SEASON OF HARVEST OF *Amaranthus cruentus* L. IN RELATION TO SEED-BORNE MYCOFLORA AND MAJOR SHOOT DISEASES

BY

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A Thesis in the Department of
AGRICULTURAL BIOLOGY

Submitted to the Faculty of Agriculture and Forestry
in partial fulfilment of the requirements
for the degree of
DOCTOR OF PHILOSOPHY
of the
UNIVERSITY OF IBADAN
IBADAN

April, 1990

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ABSTRACT

The quality of *Amaranthus cruentus* L. seeds were influenced both by seasons of harvest and varietal type. The wetness that accompanied harvests of the first (April-June) and second (August-October) seasons greatly reduced the quality of seeds of cultivars. Quality reduction were in the form of seed malformation, discolouration and mycelial growth on them. Fifteen fungal species were found to be associated with seeds of the cultivars used. There was a seasonal variability in the occurrence, distribution and the quantity of the seed-borne fungi. Least number and mean percentage seed infection were recorded for $NHAc_{100}$ while $NHAc_{30}$ and $NHAc_{33}$ carried the highest percentage inoculum on the seed testa. Three of the seed-borne fungi were highly pathogenic on seedlings of two of the cultivars while two of the fungi were seed transmitted. Six of these seed-borne mycoflora are new records on *Amaranthus* seeds in Nigeria.

For the two year trials, significantly (P=0.05) least incidence (0%) of dieback, stem blight (8%) were recorded during the first season for $NHAc_{33}$ and $NHAc_{30}$ while 4% mean leaf blight was obtained from $NHAc_{100}$ in the third (December-February) season. Least mean percentage leaf blight was

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recorded for all the cultivars also by the third season. Conversely, the second season recorded the highest incidence of all these aerial symptoms of the disease caused by Choanephora cucurbitarum (Berk & Rav.) Thaxt. in all the cultivars.

Hot water treatment of infected seeds of all cultivars at 60°C for 6 to 8 minutes completely disinfested them of the seed-borne fungi with consequent increase ($>$ 95%) in seedling emergence. Also soaking in or dusting of infected seeds with a mixture of Benlate-Captafol or single Captafol at 10g a.i./kg seed controlled the seed-borne fungi. Pre-harvest sprays of Benlate-Captafol mixture or Captafol at 3.3 kg/ha controlled inflorescence infection and seed-borne fungi with resultant yield increases and seedling emergence over the non-treated control.

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ACKNOWLEDGEMENT

I thank my supervisors Professor M.O. Adeniji and Dr. Tunde Ikotun most sincerely for their good dispositions, expertise advice and very highly useful technical suggestions. Their wealth of experience made tremendous impact on the standard of this work.

The experts on my Supervisory Committee: Drs. E.J.A. Ekpo, G.I. Atiri and A.O. Oladiran did a very thorough job. Their individual and collective suggestions, objective criticisms right from the proposal stage till the final write-up had helped a lot in raising the standard of this work. They, also along with my supervisors assisted in reading through all stages of the work in good time on any of the occasions. I appreciate their input and express my profound gratitude to them all.

I am grateful to the Ogun State University, Ago-Iwoye, Nigeria, for financial support during the course of this programme.

The Management of the National Horticultural Research Institute (NIHORT), Ibadan, Nigeria, assisted by allowing the use of their field, nursery and screen-house spaces. For this, I am indeed grateful.

The assistance in statistical analysis rendered by

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Dr. Kunle Ogunfidodo (and his staff) of NIHORT and the computer facilities provided by the International Institute of Tropical Agriculture, IITA, are acknowledged with thanks.

I also thank Mr. M.A. Shittu of NIHORT for handling the typing of this work at every stage.

I thank my colleagues at the Department of Biological Sciences of the Ogun State University, Ago-Iwoye most sincerely and the Ajayis, Adebanjos of Oru Ijebu, Dosumus and others for sharing with me and soothing the anxiety experienced during this programme.

Thanks to the International Mycological Institute, IMI, of the C.A.B., Kew, for the identification and confirmation of fungi isolated from the seeds.

I am also grateful to mother, Chief (Mrs.) S. Nuga-Adebanjo and my brother Mr. A.O. Adebanjo for my upbringing and encouragement.

My devoted wife Adenike Oluwafunmilayo Adebanjo and all my children encouraged and supported me in all ways while on this programme. I am indeed ever grateful to them.

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DEDICATION

This thesis is affectionately dedicated to my eldiest sister Mrs. Esther Adedoja Onafeko (Nee Adebanjo) who thought it fit to send me to school at a very tender age despite the fact that we lost our father at a very tender age.

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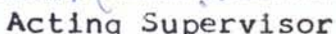
CERTIFICATION

This is to certify that this work now submitted as a thesis for the degree of DOCTOR OF PHILOSOPHY of the University of Ibadan, is the result of investigations (except where reference is made to published literature and where assistance is acknowledged) by

MR. AFOLABI ADEBANJO

of the Department of Agricultural Biology, of the University of Ibadan, Nigeria and has not been part of any presentation for any other qualification.



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CHAPTER 1

INTRODUCTION

Amaranthus is widely grown as a green leafy vegetable or as a grain crop in parts of sub-tropical and tropical Asia, Africa, China and Central America (Herklots, 1972; Harwood, 1980). The species commonly grown are *A. cruentus* and the cereal Amaranth, *A. caudatus* (Sauer, 1967).

Amaranthus is used for the treatment of toothache, acute bronchitis, boil and liver diseases (Heyre, 1950; Berhaut, 1971) and for the preparation of red dye (Sauer, 1967). *A. tricolor* has been used for ornamental purposes in parks and gardens (Wit, 1963). *Amaranthus* is used for the preparation of soups and stews in Nigeria. It contains a wide assortment of minerals, vitamins, carbohydrates, lipids, proteins and fibre which are the nutrients required by various age groups (Oyenuga, 1968; Oyenuga and Fetuga, 1975). *Amaranthus* is rich in calcium, iron and potassium, zinc and other micro minerals (FAO, 1988). Grain *Amaranthus* still remains an important crop in some parts of India (Vavilov, 1951). With protein contents of about 16%, *Amaranthus* seeds compare well with conventional varieties of wheat (12 - 14%); rice (7 - 10%), maize (9 - 10%) and other widely consumed cereals (Anon, 1984).

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Amaranthus is grown from seed and can yield many seeds to guarantee survival (Anon, 1984). The seed is the primary and essential foundation for the success of the future crop. Good quality seed is essential because it frequently produces higher yields per hectare, fewer off-type plants and a high quality crop which may bring a higher price (Mac-Gillivary, 1961). The overall quality of the seeds are those qualities acquired during growth, harvesting, processing and storage and these determine the quality of the seedling produced (Okoro and Jones, 1977; Nwoboshi, 1982). Seed quality also embraces potential germination (including vigour), genetical quality, mechanical purity and freedom from seed transmitted pathogens and pest (George, 1987). Many seeds harbour a great variety of microflora especially fungi. This is particularly true of those seeds that are exposed to contaminations by air-borne inoculum and those infected through the mother plant.

In Nigeria, seed pathology started a few years back and emphasis on seed-borne mycoflora has been on cowpea (Onesirosan, 1978), rice, maize, wheat and soya-bean of late (Kilpatrick, 1957; Sinclair, 1981). The vegetables appear to have received very little attention.

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Observations at the National Horticultural Research Institute (NIHORT), Ibadan, Nigeria, have shown that the establishment of *Amaranthus* seeds directly in the field could be difficult. Even when attempts are made to raise seedlings in the nursery, the number of established seedlings do not justify the quantity of seeds planted in most cases (Anon., 1983 and 1985).

Many reports have indicated that the major field fungal disease of *Amaranthus* is apical shoot blights, dieback and leaf disease caused by *Choanephora cucurbitarum* (Irvine, 1969; Maduewesi, 1970; Ikediugwu, 1981). Season of harvest may likely play an important role in the above-ground shoot and inflorescence hence seed infection of *Amaranthus* (Anon., 1985). Furthermore, there have been no report on the control of *Amaranthus* inflorescence infection by *C. cucurbitarum*. Although, efforts have been made to control seed-borne fungi mostly for rice, maize and wheat by thermotherapy (Baker, 1962, 1972) and by the use of chemicals (Neergaard, 1977) but to date, these treatments have not been applied on *Amaranthus* seeds. There is a dearth of information on the nature of seed-borne fungal flora of *Amaranthus* and their control, control of *Amaranthus* inflorescence infection

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and the effect of environmental conditions at the time of harvest on the shoot diseases severity, quality and viability of seeds.

The objectives of this study were:

(a) to study the effect of season of harvest on seed-borne mycoflora of *Amaranthus*;

(b) to investigate the effect of season of harvest on the incidence of the major *Amaranthus* shoot diseases;

(c) to establish the pathogenicity of the detected seed-borne and shoot-borne fungi;

(d) to screen seed treatment measures for the control of the seed-borne fungi; and

(e) to investigate pre-harvest chemical treatments for control of inflorescence infection and field-acquired mycoflora of the seeds.

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CHAPTER 2

2. LITERATURE REVIEW

2.1 Fungi infecting growing Amaranthus plants

A review of the fungi infecting growing Amaranthus plant is necessary because there is a relationship between seed-borne fungi and field diseases. Pathogens isolated from diseased plant parts were also detected on the seeds (Joaquim, 1976).

Species of Amaranthus have a range of fungi associated with the growing plant. A. caudatus has been reported to be susceptible to Alternaria alternantherae in Pennsyslvania, causing much leaf damage that drastically reduces plant vigour (Anon., 1984). A similar blight of the leaves and flowers caused by Alternaria amaranthi (Peck) was reported in India on other Amaranthus spp. White rust, caused by Albugo bliti, showing white pustles on the under-surface of the leaves has been reported to reduce the market value and quality of Amaranthus (Nicholas and Aggery, 1952; Mishra and Chona, 1963; Anon., 1984). Solheim and Stevens (1931) isolated Cercospora canescens from diseased leaves while C. beticola was isolated from infected leaves of Amaranthus retroflexus (Anon., 1933). The crop was also reported to be a host plant of Verticillium dahliae (Martinson, 1964), Rhizoctonia spp. and Verticillium albo-atrum (Oshinma et al.,

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1963) and V. nigrescens (Evans, 1968). Infection by Rhizoctonia solani (Deighton, 1931) and V. amaranthi (Verona and Ceccareli, 1935) were also recorded.

Taubenhaus and Ezekiel (1931) reported a wilt caused by Sclerotinia sclerotiorum in A. retroflexus. Sharples (1929) isolated Pythium aphanidermatum from diseased seedlings of Amaranthus spp. Kusakari et al. (1979) reported the damping off of spinach seedlings by Pythium sp. Also Sealy (1988) evaluated one hundred and twenty-six Amaranthus accessions for resistance to damping-off disease caused by Pythium myriotylum. Palm and Jochems (1924) associated C. cucurbitarum with an Amaranthus sp. Bremer (1954) isolated Macrophomina phaseoli, Fusarium solani, Rhizoctonia solani, Colletotrichum altramentarium from the roots of healthy tomato and Amaranthus retroflexus growing in the same field as the diseased egg-plant, potato and chilli. Irvine (1969) reported that the pathogen only infect Amaranthus leaves while Maduewesi (1970) listed C. cucurbitarum as causing a dieback of an Amaranthus sp. Odebunmi-Osinkanlu (1977) isolated a Choanephora spp. from Amaranthus and observed that such an infection could result in total crop failure.

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2.2. Seed-borne pathogens

The incidence of seed-borne pathogens and of pathogens which may be seed-borne but cause diseases of varying intensities in the growing plants have been reported for other crops by (Prasad and Sinha, 1963; Bisht and Mathur, 1964; Aluko, 1969; Sharma and Mukherji, 1970; Vir and Gaur, 1970). Some of these seed-borne mycoflora may reduce the germinability of the seeds, speed of germination, seedling vigour, yield and quality of product. Fungal pathogens have been implicated in soybean seed deterioration before harvest (Wilcox *et al.*, 1974; Roy and Abney, 1977; Paschal and Ellis, 1978).

Only a few accounts of the association of a fungus with *Amaranthus* seeds were obtained in general throughout the exhaustive literature search. Venkatakrishniah (1952), reported the severe attack by *Alternaria amaranthi* on the flowers of *Amaranthus paniculatus* in India. He stated that the fungus caused the blackening of *A. paniculatus* flowers on the central spike presenting the appearance similar to smut. He concluded that such an infection can spread to colonize the seed since conidia and conidiophores were detected in the stigma and ovary of infected flowers.

The presence of mycelium and oospores of *Cystopus*

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bliti in the seeds of Amaranthus retroflexus was reported by Melhus (1931). The fungus caused a reddish or light brown discolouration of the normally green flowers accompanied by hypertrophy and distortion of the flowers and stem. Joaquim (1976) did some work with Amaranthus hybridus and found the seed to harbour Fusarium moniliforme, F. semitectum, F. solani, Alternaria amaranthi and Cystopus bliti. She reported further the presence of Curvularia lunata and Aspergillus flavus on seeds of A. hybridus. Out of these seed-borne fungi, F. semitectum was recorded at highest percentage (8.75%) while the rest were recorded at very low levels. All these seed-borne fungi were not found to contribute to disease development in the field although F. semitectum and C. bliti were found to be pathogenic on Amaranthus seedlings in the laboratory. A. flavus and C. lunata she concluded were mere saprophytes.

