F
Treatment	10	2616.8236	261.6823	0.5124^{NS}
Error	22	11234.0001	510.6363	
	32	13850.8237	772.3186	

Appendix 7. ANOVA procedure for shoot length (Experiment I).

Source	DF	SS	MSS	F
Treatment	10	86.9073	8.6907	1.6781^{NS}
Error	22	113.9334	5.1787	
	32	200.8407	13.8694	

Appendix 8. ANOVA procedure for root length (Experiment I).

Source	DF	SS	MSS	F
Treatment	10	17.9521	1.7952	1.4614^{NS}
Error	22	27.0267	1.2284	
	32	44.9788	3.0236	

Appendix 9. ANOVA procedure for stem diameter (Experiment I).

Source	DF	SS	MSS	F
Treatment	10	0.6352	0.0635	0.5461[NS]
Error	22	2.5600	0.1163	
	32	3.1952	0.1798	

Appendix 10. ANOVA procedure for germination in the glasshouse (Experiment II).

Source	DF	SS	MSS	F
Treatment	10	1531.7812	153.1781	0.4333[NS]
Error	22	7777.2224	353.5101	
	32	9309.0036	506.6882	

Appendix 11. ANOVA procedure for seedling stand establishment in the glasshouse (Experiment II).

Source	DF	SS	MSS	F
Treatment	10	3052.7394	305.2739	0.0405[NS]
Error	22	1666.0742	752.2761	
	32	4718.8136	1057.5500	

Appendix 12. ANOVA procedure for shoot length (Experiment II).

Source	DF	SS	MSS	F
Treatment	10	249.3333	24.9333	2.4657*
Error	22	222.4591	10.1117	
	32	471.7924	35.0450	

LSD = 5.3847.

Appendix 13. ANOVA procedure for root length (Experiment II).

Source	DF	SS	MSS	F
Treatment	10	18.5230	1.8323	0.6967^{NS}
Error	22	57.8534	2.6297	
	32	76.3764	4.4620	

Appendix 14. ANOVA procedure for stem diameter (Experiment II).

Source	DF	SS	MSS	F
Treatment	10	0.2940	0.0294	0.6433^{NS}
Error	22	1.0467	0.0457	
	32	1.3407	0.0751	

Appendix 15. ANOVA procedure for germination in the field 1999/2000 season.

Source	DF	SS	MSS	F
Treatment	10	507.0073	50.7007	1.164^{NS}
Error	22	957.4539	43.5206	
	32	1464.4612	94.3213	

Appendix 16. ANOVA procedure for stand establishment in the field 1999/2000 season.

Source	DF	SS	MSS	F
Treatment	10	672.7273	67.2727	0.6436^{NS}
Error	22	2299.2727	104.5123	
	32	2972.0000	171.785	

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Institutions Attended	**From**	**To**	**Cert/Degree**
St. John's Prim. School, Gboko	1975	1981	1st School Cert.
St. Gabriel's Sec. Sch. Makurdi	1981	1986	G.C.E. O'Level
School of Basic Studies, A.B.U., Zaria	1987	1988	I.J.M.B. A'Level
Ahmadu Bello University, Zaria	1988	1994	B. Agric.

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Community Sec. Sch. Ayere, Ijumu, Kogi State	1994	1995	Agric. Master
Akperan *Orshi College* of Agric. Yandev, Benue State	1995	1996	Lecturer (Part time).
Sunseed Nigeria Plc, Dakace, Zaria	1997	1998	Extension Officer
Sunseed Nigeria PLC, Dakace, Zaria	1998	1999	Extension Supervisor
Dept. of Crop Protection, Ahmadu Bello University, Zaria	1999-Date		Assistant Lecturer

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Appendix 17. ANOVA procedure for seedling bacterial blight 1999/2000 season.

Source	DF	SS	MSS	F
Treatment	10	621.2121	62.1212	2.2162*
Error	22	616.6667	28.0303	
	32	1237.8788	90.1515	

LSD = 2.15

Appendix 18. ANOVA procedure for germination, 2000/2001 season..

Source	DF	SS	MSS	F
Treatment	10	1851.5926	185.1592	0.4489^{NS}
Error	22	9073.9262	412.4511	
	32	10925.5188	597.6103	

Appendix 19. ANOVA procedure for crop stand establishment, 2000/2001 season.

Source	DF	SS	MSS	F
Treatment	10	3146.1813	314.6181	16.8604**
Error	22	410.5233	18.6601	
	32	3556.7046	333.2782	

LSD = 7.3149.

Appendix 20. ANOVA procedure for seedling bacterial blight, 2000/2001 season.

Source	DF	SS	MSS	F
Treatment	10	5438.0775	543.8077	3.4**
Error	22	3518.7039	159.9510	
	32	8956.7814	703.7487	

LSD = 21.4161.