Sharma et al. (1980) found the following fungi to be associated with the seeds of Amaranthus caudatus in India - Alternaria alternata; A. amaranthi; A. tenuissima; Aspergillus flavus; Botrytis cinera; Cephalosporium curtipes; Cercospora sp.; Cladosporium cladosporioides; C. sphaerospermum; Coniothecium atrum; Curvularia

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pallescens; Fusarium sp.; F. culmorum; F. moniliforme; Memnoniella echinata; Mucor globosus; Oedocephalum glomerulosum; Phoma amaranthi; P. glomerata; Rhizopus oryzae; Stachybotrys atra; Stemphylium botryosum; Stysanus medius; Trichothecium roseum and Ulocladium botrytis. He reported that the following seven fungi, out of all, A. alternata, Cladosporium, F. moniliforme, Penicillium sp., Phoma amaranthi, R. oryzae and Trichoderma viride significantly reduced the number of normal seedlings over control and caused stem rot and seedling blight.

2.3. Effect of seasons of harvest on seed-borne mycoflora

There is little information on the effect of environmental conditions at the time of harvest on the level of associated mycoflora, quality and germinability of well studied seeds of maize, rice, wheat and sorghum.

The only documented information obtained on the effect of season of harvest on seed-borne mycoflora was on cowpea which is also regarded as a vegetable crop. In a study carried out by Onesirosan (1983), the following fungi were isolated from cowpea seeds at harvest: Fusarium equiseti; Botryodiplodia theobromae; Cladosporium spp.; Rhizopus stolonifer; R. arrhizus and a species of Colletotrichum.

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He reported that the level of internally seed-borne microorganisms was much higher in the wet season harvest than in the dry season. Some of the microorganisms isolated during the wet season were completely absent in the dry season. The aesthetic quality of wet season seeds was also much poorer due primarily to invasion by F. equiseti than that of dry season seeds. Furthermore, he concluded that seeds harvested in the wet season generally had a low germination percentage than those harvested in the dry season.

Nangju (1977) also reported that the poor quality and low viability of cowpeas and soyabeans in the wet season were accompanied by a high level of microbial infection.

2.4. Control of field acquired and seed-borne pathogens

2.4.1. Seed treatments

The value of treating vegetable seeds to destroy disease-producing organisms carried with the seed has been proven repeatedly. Protection is also provided against certain seed-rotting and seedling blight fungi in the soil. Seed treatment gives maximum insurance benefits when cold wet weather follows planting or when seeds germinate

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slowly (Shurtleff and Linn, 1971).

Seed treatments are of two general types: eradicative, which destroys disease-causing fungi and bacteria carried on and within the seed, and protective, which apply a chemical coating to the surface of the seed to protect it against decay and damping-off caused by soil organisms (Shurtleff and Linn, 1971). Both types of treatment are important in producing healthy vigorous vegetable plants.

Hot water treatment (thermotherapy) can be used as an eradicative treatment to control disease on a number of kinds of vegetable seeds (Shurtleff and Linn, 1971). A hot water treatment properly applied just before planting, kills most of the internal and external disease causing organisms but is recommended for the seeds of a few of the vegetable including spinach (Shurtleff and Linn, 1971). Seed can be treated against seed rot, damping-off, seedling blight, root rot and _Fusarium_ wilt. Baker (1962, 1972) reviewed the use of thermotherapy for the control of plant diseases and seed-borne pathogens. Many media have been used to heat-treat seed: water, steam, air, carbon tetrachloride (CCl_4), petroleum oils, vegetable oils, and microwave irradiation (Watson _et al._, 1951; Baker, 1962 and Matthews, 1970). Hot water is the most commonly

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used medium (Zinnen and Sinclair, 1982). However, seeds of large seeded legumes, such as soybean (Glycine max (L.) Merr) and green beans (Phaseolus vulgaris (L.), rarely are heated in hot water because they quickly imbibe water, slough off their seed coats, hence non-aqueous fluids (e.g. CCl_4) are used (Watson et al., 1951). Information is lacking on the application of hot water for the control of seed-borne pathogens of Amaranthus. However, Joaquim (1976) reported that treatment of Corchorus olitorius (a leafy vegetable) in hot water at 70°C for 10 mins broke its dormancy. This treatment also reduced infection by Cercospora corchori from 49% in untreated seeds to 4% in the treated seeds and the seed germination rose to well over 80%. Similarly, Neergaard (1977) reported on a variety of non-chemical seed treatment methods as means that have been practised for many centuries by agriculturists.

Apart from the non-chemical methods of seed treatment, chemical method of seed protection (Seed-dressing) has also been in practice for long (Neergaard, 1977). Chemical seed treatments are applied to control externally - or internally-borne pathogens intimately associated with seeds or soil-borne pathogens of seed and seedlings (Sinclair, 1981). Seed treatment has been carried out

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with fungicides belonging to the group of organo-mercurials, dithiocarbamates, antibiotics and systemic fungicides (Vir, 1983). Most studies have shown that fungicidal seed treatment is fairly effective against a number of seed-borne diseases. In a few instances, seed treatment also affords some protection against soil-borne pathogens, during pre- and post-emergence phases of the plant (Vir, 1983). The net gains achieved as a result of improved germination, disease control and higher yields are many.

Sharma et al. (1980) evaluated ten different fungicides and six different plant extracts (as seed-dressings) for the control of seed-borne fungi of Amaranthus caudatus in India. Out of the ten fungicides tested, 0.2% Agrosan GN (Ethyl mercury acetate) and Ceresan (Phenyl mercury chloride and phenyl mercury acetate) proved to be effective and controlled almost all of the common seed-borne fungi. Although the percent incidence of different fungi was less when the seeds were treated with various plant extracts but none of them proved to be effective in inhibiting the growth of common seed-borne fungi over chemical seed treatment. Apart from this study on A. caudatus, the other information obtained was the effectiveness of 4g/kg seed of each of Orthocide, Diquinone and Phygon dust for

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the control of seed-borne fungi of tomato, pea, pepper and carrot (Nakamura *et al.*, 1972).

2.4.2. Pre-harvest chemical treatment

Seed-borne fungi can reduce seed quality and serve as a source of primary inoculum for pathogens, which affect disease incidence and spread of certain pathogens (Leach, 1979). The benefits of controlling seed-borne pathogens go beyond increasing yield, the parameter most frequently used to justify fungicide sprays (Sinclair, 1983). The use of pre-harvest fungicide sprays to control diseases caused by seed-borne pathogens are on control of symptoms, effect on yield and other agronomic parameters and rarely on results from seed-assays for the effect on internally seed-borne fungi (Sinclair, 1983). It is only through the use of assays of seeds can internally seed-borne fungi be detected and the effect of pre-harvest sprays be evaluated. The use of fungicide sprays for control of internally seed-borne fungi as measured by the assay of seeds is recent in origin (Sinclair, 1983). Therefore, studies on the use of fungicide sprays to control seed-borne pathogens in order to increase the quality (pathogen-free seeds) and quantity have not been done for *Amaranthus*. However, the

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only information available on fungicidal sprays for control of seed-borne fungi of one of the vegetables was on okra. Vindhyasekaran and Kandaswamy (1980) reported that pre-harvest spray with monocrotophos plus carbendazim effectively controlled some seed-borne fungi of okra in India.

The first report on the use of fungicide sprays to control seed-borne fungi as determined by seed assay was by Crittenden and Yelen (1967). There are however, many reports on the use of pre-harvest fungicide sprays to control diseases caused by pathogens that are seed-borne in rice, soybean, wheat (Sinclair, 1981); wheat (Cook, 1977; Eberle and Mayr, 1979).

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CHAPTER 3

3. MATERIALS AND METHODS

3.1. Seed source

The seeds of Amaranthus cruentus, cultivars $NHAc_{33}$, $NHAc_{30}$ and $NHAc_{100}$ were collected from the breeders stock at the National Horticultural Research Institute (NIHORT), Ibadan, Nigeria. The choice was based on susceptibility to diseases and pests (Anon., 1983; 1985) and Denton (Personal Communication) and general acceptability by consumers (Badra and Denton, Personal Communication).

3.2. Detection of mycofloral infection of seeds at seasons of harvest

Apparently healthy seeds of three susceptible Amaranthus cultivars, $NHAc_{33}$, $NHAc_{30}$ and $NHAc_{100}$ were raised in seed trays in the nursery. After three weeks, the seedlings were transplanted onto $1m^2$ plots in the field. The plots comprised three rows spaced 25cm within and between row and there were a total of fifteen plants per plot of each cultivar replicated four times in a randomized complete block design. Manual weeding was done when necessary.

The plantings were done both in the first, second and

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third seasons of 1987 and 1988. The first (early) season crop was transplanted in April of each year and was terminated in June while the second (late) season crop was transplanted in August to terminate in October. The third (dry) season cropping was transplanted in December and was terminated in February each year. The dry season crops were fed with irrigation water because of lack of rainfall at the period. In all the seasons, the plants received a basal dressing of N.P.K. 15:15:15 two weeks after transplanting. The plants were exposed to natural infection by pathogens.

To detect as many different associated fungi as possible, five mature inflorescences were randomly selected and harvested from each plot. The inflorescence of each cultivar was shaken separately into 41 x 30 cm brown envelopes and the seeds were separated from the chaff using sterile hand rubber gloves. The processed seeds were packed into sterile 13 x 9 cm polyethylene envelope (steriline). Seed health was determined using the standard procedure set out by the International Seed Testing Association (ISTA, 1966) as follows on the day of harvest:

(a) dry inspection method of the seeds;

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(b) plating of the seeds and incubation under specified conditions of light and temperature.

3.2.1. <u>Dry inspection method</u>

The seed samples for each season were examined on filter paper first with the unaided eye and later under the stereoscopic binocular microscope to establish their homogeneity and to detect fungal structures such as hyphal fragment on them; malformed and discoloured seeds and the number that were clean. Four replicate samples of one hundred seeds per cultivar were examined. The data were analysed using factorial analysis (2 x 3 x 3) and the means were separated by the Duncan's multiple range test. Infected seeds were separated and kept aside for subsequent use.

3.2.2. <u>Incubation methods</u>

The "Blotter" and the "Agar Plate" methods were used. The former was used for the detection of the seed-borne fungi while the latter was used for the study of the cultural characteristics of the fungi.

(i) <u>Blotter method</u>

(a) <u>Working sample:</u> Four hundred (400) seeds

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were used as the working sample.

(b) Procedure: Three layers of filter papers placed in 9-cm diameter sterile Petri dishes moistened with sterile distilled water served as the growth medium. The Petri dishes were earlier on sterilized in the oven at 160°C for three hours and allowed to cool before use.

(c) Plating: The four hundred seeds obtained by the dry inspection method (in 3.2.1. above), were surface sterilized in 1% sodium hypochlorite solution for one minute. The seeds were rinsed in a change of sterile distilled water. One hundred (100) seeds of each cultivar were spaced out in the moistened filter paper in Petri dishes and this was replicated four times. The plates were incubated for 10 days under alternating cycle of 12 hours near ultra violet (N.U.V.) light and 12 hours darkness. Constant power was supplied from a standby big generator any time there was power failure.

(d) Examination: The seeds were examined directly for fungal infection counts with the aid of

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stereoscopic microscope and the fungi associated with the seeds of each cultivar were identified, counted and recorded. The values were transformed by arc-sin before proceeding with the analysis of variance, and the means were separated and recorded for early, late and dry seasons of both 1987 and 1988.

(ii) <u>Agar plate method</u>

(a) <u>Preparation of culture medium:</u> Potato dextrose agar powder (PDA), Oxoid, was prepared by dissolving thirty-nine grammes (39g) in one litre of distilled water by heating on a hot plate. Thereafter, the medium was autoclaved at $121^{o}C$ kg/cm^{2} for 15 minutes and allowed to cool (Tuite, 1969). Petri dishes were washed, air-dried and sterilized in a hot air oven at $160^{o}C$ for three hours and also allowed to cool. The molten PDA was poured aseptically into the sterile plates to solidify.

Four hundred (400) infected seeds of each of the cultivars were immersed separately for 60 seconds in a

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solution of sodium hypochlorite containing approximately 1% w/w available chlorine. Excess chlorine was washed off in three changes of sterile distilled water and seeds were blotted dry with sterile tissue paper.

One hundred seeds of each cultivar were plated onto the PDA. This was replicated four times and the plated seeds incubated for 10 days under the conditions stated in 3.2.2. (i).

The fungal colonies were examined by the naked eye, stereoscopic and compound microscopes (ISTA, 1966). The blotter method was used for the detection of seed-borne fungi for each season during 1987 and 1988 while the agar plate method was used for the cultural characteristics. Identification of the seed-borne fungi was by cultural and morphological characteristics of the spores observed under the microscope and reference to "illustrated Genera of Imperfect Fungi" (Barnett and Hunter, 1972). Confirmation of identified organisms was further carried out by the Commonwealth Mycological Institute (CMI), Kew, London.

3.2.3. Pathogenicity test of seed-borne fungi on seedlings

Healthy seedlings of *Amaranthus* each of cultivars $NHAc_{33}$, $NHAc_{30}$ and $NHAc_{100}$, were raised in polyethylene

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bags filled with sterile top soil in the nursery at NIHORT, Ibadan, in 1988 and 1989. The bags were watered regularly before and after seedling emergence. The set up was done at the rate of 350 seedlings/fungus/cultivar each time. This gave a total of twenty-five seedlings per cultivar for each test fungus. At three weeks, the seedlings were inoculated with ten 4-mm diameter agar discs of a 10-day old sporulating cultures of each of the test fungi macerated in 100 ml of sterile distilled water in a Waring blender. The following fungi isolated and identified from seeds in 3.2. - Choanephora cucurbitarum; Alternaria amaranthi; Rhizoctonia solani; Aspergillus niger; A. flavus; Fusarium moniliforme; F. pallidoroseum; Rhizopus oryzae; Pythium aphanidermatum; Aspergillus fumigatus; Thielavia terricola; Bipolaris zeae; A. tamarii and Phoma sorghina constituted the test fungi. Mycelial discs of Rhizoctonia solani (which did not sporulate easily) were used for inoculating plants whereas seedlings were sprayed with spore suspensions of other test fungi until run-off. The plants were kept in the greenhouse.

Healthy seedlings inoculated with pure agar discs or agar discs macerated in the Waring blender were used as controls. All seedlings were each covered in separate

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moist polyethylene bags for 48 hours as described under section 3.2.8.

The number of seedlings showing infection out of the total inoculated was recorded and the percentage infection calculated. Re-isolations were made from any of the seedlings showing symptoms to conform with Koch's postulates.

3.2.4. Plating component parts of the seeds

This study was carried out to find out the actual location of the pathogens isolated from the seeds. Twenty seeds of each of the cultivar were soaked in 6 ml sterile distilled water in each of three test-tubes for 24 hours and then dissected out under the stereoscopic microscope to separate the testa from the cotyledons. Four replicates of each part were plated separately on blotter in Petri dishes and incubated as described for the blotter method in 3.2.2. (i). After a week, the percentage infection by various fungi was calculated. The study was carried out on the seeds from the early and late season plantings of 1987 and 1988.

3.2.5. Seed transmission test

One hundred (100) apparently healthy looking and surface sterilized seeds of each *Amaranthus* cultivars

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used - $NHAc_{30}$, $NHAc_{33}$ and $NHAc_{100}$, were inoculated with ten 4-mm diameter agar discs of sporulating cultures of each of the seed-borne fungi or mycelia disc as in the case with Rhizoctonia solani after maceration in 100 ml sterile water. Inoculation was done by soaking the seeds in the spore suspension of each of the test fungus in sterile Petri dishes for sixty minutes (Joaquim, 1976). Thereafter, the seeds were drained through muslin and incubated for 5 days at ambient temperature in sterile empty Petri dishes. Seeds soaked in macerated sterile agar served as control. After 5 days, the seeds were removed and germinated in sterile soil in the greenhouse.

Four 10-l capacity buckets of each cultivar were sown with one hundred each of fungal inoculated seeds and the control. The seedlings were watered regularly and observed over a period of four weeks in the greenhouse for disease development. The total number of seedlings showing infections for both set up were recorded and percentage infection was calculated. Re-isolations were made from seedlings showing infection. The study was carried out in 1988 and 1989.

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3.2.6. Investigating the effect of season of harvest on the incidence of shoot infections

The experimental layout and plantings were as set up in section 3.2. Plants were exposed to natural infection by pathogens and later screened for C. cucurbitarum each season each year. Ratings were done at weekly intervals on all the plants in each plot and the number with a shoot dieback, stem blight and leaf blight were recorded and the percentage infection was calculated. This was done over the three seasons. Isolations were also made from the diseased plant parts each season as described below.

3.2.7. Isolation of the pathogen from shoot tip, stem and leaf

Diseased Amaranthus stem, leaf and shoot tip were obtained from infested plots described in section 3.2.6. above each season of the two years of investigation. The infected parts were cut into small pieces (4 cm each) to include a little of the healthy portions. The pieces were surface-sterilized in 50% "Milton" (0.5% sodium hypochlorite) solution for 60 seconds. Four each of the cut pieces of each part were then rinsed separately in three changes of sterile distilled water; blotted dry in sterile tissue paper and plated out onto solidified potato dextrose agar

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(PDA) or corn meal agar (CMA). The tissue paper was sterilized in a big beaker (1000cc capacity) in an autoclave at 121°C for 15 minutes kg/cm^2.

Potato dextrose agar was prepared as indicated in 3.2.1. (iia) poured and allowed to solidify. Four pieces of each surface sterilized plant parts were plated on the solidified PDA. The plates containing the cut pieces of the diseased plant materials were incubated at room temperature (28 ± 2°C) for 48 hours. The organism growing on the plate was subcultured by cutting agar blocks from the advancing margin of the growing mycelia and transferring these onto PDA in which 150mg per litre of streptomycin sulphate had been added prior to pouring to inhibit bacterial growth (Schneider et al., 1973). Pure cultures were obtained from the parts of the plant stated above through further aseptic transfers to sterile PDA at room temperature. The cultures were identified on the basis of their cultural and morphological characteristics observed under the microscope and by reference to Illustrated Genera of Imperfect Fungi (Barnett and Hunter, 1972). Identified cultures were sent to the CMI, Kew, for confirmation.

3.2.8. Pathogenicity tests

Top soil was sterilized in zinc buckets in a hot air

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oven at 180°C for 36 hours. Polyethylene bags were half filled with this sterile soil and planted with seeds of A. cruentus to obtain seedlings.

Mycelial/spore suspension of the organism isolated from each plant part was prepared by homogenising 6-day old cultures in a Waring blender. The suspension was filtered through a sterile cheese cloth and adjusted to 3.0×10^5 spores per ml. by using a haemocytometer kit (Ikediugwu, 1981). Four seedlings each were inoculated at four weeks at the lamina, stem, petiole, mid-rib and at the apical growing region (shoot tip). Inoculations were done by spraying the inoculum on the plants until run-off using a hand sprayer. Each inoculated seedling was replicated three times. Comparable parts inoculated with sterile water only, served as the control and these were also replicated three times. All inoculated seedlings were covered in separate moist polyethylene bags for 48 hours to create a humid environment for disease development and to prevent the inoculum from drying out. Thereafter, the seedlings were watered regularly and observed over 21 days for disease development. Re-isolation was made from infected plant parts to conform with Koch's postulate.

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3.3 Control of seed-borne fungi by hot water treatment

The effects of hot water treatment on both seed germination and control of all possible seed-borne pathogens as indicated in 3.2.3. were studied. Infected seeds (400 each) of the cultivars were sorted out as indicated under 3.2.1. tied in muslin cloths and immersed in a thermoregulated water-bath for 2,4,6,8 and 10 minutes at each of 40°, 50°, 60°, 70° and 80°C. The temperatures of treatment and duration were based on the results of preliminary investigations. A thermometer was inserted in the water baths to confirm the temperatures stated above. The effectiveness of these treatments on seedling emergence was carried out thus: one hundred treated seeds of each cultivar were germinated in sterile Petri dishes containing moist sterile top soil. These were replicated four times. Control of seed-borne fungi by this method was assessed by the "Blotter" method earlier described under 3.2.2. Infected seeds (400 each) from the same lot immersed in cold water at room temperature (28°C) for 10 minutes served as controls.

3.4. Control of seed-borne fungi by chemical treatment

Four hundred seeds of each cultivar found to be

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naturally infected were separated from the clean ones as indicated under the dry inspection method of study 3.2.1. and used for this study. Such infected seeds were stirred for 2 hours in aqueous fungicide suspension (2g, 5g and 10g a.i. each/l water/kg seed) for each of Iprodione, (3-3,5-dichlorophenyl-N-(1-methylethyl)2,4-dioxo-1-imidazolidinecarboxamide); Tecto(2-(4-Thiazolyl benzimidazole); Captafol (N,1,1,2,2 tetrachloroethyl)-4-cychlohexane 1-2 dicarbaximide) and a mixture of Benomyl (Methl-N(-1-butylcarbamoyl)-2 benzimidazolele carbamate) and Captafol. Each batch of treated seeds was then air-dried separately on separate sterile tissue paper for each treatment. The list of chemicals used was increased to include a mixture of Iprodione-Tecto during 1988. The choice of the chemicals and concentrations used were based on preliminary screening exercises (Anon., 1985). The same chemicals and the concentrations used during 1987 were repeated in 1988. Information on the level of mammalian toxicity and the half life of the chemicals used are contained in Appendix XIX.

Similarly, infected seeds as used above were also dusted with the fungicides stated above at the same concentrations and were kept in sterile McCartney bottles.

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The bottles were incubated at room temperature for 10 days. The effectiveness of dusting and soaking with fungicides for the control of the seed-borne fungi was assessed by the "Blotter" method stated in 3.2.2. (i) above. The percentage seedling germination and infection were recorded by the "Blotter" method. Four hundred treated seeds of each cultivar were used for the evaluation of percentage seedling emergence and seedling infection. The experiments were carried out once for two years - 1987 and 1988.

3.5. Control of inflorescence infection and field acquired mycoflora of seeds by pre-harvest spray of chemicals

(i) Control of inflorescence infection

The efficiency of pre-harvest spray of chemicals on *Amaranthus* sp. to control *C. cucurbitarum* and other field-acquired mycoflora of the seeds was investigated. The field lay-out was as described in section 3.2. and cultivar $NHAc_{33}$ was used. When 50% of the plants in each plot had produced inflorescences, three of such plants from the middle rows were artificially inoculated. Inoculation was done by pressing firmly two 4mm diameter agar discs of the sporulating culture of *C. cucurbitarum* on each side

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of the main flower head 5-cm from the tip (Adebanjo, 1989). Inoculations were done in the evening and inoculated plants were covered with moist polyethylene bags to prevent the inoculum both from drying off and from being washed off by rain. The polyethylene bags were removed after 48 hours and the inoculated plants served as spreader of the pathogen for each row.

The following chemicals: Iprodione; Tecto; Captafol and a mixture of Benomyl and Captafol at 1.25g, 2.5g and 5.0g a.i. each/plot were applied in 1987 with a Knapsac sprayer onto all the inflorescences 12 days after artificial inoculation of the inflorescences. A mixture of Iprodione and Tecto at the same rate was added to the list of the chemicals used upon suggestion in 1988. The second chemical application was done 15 days after the first one. The chemical action was evaluated by rating 10 plants randomly selected from each plot for the number of infected inflorescences out of the total planted. Artificially inoculated inflorescences that were sprayed with sterile water each season each year served as controls. The experiment was carried out during the early and late seasons of 1987 and 1988. Early season transplantings were done each year in May and harvested by August while that of the

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late season for both years were done in August and terminated in November. The choice of the chemicals was based on the results of a preliminary study on chemical control of dieback and stem rot of *Amaranthus* caused by *C. cucurbitarum* (Anon., 1985).

(ii) Effect of chemical application on seed yield

At maturity, five inflorescences randomly selected from each plot (as in 3.5) for each treatment were harvested by cutting and the seeds were removed as described under 3.2. weighed and recorded both for the sprayed and the control plots. The collected data was analysed statistically and the means separated by the Duncan's multiple range test (DMRT). This study was also carried out for the early and late seasons of 1987 and 1988.

(iii) Chemical control of seed-borne mycoflora and seedling emergence

Detection of seed-borne mycoflora of seeds harvested at maturity in study 3.5 (ii) above was carried out on the day of harvest using the "Blotter" method as earlier described. The emergence ability of the seedlings were also evaluated as described in 3.3. Detection of seed-borne fungi and seedling emergence ability were also

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carried out with seeds harvested from the control plots. The values obtained were subjected to factorial analysis and their means were separated by DMRT. The experiment was also carried out during the early and late seasons of 1987 and 1988.

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CHAPTER 4

4. RESULTS

4.1. Detection of mycofloral infection of seeds harvested at different seasons

4.1.1. Dry inspection method

This method revealed that the seeds were normally contaminated with mycelial growth in varying quantity depending on the cultivar and the season of harvest. Similarly, the seeds of the cultivars were either discoloured, had mycelial growth or were malformed depending on the season of harvest. Malformation of the seeds involved seeds showing dents/irregular shape when compared to others. Discoloured seeds were those few ones with yellowish or light brown colouration when compared to the predominant dark or blackish normal types. Healthy seeds on the other hand were without mycelial growth on them, not malformed or discoloured.

The mean percentage mycelial growth on seeds were significantly higher (P=0.05) during the first (18%) and second (20%) seasons of 1987 (Fig. 1a). Similarly, higher mycelial growth were also recorded on seeds in the first (19%) and second (20%) seasons of 1988 regardless of cultivars (Fig. 1b). In other words, seeds produced during the third season of the two years had significantly low percentage

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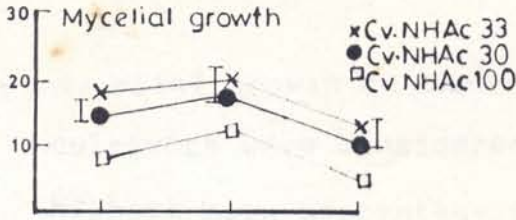

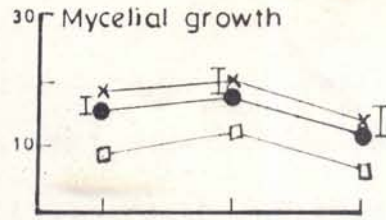

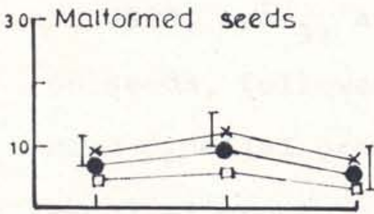

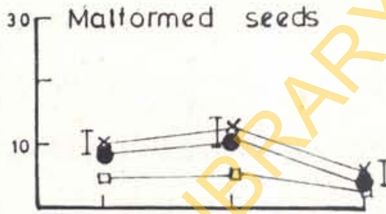

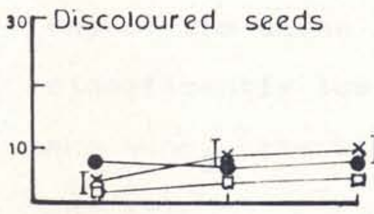

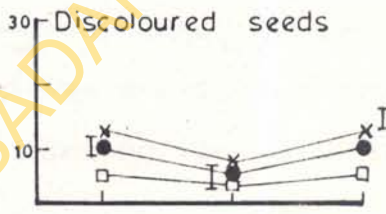

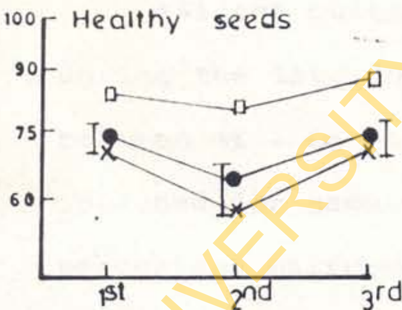

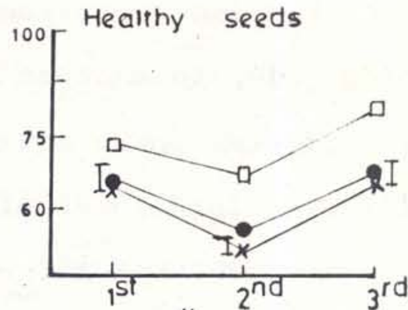

(a) S E A S O N S* (b)

Fig. 1. The effect of season of harvest on the health of three cultivars of Amaranthus seeds during 1987 (a) and 1988 (b) I LSD at 0·05

* 1st season, April - June, 2nd season, August - October, 3rd season, December - February.

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mycelial growth on them (Figs. 1a and 1b). When all the cultivars were considered in 1987, $NHAc_{33}$ recorded the highest mean percentage mycelial growth on seeds (20%) followed by $NHAc_{30}$ (18%) and $NHAc_{100}$ had 12% only (Fig. 1a). In 1988, $NHAc_{33}$ also had 20% mean percentage mycelial growth on seeds, followed by $NHAc_{30}$ and $NHAc_{100}$ with 19% and 13% mean mycelial growth respectively (Fig. 1b). The highest mycelial growth on seeds was recorded during the 2nd season of the two years for all cultivars (Figures 1a and 1b). At any of the three seasons, seeds of $NHAc_{100}$ recorded significantly low mean percentage mycelial growth (5% and 7%) and $NHAc_{33}$ the highest (20%) for the two years (Figures 1a and 1b).

All the cultivars had malformed seeds in all the seasons. During the 1st, 2nd and 3rd seasons of 1987, $NHAc_{100}$ had between 4% - 6% malformed seeds (Fig. 1a) while 4 - 8% was obtained for same cultivar in 1988 (Fig. 1b). The highest percentage malformed seeds - 12% and 14%, were recorded during the second season of both years. When all the cultivars were considered, $NHAc_{100}$ produced significantly least malformed seeds during the third (4%) and first (5%) seasons of 1987 and 1988 (Figures 1a and 1b).

Except for $NHAc_{100}$, the other two cultivars produced significantly higher percentage (9%, 10% and 10% 13%)

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discoloured seeds during the third season. Low percentages discoloured seeds (3 - 6%) were recorded for $NHAc_{100}$ when compared with 13% for $NHAc_{33}$ or 11% for $NHAc_{30}$ (Figures 1a and 1b). That is among the cultivars, $NHAc_{100}$ consistently produced least mean percentage discoloured seeds each season of the two years. Generally, $NHAc_{30}$ was next to $NHAc_{100}$ in terms of production of fewer discoloured seeds each season (Figures 1a and 1b).

The healthiest (clean) seeds of all cultivars were produced during the 3rd and 1st seasons of each year. Seeds of all cultivars produced during the 2nd season were significantly least healthy than the 1st or 3rd season (Figures 1a and 1b). Again, high quality seeds (87%) were obtained from $NHAc_{100}$ in 1987, 78% in 1988; $NHAc_{30}$ had 75% (1987) and 68% (1988) mean healthy seeds. $NHAc_{33}$ recorded between 60 - 71% and 51 - 66% healthy seed during 1987 and 1988 respectively (Figures 1a and 1b).

4.1.2. Incubation method

Both the blotter and the agar plate methods produced same organisms. But because of ease of set-up, (no auto-claving, pouring of agar) the blotter method was used for the detection of seed-borne fungi while the agar plate method was used for culturing of fungi obtained by the blotter for

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subsequent identification.

A total of fifteen fungal species were isolated from the seeds of three *Amaranthus* cultivars used for this study. These fungi were *Choanephora cucurbitarum* (Berk. and Rav.) Thaxt. Herb. IMI No. 332106; *Alternaria amaranthi* (PK) Van Hook IMI No. 332118; *Rhizoctonia solani* Kuhn IMI No. 332116; *Aspergillus niger* van Tieghen IMI No. 33210; *A. flavus* Link ex Fries IMI No. 332100; *Fusarium moniliforme* var. *intermedium* Neish & Leggett IMI No. 332113; *F. pallidoroseum* (Cooke) Sacc. IMI No. 332111; *Rhizopus oryzae* Went & Prinsen Geerligs IMI No. 332119; *Pythium aphanidermatum* (Ed.) Fitz IMI No. 332108; *Aspergillus fumigatus* Fres. IMI No. 332104; *Thielavia terricola* (Gilman & Abbot) Emmons IMI No. 332112; *Bipolaris zeae* Sivan IMI No. 332110; *Curvularia geniculata* (Tracy & Earle) Boedijn anamorph of *Cochliobolus geniculatus* Nelson IMI No. 332102; *Aspergillus tamarii* Kita IMI No. 332099 and *Phoma sorghina* (Sacc.) Boerema *et al.*, IMI No. 332109 (Tables 1a and 1b).

The greatest number, types and highest mean percentage mycofloral infection of the seed were recorded for all cultivars during the second season each year. For example *C. cucurbitarum* (10%); *A. amaranthi* (7%); *F. pallidoroseum* (1%); *F. moniliforme* (2%); *A. niger* (4%); *A. fumigatus* (3%); *A. tamarii* (3%) and *B. zeae* (2%) were isolated from $NHAc_{33}$

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Table 1a: The effect of season** of harvest on the level* of mycofloral infection of three cultivars of *Amaranthus* seeds during 1987

Cultivar	Seasons/Organisms Isolated								
	Season 1:	IMI No.	% infection	Season 2:	IMI No.	% infection	Season 3:	IMI No.	% infection
$NHAC_{33}$	*C. cucurbitarum*	332106	8.3±0.03	*C. cucurbitarum*	332106	10.0±1.15	*C. cucurbitarum*	332106	3.8±0.27
	A. amaranthi	332118	5.0±0.29	*A. amaranthi*	332118	6.7±1.01			
	R. solani	332116	2.9±0.41	*F. pallidoroseum*	332111	1.3±0.09	*P. aphanidermatum*	332108	6.7±0.17
	A. niger	332103	1.0±0.12	*F. moniliforme*	332101	2.1±0.31	*R. solani*	332116	7.0±0.29
	F. pallidoroseum	332111	1.0±0.13	*A. niger*	332103	4.2±0.27	*F. pallidoroseum*	332111	1.7±0.24
				A. fumigatus	332104	3.3±0.27			
				A. tamarii	332099	2.5±0.12			
				B. zeae	332110	2.1±0.32			
$NHAC_{30}$	*C. cucurbitarum*	332106	6.7±1.01	*C. cucurbitarum*	332106	11.7±1.85	*C. cucurbitarum*	332106	3.8±0.27
	P. aphanideratum	332108	3.3±0.27	*A. amaranthi*	332118	5.3±0.64			
	R. solani	332116	3.2±0.56	*C. geniculata*	332102	1.7±0.24	*P. aphanidermatum*	332108	5.0±0.29
	A. niger	332103	1.5±0.17	*A. niger*	332103	3.2±0.56	*R. solani*	332116	4.1±0.21
	F. pallidoroseum	332111	1.0±0.12	*A. flavus*	332100	4.1±0.20	*F. pallidoroseum*	332111	1.9±0.23
	A. amaranthi	332118	4.6±0.38	*F. pallidoroseum*	332111	1.2±0.07			
	R. oryzae	332119	1.0±0.14	*A. tamarii*	332099	2.8±0.43			
$NHAC_{100}$	*C. cucurbitarum*	332106	5.4±0.58	*C. cucurbitarum*	332106	7.0±0.29	*C. cucurbitarum*	332106	1.7±0.24
	P. aphenidermatum	332108	3.8±0.25	*A. niger*	332103	1.3±0.09	*A. amaranthi*	332118	1.5±0.12
	A. amaranthi	332118	3.2±0.56	*A. flavus*	332100	2.3±0.33	*P. aphanidermatum*	332108	7.3±0.37
	A. niger	332103	0.7±0.17	*A. amaranthi*	332118	3.8±0.33			
				A. tamarii	332099	1.9±0.23			

*Percentages along or within collumn are mean values of four replications ± standard error.

**Season 1 = April-June; Season 2 = August-October; Season 3 = December-February.

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Table 1b: The effect of season** of harvest on the level* of mycofloral infection of three cultivars of *Amaranthus* seeds during 1988

Cultivar	Seasons/Organisms Isolated								
	Season 1:	IMI No.	% infection	Season 2:	IMI No.	% infection	Season 3:	IMI No.	% infection
NHAC 33	*C. cucurbitarum*	332106	10.0±2.00	*C. cucurbitarum*	332106	13.3±0.67	*C. cucurbitarum*	332106	5.6±1.2
	A. amaranthi	332118	7.0±0.58	*A. amaranthi*	332118	9.7±0.88	*A. amaranthi*	332118	5.0±0.58
	R. solani	332116	4.3±0.23	*A. flavus*	332100	5.3±1.20	*P. aphanidermatum*	332108	8.3±0.88
	A. niger	332103	2.7±0.88	*A. niger*	332103	5.7±1.45	*R. solani*	332116	9.0±0.58
	A. flavus	332100	4.0±0.58	*B. zeae*	332110	2.1±1.10	*C. geniculata*	332102	2.7±0.33
	F. moniliforme	332113	2.3±0.67	*C. geniculata*	332102	4.0±0.00	*F. moniliforme*	332113	3.0±0.00
	F. pallidoroseum	332111	2.0±0.58	*F. moniliforme*	332113	3.7±0.67	*A. tamarri*	332099	1.3±0.30
	R. oryzae	332119	1.7±0.33	*F. pallidoroseum*	332111	2.6±0.68	*B. zeae*	332110	4.0±0.58
	P. aphanidermatum	332108	4.7±0.88	*A. tamarri*	332099	2.7±0.67	*P. pallidoroseum*	332111	3.6±0.62
				P. sorghina	332109	3.3±0.33			
				R. oryzae	332119	2.7±0.68			
				T. terricola	332112	5.0±0.58			
NHAC 30	*C. cucurbitarum*	332106	8.3±1.2	*C. cucurbitarum*	332106	12.7±0.88	*C. cucurbitarum*	332106	7.3±1.20
	P. aphanidermatum	332108	4.7±0.33	*A. amaranthi*	332118	6.7±0.33	*A. amaranthi*	332118	3.3±0.33
	A. niger	332103	2.0±1.15	*C. geniculata*	332102	5.0±0.58	*P. aphanidermatum*	332108	6.7±0.33
	A. fumigatus	332104	2.7±0.33	*A. niger*	332103	4.3±0.33	*R. solani*	332116	6.3±0.33
	R. solani	332116	4.0±0.58	*A. fumigatus*	332104	6.0±0.00	*A. fumigatus*	332104	4.0±0.00
	F. moniliforme	332113	1.3±0.33	*A. tamarii*	332099	2.0±0.58	*C. geniculata*	332102	1.3±0.67
	T. terricola	332112	3.3±0.33	*F. pallidoroseum*	332111	3.5±0.33	*F. moniliforme*	332113	1.7±0.33
	F. moniliforme	332113	1.3±0.33	*T. terricola*	332112	5.0±0.58	*F. pallidoroseum*	332111	2.0±0.58
	F. pallidoroseum	332111	2.5±1.10	*P. sorghina*	332109	1.7±0.33			
				F. moniliforme	332113	2.7±0.33			
				B. zeae	332110	1.7±0.33			
NHAC 100	*C. cucurbitarum*	332106	5.3±0.33	*C. cucurbitarum*	332106	8.7±1.70	*C. cucurbitarum*	332106	4.0±0.58
	P. aphanidermatum	332108	2.7±0.33	*A. niger*	332103	3.0±0.00	*P. aphanidermatum*	332108	7.3±0.33
	A. niger	332103	1.7±0.88	*A. flavus*	332100	2.7±0.33	*A. niger*	332103	2.0±0.58
	R. oryzae	332119	1.3±0.67	*A. tamarii*	332099	1.7±0.67	*A. flavus*	332100	1.3±0.58
	A. amaranthi	332118	3.3±0.33	*P. aphanidermatum*	332108	2.0±0.58	*A. amaranthi*	332118	4.0±0.00
	F. pallidoroseum	332111	1.0±0.14	*R. oryzae*	332119	2.0±0.58			
				A. amaranthi	332118	5.0±0.00			

*Percentages along or within column are mean values of four replications ± standard error.
**Season 1 = April-June; Season 2 = August-October; Season 3 = December-February.

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during the 2nd season of 1987 (Table 1a). These organisms were more in number and the % infection in them were significantly higher than those of C. cucurbitarum (8%); A. amaranthi (5%); R. solani (3%); A. niger (1%) and F. pallidoroseum (1%) obtained from the seeds of same cultivar in the 1st season (Table 1a). During the 2nd season of 1988, a total of twelve fungi were isolated from $NHAc_{33}$ compared to nine each in the 1st and 3rd seasons (Table 1b). C. cucurbitarum was isolated from 10% of the plated seeds (1987) and 13% (1988) during the 2nd season while A. amaranthi infested 7% and 10% of such seeds in 1987 and 1988 respectively. These values were significantly (P=0.05) higher than for any of the other fungi isolated from these cultivars in either of the two years. In general, C. cucurbitarum and A. amaranthi were isolated from the seeds of all cultivars used for the two years in significantly higher proportions during 2nd and 1st seasons (in order of magnitude) than the rest of the seed-borne fungi isolated (Tables 1a and 1b). Unlike the other two seasons, significantly higher seed infection of all cultivars by P. aphanidermatum ranging between 5% - 7% (1987); 7% - 8% (1988) and R. solani between 4% - 7% (1987); 6% - 9% (1988) were noticed during the 3rd season. C. cucurbitarum and A. amaranthi that were isolated in high proportions from the

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seeds in the 2nd and 1st seasons were recorded only on $NHAc_{33}$ and $NHAc_{30}$ for the two years in least proportions in the 3rd season.

Cultivar $NHAc_{30}$ is not markedly different from $NHAc_{33}$ in terms of the number and types of fungi isolated from the seed each season. C. cucurbitarum and A. amaranthi were on top of the fungi isolated in the 2nd and 1st seasons while P. aphanidermatum and R. solani were detected in higher percentages for the two years in the 3rd season only (Tables 1a and 1b).

The third cultivar, $NHAc_{100}$ was strikingly different from the other two in that the number of fungi detected from the seed were fewer and were recorded at between 1% - 7% each year (Tables 1a and 1b). An example is C. cucurbitarum that was associated with 13% each of $NHAc_{33}$ and $NHAc_{30}$ seeds during the 2nd season of 1988 only occurred in 9% of $NHAc_{100}$. However the percentage infection by P. aphanidermatum in seeds of $NHAc_{100}$ during the 3rd season was significantly higher than percentage infection by each of the other fungi. Some of the fungi isolated from Amaranthus seeds are shown in Plates 1&2).

4.1.3. Pathogenicity test of seed-borne fungi on seedlings

The seedlings of $NHAc_{33}$ and $NHAc_{30}$ were highly susceptible to C. cucurbitarum, A. amaranthi, R. solani and P.

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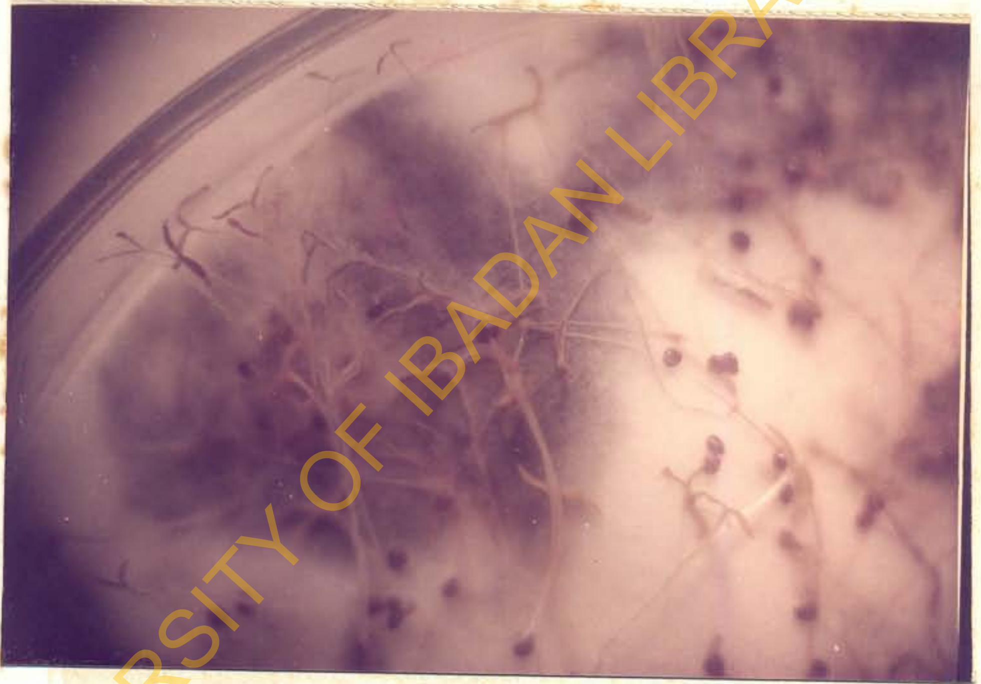

x 50

Plate 1: Choanephora cucurbitarum found associated with seeds of Amaranthus sp.

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x 500

Plate 2: Photomicrograph of conidia of *Alternaria amaranthi* isolated from the seed of *Amaranthus* sp.

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aphanidermatum. However, seedlings of $NHAc_{100}$ were slightly susceptible to these organisms. All the inoculated seedlings of $NHAc_{33}$ and $NHAc_{30}$ were completely infected (100%) by C. cucurbitarum while only 20% infection by this fungus was recorded for $NHAc_{100}$ (Table 2). Seedlings of such susceptible plants either show symptoms of shoot-tip dieback or severe leaf and/or stem tissue necrosis (Plate 3). Infections as these ultimately results in complete death of seedlings as the inoculation days increased (Plate 4). Similarly, P. aphanidermatum was recorded on 88% of inoculated seedlings of $NHAc_{33}$, 84% of $NHAc_{30}$ and 60% of $NHAc_{100}$. R. solani infected 92% seedlings of $NHAc_{33}$, 88% of $NHAc_{30}$ and 25% of $NHAc_{100}$ (Table 2). Both P. aphanidermatum and R. solani caused damping off of inoculated seedlings. Symptoms of the former were small water-soaked discoloured areas at soil level on the younger stem resulting in softening and shrivelling of the stalk. Consequently, the seedlings wilt, collapsed and fell over (Plate 5). However, R. solani caused a more general rot of the seedlings and small black sclerotia on the dead leaves.

Alternaria amaranthi infected 40% of the inoculated seedlings of $NHAc_{33}$, 32% of $NHAc_{30}$ and 8% of $NHAc_{100}$. Fusarium moniliforme produced 8% and 4% infection of inoculated seedlings of $NHAc_{33}$ and $NHAc_{30}$ respectively

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Table 2: The effect of *Amaranthus* seed-borne fungi on healthy seedlings of three *Amaranthus* cultivars

Cultivar	Isolated fungi	No. of Seedlings inoculated	No. infected	% infection*
NHAC 33	*Choanephora cucurbitarum*	25	25	100
	Alternaria amaranthi	25	10	40
	Rhizoctonia solani	25	23	92
	Aspergillus niger	25	0	0
	Aspergiluus flavus	25	0	0
	Fusarium moniliforme	25	2	8
	Fusarium pallidoroseum	25	0	0
	Rhizopus oryzae	25	0	0
	Pythium aphanidermatum	25	22	100
	Aspergillus fumigatus	25	0	0
	Thielavia terricola	25	0	0
	Bipolaris zeae	25	1	4
	Curvularia geniculata	25	1	4
	Aspergillus tamarii	25	0	0
	Phoma sorghina	25	0	0
	Sterile water	25	0	0
NHAC 30	*C. cucurbitarum*	25	25	100
	A. amaranthi	25	8	32
	R. solani	25	22	88
	A. niger	25	0	0
	A. flavus	25	0	0
	F. moniliforme	25	1	4
	F. pallidoroseum	25	0	0
	R. oryzae	25	0	0
	P. aphanidermatum	25	21	84
	A. fumigatus	25	0	0
	T. terricola	25	0	0
	B. zeae	25	0	0
	C. geniculata	25	1	4
	A. tamarii	25	0	0
	P. sorghina	25	0	0
	Sterile water	25	0	0

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Table 2 (Cont'd)

Cultivar	Isolated fungi	No. of Seedlings inoculated	No. infected	% infection*
NHA C_{100}	*C. cucurbitarum*	25	5	20
	A. amaranthi	25	2	8
	R. solani	25	6	24
	A. niger	25	0	0
	A. flavus	25	0	0
	F. moniliforme	25	0	0
	F. pallidoroseum	25	0	0
	R. oryzae	25	0	0
	P. aphanidermatum	25	15	60
	A. fumigatus	25	0	0
	T. terricola	25	0	0
	B. zeae	25	0	0
	C. geniculata	25	0	0
	A. tamarii	25	0	0
	P. sorghina	25	0	0
	Sterile water	25	0	0

*Values are averages of different inoculations done on two different occasions.

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Plate 3: Artificially inoculated Amaranthus seedling showing infection of apical region (dieback A) leaf infection (leaf blight S) and stem infection (stem blight B) caused by *Choanephora cucurbitarum*.

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× 3

Plate 4: Almost completely dead Amaranthus seedling resulting from inoculation with Choanephora cucurbitarum. Note the severely damaged portion of stem E.

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Plate 5 : Damping off of *Amaranthus* seedling (Left) inoculated with *Pythium aphanidermatum* isolated from the seed. The control seedling (Right) remained healthy.

(Table 2). *Bipolaris zeae* recorded 4% seedling infection on $NHAc_{33}$ only. *Curvularia geniculata* also recorded 4% infection each on seedlings of $NHAc_{33}$ and $NHAc_{30}$ (Table 2). *A. amaranthi* and *F. moniliforme* produced many brown necrotic spots on the leaves of seedlings which coalesce to form lesions (Plate 6). *B. zeae* and *C. geniculata* on the other hand produced few (4%) pin-head brown spots on the leaves of inoculated seedlings of $NHAc_{33}$ and $NHAc_{30}$ only (Table 2). All the other seed-borne fungi inoculated on seedlings of all cultivars produced no noticeable infection. All the control seedlings remained healthy (Table 2).

All the organisms re-isolated from the inoculated seedlings resembled the ones used for inoculation in cultural and morphological appearance.

4.1.4. Plating component parts of the seed

Results indicated that the seed testa of all cultivars harboured the highest percentage and number of fungi during the 1st and 2nd seasons of both years. *Aspergillus tamarii* and *A. fumigatus* were recovered from the cotyledon of $NHAc_{30}$ and $NHAc_{33}$ both years at between 1.6 - 6.7% and 2.7 - 3.3% respectively. The cotyledon of $NHAc_{100}$ was found to be fungi-free during the first season of both years (Tables 3a and 3b).

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x3

Plate 6: Brown leaf spots (C) and lesions (D) produced on *Amaranthus* leaf inoculated with *Alternaria amaranthi*.

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Table 3a: The occurrence of seed-borne fungi in different parts of the seed of three *Amaranthus* cultivars during 1987

Part of Seed	Fungi isolated	Cultivar/Mean % fungi recovery*			
		NHAC 30	NHAC 33	NHAC 100	
Testa	*A. niger*	10.1±1.16	6.7±0.44	1.7±0.17	1st Season
	A. flavus	6.7±0.44	5.0±0.58	1.7±0.33	
	C. cucurbitarum	16.7±0.67	11.7±0.17	1.7±0.17	
	P. aphanidermatum	6.7±1.01	3.3±0.60	1.7±0.17	
Cotyledon	*A. tamarii*	6.7±0.88	1.67±0.17	0.0±0.00	
Testa	*A. fumigatus*	13.3±0.73	10.0±1.15	3.3±0.60	2nd Season
	A. flavus	10.0±1.06	6.7±1.01	1.7±0.17	
	C. cucurbitarum	21.7±0.60	20.2±0.33	6.7±0.44	
	P. aphanidermatum	8.3±0.03	6.7±1.01	1.7±0.73	
Cotyledon	*A. tamarii*	3.3±0.60	3.3±0.17	1.7±0.73	
	A. niger	8.3±0.03	5.0±0.58	1.6±0.53	

*Values are mean percentages of four replications, each made up of 4 component parts/replicates ± standard error.

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Table 3b: The occurrence of seed-borne fungi in different parts of the seed of three *Amaranthus* cultivars during 1988

Part of seed	Fungi isolated	Cultivar/Mean % fungi recovery*			
		$NHAc_{30}$	$NHAc_{33}$	$NHAc_{100}$	
Testa	*A. fumigatus*	10.0±0.76	11.7±0.17	3.3±0.60	1st Season
	A. flavus	6.7±1.01	5.0±0.29	3.3±0.17	
	C. cucurbitarum	13.3±0.37	10.0±1.50	0.0±0.00	
	C. geniculata	9.3±0.44	5.0±0.29	2.7±0.44	
	F. moniliforme	5.0±0.58	0.0±0.00	0.0±0.00	
	T. terricola	4.0±0.29	3.3±0.27	0.0±0.00	
Cotyledon	*A. tamarii*	7.3±0.60	3.3±0.17	0.0±0.00	
	A. fumigatus	3.3±0.17	2.7±0.44	0.0±0.00	
Testa	*A. fumigatus*	12.7±0.44	10.0±1.15	6.0±0.29	2nd Season
	A. flavus	10.0±1.15	8.3±0.03	3.3±0.17	
	C. cucurbitarum	22.7±0.73	23.3±0.44	8.5±0.60	
	P. aphanidermatum	9.3±0.60	10.0±1.15	0.0±0.00	
	F. moniliforme	5.0+0.58	3.3+0.44	0.0+0.00	
Cotyledon	*A. tamarii*	8.0±0.50	6.7±1.01	2.7±0.44	
	A. flavus	4.0±0.29	4.0±0.29	0.0±0.00	
	A. niger	5.0±0.50	4.0±0.76	0.0±0.00	

*Values are mean percentages of three replications each made up of 4 component parts/replicate ± standard error.

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In general, the component parts of seeds of cultivars harboured fungi in varying quantities. In 1987, seeds of $NHAc_{30}$ carried the highest percentage infection (21.7%) on the testa and had 8% infection of the cotyledon during the 2nd season. Cultivar $NHAc_{33}$ followed with 20% testa and 5% cotyledon infection while $NHAc_{100}$ had 6.7% and 1.7% infection of these seed parts respectively (Table 3a). These highest values of seed infection were also recorded in the 2nd season. Similarly, during 1988, $NHAc_{30}$ and $NHAc_{33}$ had the highest mean (23% each) seed testa infection, 8% and 7% cotyledon infection respectively also in the 2nd season (Table 3b). The range of infection of the testa of the cultivars by other organisms for both years were 16.7 - 22.7% for C. cucurbitarum, 10 - 12.7% for A. fumigatus and 1.7 - 10% for P. aphanidermatum (Tables 3a and 3b).

4.1.5. Seed transmission test

High percentage seedling infection (75%) by P. aphanidermatum and C. cucurbitarum (12%) were recorded for inoculated seeds of $NHAc_{30}$. These fungi also caused 70% and 15% seedling infection in $NHAc_{33}$. Choanephora cucurbitarum had 8% and P. aphanidermatum recorded 25% seedling infection for $NHAc_{100}$ (Table 4). The seedlings produced by P. aphanidermatum inoculated seeds had brown

Table 4: Seed transmission of some seed-borne fungi isolated from three cultivars of *Amaranthus* seed during 1988/89

Cultivar	Isolated fungi	No. inoculated seeds planted	No. seedlings showing symptoms	% infection
$NHAc_{33}$	*Choanephora cucurbitarum*	100	15	15
	Alternaria amaranthi	100	0	0
	Aspergillus flavus	100	0	0
	Aspergillus niger	100	0	0
	Bipolaris zeae	100	0	0
	Curvularia geniculata	100	0	0
	Fusarium moniliforme	100	0	0
	Fusarium pallidoroseun	100	0	0
	Aspergillus tamarii	100	0	0
	Phoma sorghina	100	0	0
	Thielavia terricola	100	0	0
	Rhizopus oryzae	100	0	0
	Pythium aphaniderатum	100	70	70
	Rhizoctonia solani	100	0	0
	Control	100	0	0
$NHAc_{30}$	*C. cucurbitarum*	100	12	12
	P. aphanidermatum	100	75	75
	A. niger	100	0	0
	A. fumigatus	100	0	0
	R. solani	100	0	0
	F. moniliforme	100	0	0
	T. terricola	100	0	0
	F. pallidoroseum	100	0	0
	A. amaranthi	100	0	0
	C. geniculata	100	0	0
	A. tamarii	100	0	0
	P. sorghina	100	0	0
	B. zeae	100	0	0
	Control	100	0	0
$NHAc_{100}$	*C. cucurbitarum*	100	8	8
	P. aphanidermatum	100	25	25
	A. niger	100	0	0
	R. oryzae	100	0	0
	A. amaranthi	100	0	0
	F. pallidoroseum	100	0	0
	A. flavus	100	0	0
	A. tamarii	100	0	0
	R. solani	100	0	0
	Control	100	0	0

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roots which shrivelled and damped off, turned black and resulted in death. Seedlings produced from seeds inoculated with *C. cucurbitarum* turned brown while the radicle and plumule became water-soaked and rot (Plate 1).

The fungi inoculated onto the seeds were re-isolated from the seedlings showing infection to satisfy Koch's postulates. All the other fungi showed no evidence of seedling infection hence seed transmission. The seedlings produced by the seeds inoculated with sterilized agar (controls) remained healthy.

4.2. Effect of season of harvest on the incidence of shoot infections

The season of harvest affected the percentage dieback, stem and leaf blights differently for the two years. During 1987, significantly least percentage dieback (9%) was recorded for $NHAc_{33}$ during the 3rd season (Fig. 2a). The 1st season had the next least percentages dieback ranging between 17 - 18% for all the cultivars. The highest mean percentage dieback (24 - 25%) were recorded for two of the cultivars, $NHAc_{100}$ and $NHAc_{30}$ in the 2nd season. However, $NHAc_{33}$ had significantly least dieback (16%) during this season (Fig.2a). The incidence of stem blight also varied among the cultivars

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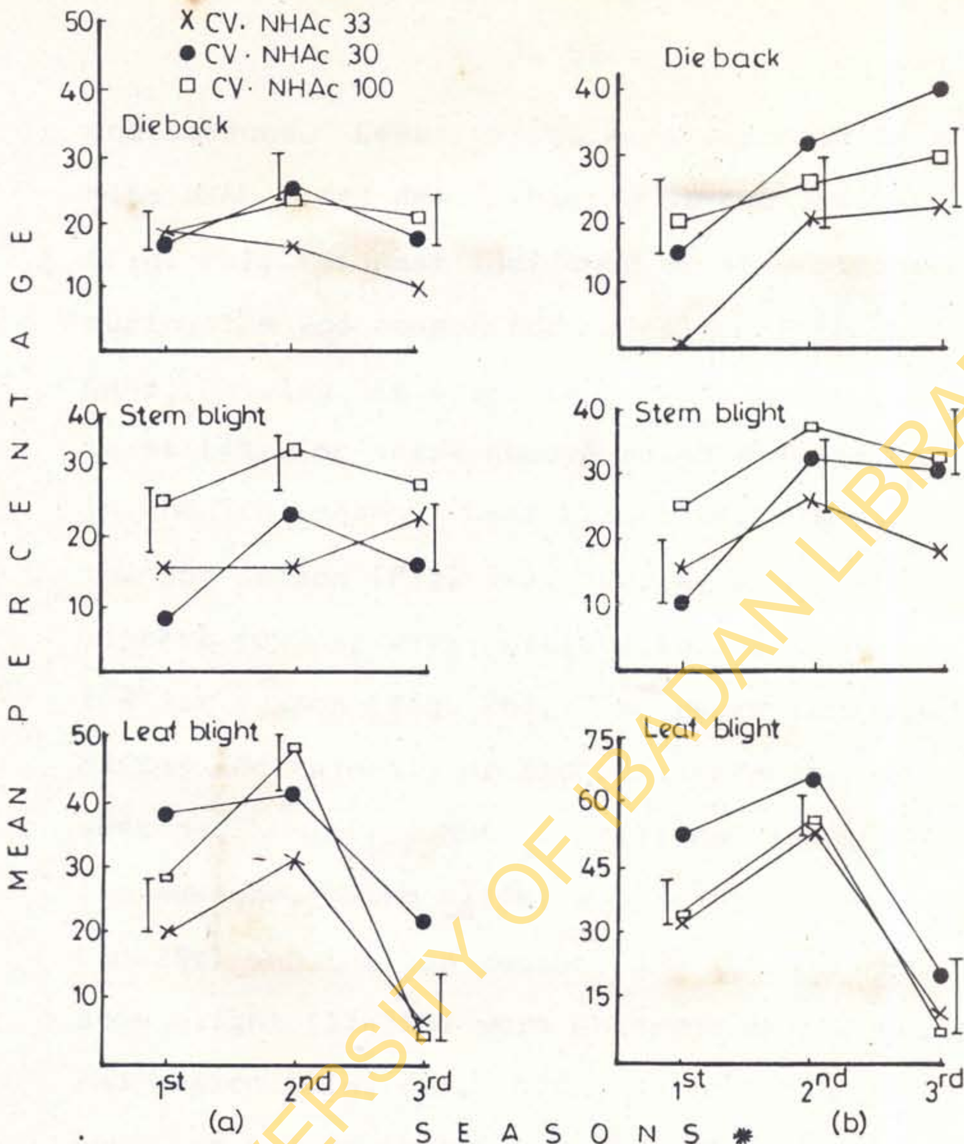

Fig. 2: The effect of season of harvest on the incidence of die back, stem and leaf blights of three *Amaranthus* cultivars during the three seasons of 1987 (a) and 1988 (b) ILSD at 0.05.
* 1st season, April - June, 2nd season, August - October, 3rd season, December - February.

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and seasons. Least values were recorded in the 1st season with $NHAc_{30}$ and $NHAc_{33}$ having 8% and 15% respectively (Fig. 2a). Highest incidence of stem blight were obtained during the 2nd season for majority of the cultivars with $NHAc_{33}$ having 15% (Fig. 2a). Percentage leaf blight was least (4%) for these above ground disease and was recorded in the 3rd season. Leaf blight was also highest (49%) in the 2nd season (Fig. 2a). During 1988, significantly least dieback (0-20%) were obtained for all the cultivars also in the 1st season (Fig. 2b). The percentage dieback did not differ for majority of the cultivars in the 2nd and 3rd seasons. However, $NHAc_{33}$ still had least values for the two seasons. Stem blight was least in the 1st season (10-25%) and the 3rd season (18-32%). Highest rates of stem blight (33-38%) were observed on all cultivars in the 2nd season (Fig. 2b). Also, during this year, the percentage leaf blight in the 3rd season was significantly the least for all cultivars. This ranged between 6% - 16% (Fig. 2b).

4.2.1. Isolation of pathogen from diseased plant parts

The fungus, *Choanephora cucurbitarum* (Berk. and Rav.) Thaxt. was isolated readily from the stem, petiole, mid-rib and the tip of infected plants (Plate 7). The spores were

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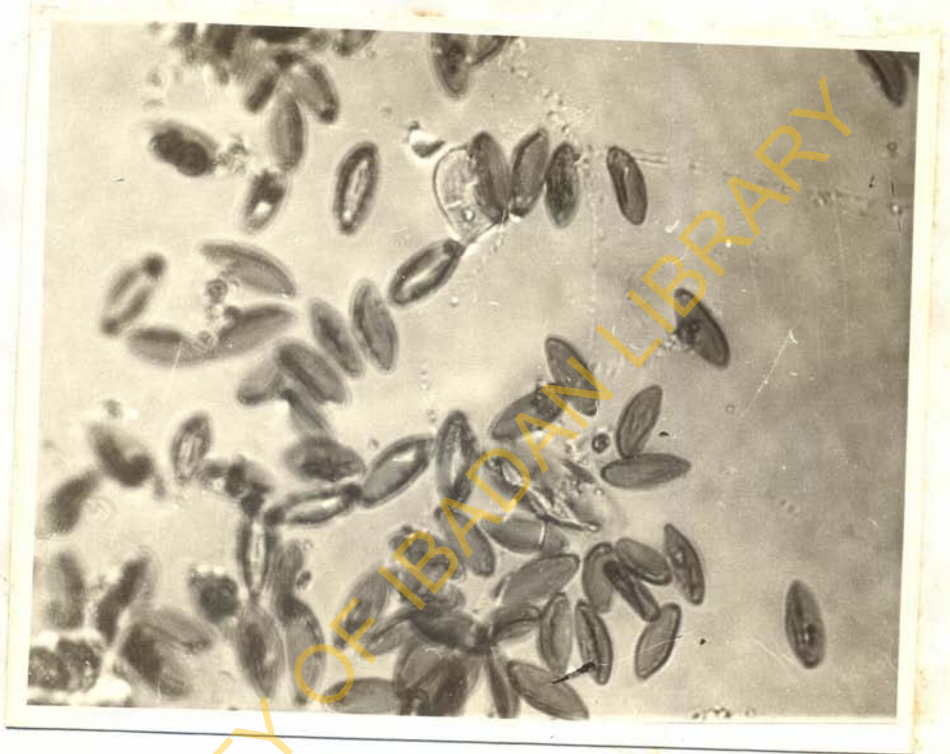

x500

Plate 7: Photomicrograph of conidia of Choanephora cucurbitarum isolated from infected Amaranthus shoot parts and seed.

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obtained within 24 hours of growth of any of these diseased plant parts either on PDA or CMA at room temperature.

4.2.2. Pathogenicity tests of fungus on seedlings

C. cucurbitarum was pathogenic on the petiole, lamina and mid-rib of the inoculated plants (Plate 3). The fungus was also pathogenic on *Amaranthus* stem forming extensive blighted areas on it (Plate 3). The young apical growing region (shoot tip) of the inoculated plants were the most susceptible to the fungus (Plate 3). Symptoms on all inoculated plant parts commenced within 24 hours usually as water-soaked areas. These are often accompanied by a discolouration of the infected zone. The water-soaked areas rapidly spread to other healthy areas causing necrosis of the tissue.

4.2.3. Control of seed-borne fungi by hot water treatment

Hot water seed treatment at 40°C for 2 and 4 minutes was slightly effective in the control of seed-borne fungi of cultivars of *Amaranthus* compared with controls. However, *C. cucurbitarum*, *A. amaranthi* and *A. fumigatus* were isolated from all cultivars at between 12-27% (Tables 5, 6, 7). Except for $NHAc_{33}$ that still had three fungi (Table 5), seed treatment at this temperature for 6, 8 and 10 minutes

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Table 5: Effect of hot water treatment on the control of seed-borne fungi and seedling emergence of Amaranthus ($NHAc_{33}$) during 1988 and 1989

Temp. °C	Dur. (mins)	Fungi isolated from seed	% fungi recovery*	% seedling emergence**
40	2	Choan. cucurbitarum, A. amaranthi, Curv. geniculata	27.00f	68.00e
50		A. fumigatus, A. tamarii	16.00de	75.00cd
60		A. tamarii, A. niger, F. moniliforme	6.00bc	77.00bc
70		A. nigeri, A. tamarii	4.00ab	79.00bc
80		A. tamarii	3.00a	20.00g
40	4	P. aphanid., R. solani, F. pallidoroseum	18.00e	68.75e
50		T. terricola, A. fumigatus	10.00d	77.00bc
60		A. tamarii, A. flavus	3.00a	83.00b
70		A. tamarii, A. niger	2.00a	84.00b
80		Nil	0.00a	16.00h
40	6	A. tamarii, A. fumigatus, F. pallidoroseum	15.00de	70.00e
50		A. fumigatus, A. niger	6.00bc	80.00bc
60		A. tamarii	2.00a	85.00b
70		Nil	0.00a	87.00a
80		Nil	0.00a	13.00h
40	8	A. fumigatus, A. niger, F. moniliforme	12.00d	72.00cd
50		A. flavus, A. tamarii	4.00ab	84.00b
60		Nil	0.00a	88.00a
70		Nil	0.00a	89.00a
80		Nil	0.00a	8.00i
40	10	A. niger, A. flavus, F. moniliforme	14.00de	72.00cd
50		Nil	0.00a	85.00b
60		Nil	0.00a	90.00a
70		Nil	0.00a	80.00bc
80		Nil	0.00a	4.00j
Control (28)		Choanephora cucurbitarum, Pythium aphanidermatum, Rhizoctonia solani, Bipolaris zeae, Alternaria amaranthi, Thielavia terricola, Aspergillus flavus, A. niger, A. fumigatus and Fusarium moniliforme	55.50g	25.00f

*% recovery of each fungus was based on 4 replicates each of 100 seeds individually examined.

**% emergence are means of 100 seeds of 4 replicates. Values followed by same alphabet(s) do not differ significantly at 5% probability level by Duncan's multiple range test.

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Table 6: Effect of hot water treatment on percentage infection by seed-borne fungi and seedling emergence of NHAc$_{100}$ during 1988 and 1989

Temp. °C	Dur. (mins.)	Fungi isolated from seed	% fungi recovery*	% seedling emergence**
40	2	C. cucurbitarum, A. amaranthi	12.00e	70.00e
50		R. oryzae, A. niger, F. pallidoroseum	10.00cd	78.75de
60		A. flavus, F. pallidoroseum	3.00ab	80.50cd
70		A. flavus, F. pallidoroseum	2.00a	85.00c
80		A. flavus, F. pallidoroseum	2.00a	30.00f
40	4	R. oryzae, Pythium	9.00cd	72.00e
50		A. niger, A. flavus	5.00b	81.00cd
60		A. tamarii, A. niger	2.00a	92.00ab
70		A. tamarii, A. niger	2.00a	94.00ab
80		Nil.	0.00a	18.00g
40	6	P. aphanid., A. niger	8.00bc	74.00e
50		A. tamarii, A. niger	2.00a	83.00cd
60		Nil	0.00a	95.00a
70		Nil	0.00a	96.00a
80		Nil	0.00a	12.00h
40	8	A. niger, A. flavus	8.00bc	76.00de
50		Nil	0.00a	85.00c
60		Nil	0.00a	96.00a
70		Nil	0.00a	92.00ab
80		Nil	0.00a	4.00i
40	10	A. tamarii	6.00b	79.00de
50		Nil	0.00a	88.00c
60		Nil	0.00a	98.00a
70		Nil	0.00a	81.00cd
80		Nil	0.00a	2.00j
Control (28)		Choanephora cucurbitarum, Pythium aphanidermatum, Alternaria amaranthi, Fusarium pallidoroseum, Rhizoctonia oryzae, Aspergillus niger, A. flavus, A. tamarii	52.00f	29.00f

*% recovery of each fungus was based on 4 replicates each of 100 seeds individually examined.
**% emergence are means of 100 seeds of 4 replications. Values followed by same alphabet(s) do not differ significantly at 5% probability level by Duncan's multiple range test.

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Table 7: Effect of hot water treatment on percentage infection by seed-borne fungi and seedling emergence of NHAc$_{30}$ during 1988 and 1989

Temp. °C	Dur. (mins.)	Fungi isolated from seed	% fungi recovery*	% seedling emergence**
40	2	C. cucurbitarum, A. amaranthi, A. fumigatus	20.00f	69.00d
50	2	P. aphanid., Curv. geniculata, C. cucurbitarum	13.00d	76.00cd
60	2	A. tamarii, A. niger, Pythium, F. moniliforme	8.00c	79.00c
70	2	A. tamarii, A. niger, P. aphanid., F. moniliforme	8.00c	80.00c
80	2	A. niger, A. flavus	3.00b	25.00e
40	4	P. aphanid., R. solani, F. moniliforme	15.00de	70.00d
50	4	T. terricola, A. fumigatus	8.00c	78.50c
60	4	A. tamarii, A. flavus	7.00c	87.50ab
70	4	A. tamarii, A. niger	6.00c	88.00a
80	4	A. niger, A. flavus	2.00a	20.00f
40	6	A. fumigatus, A. niger	11.00cd	71.50d
50	6	A. fumigatus, A. niger	5.00b	80.00c
60	6	A. niger	1.00a	88.00a
70	6	Nil	0.00a	90.00a
80	6	Nil	0.00a	12.00g
40	8	A. fumigatus, A. niger	10.00cd	73.00cd
50	8	A. flavus, A. tamarii	3.00b	84.00ab
60	8	Nil	0.00a	91.00a
70	8	Nil	0.00a	89.00a
80	8	Nil	0.00a	6.00h
40	10	A. niger, A. flavus	12.00d	75.00cd
50	10	Nil	0.00a	86.00ab
60	10	Nil	0.00a	92.00a
70	10	Nil	0.00a	80.00c
80	10	Nil	0.00a	2.00i
Control (28)		Choanephora cucurbitarum, Pythium aphanidermatum, Rhizoctonia solani, Thielavia terricola, Aspergillus flavus, A. niger, A. fumigatus, Fusarium moniliforme, Bipolaris zeae.	55.00g	26.00e

*% recovery of each fungus was based on 4 replicates each of 100 seeds individually examined.
**% emergence are means of 100 seeds of 4 replicates. Values followed by same alphabet(s) do not differ significantly at 5% probability level by Duncan's multiple range test.

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produced only two fungi notably, _A. niger_, _A. fumigatus_ or _A. flavus_ in the two other cultivars. Seed-borne fungi was reduced to 10-15% levels in $NHAc_{33}$ and $NHAc_{30}$ and 6-8% in $NHAc_{100}$ (Tables 5, 6, 7). The percentage seedling emergence also increased with treatment duration at 40°C. This ranged between 68-72% for $NHAc_{33}$; 69-75% for $NHAc_{30}$ and 70-79% in $NHAc_{100}$. The percentage seedling emergence for seeds of the control remained below 30% for all cultivars (Tables 5,6,7).

Seeds of all cultivars treated in hot water for 2 and 4 minutes at 50°C further reduced seed-borne fungi. For $NHAc_{33}$, _A. fumigatus_, _A. tamarii_ or _T. terricola_ were isolated at 10-16% levels (Table 5). Treated seeds of $NHAc_{100}$ recorded three (10%) and two (5%) fungi for 2 and 4 minutes respectively (Table 6) and $NHAc_{30}$ had three fungi isolated at 13% and two at 8% (Table 7). This resulted in a corresponding increase in percentage seedling emergence of all cultivars ranging between 77-81% (Tables 5,6,7). Seed treatment at 50°C for 6 and 8 minutes further reduced the percentage seed-borne fungi to 4-6% in $NHAc_{33}$ (Table 5); 0-2% in $NHAc_{100}$ (Table 6) and 5-3% in $NHAc_{30}$ (Table 7). _Aspergillus tamarii_, _A. niger_ or _A. flavus_ were mainly the fungi isolated from the seeds. All other fungi isolated at lower temperatures were not recovered at this temperature. It is noteworthy that no fungus was isolated from seeds

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treated for 8 minutes at 50°C for $NHAc_{100}$ and no fungus was also isolated from seeds treated at this temperature for 10 minutes in all the cultivars (Table 6). Seedling emergence at this temperature for 8 and 10 minutes was higher than 84% in $NHAc_{33}$ and $NHAc_{30}$ and above 85% for $NHAc_{100}$ (Tables 5,6,7).

Treatment of infected *Amaranthus* seeds at 60°C for any of the treatment durations was one of the best out of all temperatures evaluated. Although, treatment at this temperature for 2 minutes still recorded three of four fungal species in $NHAc_{33}$ and $NHAc_{30}$, the levels of occurrence were not more than 6% and 8% (Tables 5 and 7). Cultivar $NHAc_{100}$ had only 3% seed infection by *A. flavus* and *F. pallidoroseum* at this temperature (60°C) for 2 minutes treatment duration (Table 7). Seed treatment at 60°C for 4 and 6 minutes significantly controlled seed-borne fungi by reducing % seed infection to between 0-3% for majority of the cultivars. Seedling emergence was also increased to 85 - 88% in $NHAc_{33}$ and $NHAc_{30}$ and 92 - 95% in $NHAc_{100}$. No fungus was isolated from infected seeds of all cultivars treated at 60°C for 8 and 10 minutes. Consequently, the percentage seedling emergence for such seeds was between 88 - 92% for $NHAc_{33}$ and $NHAc_{30}$ and 96 - 98% for $NHAc_{100}$ (Tables 5,6 and 7). Seed dips in hot water at 70°C for 2, 4 and 6 minutes reduced seed-borne fungi (0-8%) and increased the percentage emergence (79 - 90%) for all the cultivars.

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However, the values obtained were not significantly different from the treatment obtained at 60°C for same duration (Tables 5,6,7). Seed-dip in water at 70°C for 8 and 10 minutes completely disinfected seeds but the % emergence declined to between 80-88% for all the cultivars. Treatment at this temperature for 10 minutes disinfected the seeds with a significant decline (2-4%) in emergence rate (Tables 5,6,7). Seed dips at 80°C for any of the durations, although significantly reduced seed-borne fungi, emergence percentages were significantly reduced to between 2-13% for all the cultivars (Tables 5,6,7). These fungi were each isolated at between 52-55% from untreated seeds of all the cultivars tested: C. cucurbitarum; P. aphanidermatum; R. solani; Bipolaris zeae; A. amaranthi; T. terricola; A. flavus; A. niger; A. fumigatus; A. tamarii; F. moniliforme; R. oryzae and F. pallidoroseum. Seedling emergence for the untreated seeds was very low (25% and 29%) for $NHAc_{33}$ and $NHAc_{30}$ and 29% for $NHAc_{100}$ (Tables 5, 6 and 7). One other interesting observation in this study was that Amaranthus seeds treated in hot water at 40°, 50° and 60°C for longer durations (4 mins. and above) emerged faster and better than the non-treated seeds. Seeds treated at 70° and 80°C for longer durations (6 mins. and above) emerged less than those treated at 60°C.

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4.2.4. Control of seed-borne fungi by chemical treatment

Soaking or dusting of infected *Amaranthus* seeds with appropriate chemicals was efficacious in controlling seed-borne fungi. No fungus was isolated from seeds treated with all the concentrations of Iprodione, Tecto, Captafol and a mixture of Benlate-Captafol during 1987. The percentage seed-borne fungi for the untreated (control) seeds were significantly high (11-14%) for all cultivars used (Table 8a). During 1988 however, it was clear that soaking infected seeds in a mixture of Benlate-Captafol or Captafol singly either at 5 or 10g a.i./kg freed seeds of seed-borne fungi in all the cultivars. Dusting infected seeds with either of these chemicals at the same rate was also good in controlling the seed-borne fungi of all the cultivars (Table 8b). Soaking or dusting of infected seeds with Tecto at 5g a.i./kg seed was next to the first two chemicals in efficiency in all cultivars by also recording zero percentage seed infection. Iprodione and a mixture of Iprodione-Tecto even at 5 or 10g a.i./kg seed were not as good as the other chemicals in the control of infected seeds. The percentage infection of seeds treated with these chemicals at this rate was 0.1% and even higher (0.2%) at lower rate (Table 8b). Non-treated seeds of all cultivars yielded significantly high (>16%) percent-

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Table 8a: Comparative effectiveness of soaking and dusting with chemicals for the control of seed-borne fungi** of three *Amaranthus* varieties during 1987

Mean* % seed infection by fungi				
Chem.	Rate (g) a.i./kg seed	Var.	Soaking	Dusting
Iprodione	2.00	$NHAc_{33}$	0.0a	0.0a
	5.00		0.0a	0.0a
	10.00		0.0a	0.0a
	0.00		11.0b	10.0b
	2.00	$NHAc_{30}$	0.0a	0.0a
	5.00		0.0a	0.0a
	10.00		0.0a	0.0a
	0.00		12.5b	13.5b
	2.00	$NHAc_{100}$	0.0a	0.0a
	5.00		0.0a	0.0a
	10.00		0.0a	0.0a
	0.00		13.0b	13.0b
Tecto	2.00	$NHAc_{33}$	0.0a	0.0a
	5.00		0.0a	0.0a
	10.00		0.0a	0.0a
	0.00		12.0b	12.5b
	2.00	$NHAc_{30}$	0.0a	0.0a
	5.00		0.0a	0.0a
	10.00		0.0a	0.0a
	0.00		11.5b	12.5b
	2.00	$NHAc_{100}$	0.0a	0.0a
	5.00		0.0a	0.0a
	10.00		0.0a	0.0a
	0.00		13.5b	14.0b

Mean* % seed infection by fungi				
Chem.	Rate (g) a.i./kg seed	Var.	Soaking	Dusting
Captafol	2.00	$NHAc_{33}$	0.0a	0.0a
	5.00		0.0a	0.0a
	10.00		0.0a	0.0a
	0.00		12.0b	12.5b
	2.00	$NHAc_{30}$	0.0a	0.0a
	5.00		0.0a	0.0a
	10.00		0.0a	0.0a
	0.00		11.5b	12.5b
	2.00	$NHAc_{100}$	0.0a	0.0a
	5.00		0.0a	0.0a
	10.00		0.0a	0.0a
	0.00		13.0b	13.5b
Benlate-Captafol	2.00	$NHAc_{33}$	0.0a	0.0a
	5.00		0.0a	0.0a
	10.00		0.0a	0.0a
	0.00		12.0b	12.5b
	2.00	$NHAc_{30}$	0.0a	0.0a
	5.00		0.0a	0.0a
	10.00		0.0a	0.0a
	0.00		12.5b	13.0b
	2.00	$NHAc_{100}$	0.0a	0.0a
	5.00		0.0a	0.0a
	10.00		0.0a	0.0a
	0.00		13.0b	13.5b

*Means of each treatment followed by a common letter are not significantly different at 5% level by Duncan's multiple range test.

**Fungi most frequently isolated from the control of all varieties either by soaking or dusting were *C. cucurbitarum*, *P. aphanidermatum*, *R. solani*, *B. zeae*, *A. amaranthi*, *F. terricola*, *A. flavus*, *A. niger*, *A. tamarii*, *A. fumigatus* and *F. moniliforme*.

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Table 8 b: Comparative effectiveness of soaking and dusting with chemicals for control of seed-borne** fungi of three *Amaranthus* cultivars during 1988

Mean* % seed infection by fungi				
Chem.	Rate (g) a.i./kg seed	Cv.	Soaking	Dusting
Iprodione	2.00	$NHAc_{100}$	0.1b	0.2c
	5.00		0.0a	0.1b
	10.00		0.0a	0.0a
	0.00		16.5c	15.0c
	2.00	$NHAc_{33}$	0.1c	0.2c
	5.00		0.1b	0.1c
	10.00		0.0a	0.0a
	0.00		17.0c	16.0c
	2.00	$NHAc_{30}$	0.2c	0.1c
	5.00		0.1a	0.1c
	10.00		0.0a	0.0a
	0.00		18.5c	18.5c
Tecto	2.00	$NHAc_{100}$	0.1b	0.1c
	5.00		0.0a	0.0a
	10.00		0.0a	0.0a
	0.00		15.5c	16.0c
	2.00	$NHAc_{33}$	0.1c	0.1c
	5.00		0.0a	0.0a
	10.00		0.0a	0.0a
	0.00		17.0c	17.5c
	2.00	$NHAc_{30}$	0.2c	0.2c
	5.00		0.0a	0.0a
	10.00		0.0a	0.0a
	0.00		16.0c	16.5c
Captafol	2.00	$NHAc_{100}$	0.1b	0.2b
	5.00		0.0a	0.0a
	10.00		0.0a	0.0a
	0.00		15.5c	16.5c
	2.00	$NHAc_{33}$	0.1c	0.2b
	5.00		0.0a	0.0a
	10.00		0.0a	0.0a
	0.00		15.5c	16.5c
	2.00	$NHAc_{30}$	0.1c	0.2b
	5.00		0.0a	0.0a
	10.00		0.0a	0.0a
	0.00		15.5c	15.0c

Mean* % seed infection by fungi				
Chem.	Rate (g) a.i./kg seed	Cv.	Soaking	Dusting
Benlate-Captafol	2.00	$NHAc_{100}$	0.0a	0.0a
	5.00		0.0a	0.0a
	10.00		0.0a	0.0a
	0.00		15.0c	15.0c
	2.00	$NHAc_{33}$	0.0a	0.1b
	5.00		0.0a	0.0a
	10.00		0.0a	0.0a
	0.00		16.0c	16.0c
	2.00	$NHAc_{30}$	0.1b	0.1b
	5.00		0.0a	0.0a
	10.00		0.0a	0.0a
	0.00		16.2c	16.0c
Iprodione-Tecto	2.00	$NHAc_{100}$	0.0a	0.0a
	5.00		0.0a	0.0a
	10.00		0.0a	0.0a
	0.00		16.0c	16.0c
	2.00	$NHAc_{33}$	0.2c	0.2b
	5.00		0.1c	0.1b
	10.00		0.0a	0.1b
	0.00		16.0c	16.0c
	2.00	$NHAc_{30}$	0.1b	0.2b
	5.00		0.1b	0.1b
	10.00		0.1b	0.1b
	0.00		16.0c	16.0c

*Means of each treatment followed by a common letter are not significant different at 5% probability level by Duncan's multiple range test.

**Fungi most frequently isolated from treated seeds of all cultivars either by soaking or dusting were *A. flavus*, *A. fumigatus*, *A. tamarii* while *Pythium* and *R. solani* were obtained at very low levels. Fungi isolated from control of all cultivars were *C. cucurbitarum*, *P. aphanidermatum*, *R. solani*, *B. zeae*, *A. amaranthi*, *T. terricola*, *A. flavus*, *A. niger*, *A. tamarii*, *A. fumigatus* and *F. moniliforme*.

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ages of these seed-borne fungi: C. cucurbitarum; P. aphanidermatum; R. solani; B. zeae; A. amaranthi; T. terricola; A. flavus; A. niger; A. tamarii; A. fumigatus and F. moniliforme (Table 8b). The fungi occasionally isolated at very low levels from seeds treated at 2g a.i./ kg seed were A. flavus, A. fumigatus, A. tamarii, R. solani and P. aphanidermatum (Table 8b).

During 1987, soaking or dusting of infected seeds with chemicals significantly improved the rate of seedling emergence. However, soaking in a mixture of Benlate-Captafol, Tecto and Captafol in order of preference was significantly superior to dusting. Thus, between 50 - 100% seedling emergence were recorded for soaked seeds while 10 - 83% were obtained from dusted seeds of the cultivars. The control (non-treated seeds) of these cultivars had very low rate (29-39%) of seedling emergence (Table 9a). Although all the concentrations of the chemicals were effective in improving seedling emergence, the mean percentage seedling emergence was highest at 10g a.i./kg seed. No significant difference in the mean percentage seedling emergence for all the cultivars used as observed. In 1988, soaking and dusting also proved beneficial to the seeds. Benlate-Captafol mixture, Captafol and Tecto at 10g a.i./kg seed were also effective by recording between 39 -

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Table 9 a: Comparative effectiveness of soaking and dusting with chemicals on seedling emergence of three *Amaranthus* cultivars during 1987

Mean* % seedling emergence					Mean* % seedling emergence				
Chem.	Rate (g) a.i./kg seed	Cv.	Soaking	Dusting	Chem.	Rate (g) a.i./kg seed	Cv.	Soaking	Dusting
Iprodione	2.00	$NHAc_{33}$	83.7ab	80.7a	Captafol	2.0	$NHAc_{33}$	88.0a	72.0b
	5.00		77.3c	75.0ab		5.0		55.4c	70.0b
	10.00		87.0a	77.0a		10.0		84.3a	77.3a
	0.00		32.0d	32.0d		0.0		37.5d	37.8d
	2.00	$NHAc_{30}$	93.7a	72.3b		2.0	$NHAc_{30}$	71.3b	65.7c
	5.00		90.7a	69.0b		5.0		74.3b	71.0b
	10.00		73.7c	76.7ab		10.0		82.7a	78.7a
	0.00		32.5d	34.0d		0.0		36.3d	36.0d
	2.00	$NHAc_{100}$	72.8c	59.0c		2.0	$NHAc_{100}$	64.7b	58.0c
	5.00		83.3ab	83.0a		5.0		87.3a	71.0b
	10.00		79.7ab	74.0ab		10.0		75.0b	65.0c
	0.00		36.0d	35.0d		0.0		42.5d	43.0d
Tecto	2.00	$NHAc_{33}$	94.0a	64.0b	Benlate-Captafol	2.0	$NHAc_{33}$	75.0a	83.7a
	5.00		95.7a	69.0b		5.0		80.0a	83.7a
	10.00		83.0ab	70.0b		10.0		86.7a	78.7a
	0.00		33.5d	34.5d		0.0		38.0c	36.5b
	2.00	$NHAc_{30}$	80.0b	72.7b		2.0	$NHAc_{30}$	35.3b	11.0c
	5.00		71.7c	22.0c		5.0		50.0b	9.7c
	10.00		91.7a	81.7a		10.0		74.0a	30.0b
	0.00		34.0d	35.5d		0.0		31.5c	29.5b
	2.00	$NHAc_{100}$	75.0b	68.0b		2.0	$NHAc_{100}$	88.0a	9.7c
	5.00		98.0a	80.0a		5.0		72.0ab	18.0c
	10.00		81.7b	77.7a		10.0		100.0a	30.0b
	0.00		38.5d	39.5d		0.0		38.51c	38.0b

*Means of each treatment followed by a common letter(s) are not significantly different at 5% level by Duncan's multiple range test. One hundred seedlings each of 4 replicates were examined for seedling emergence.

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98% seedling emergence for all cultivars either by soaking or dusting (Table 9b). Soaking the seeds was equally as good as dusting with chemicals. The third concentration (10g a.i./kg seed) of the chemicals was also more effective in improving seedling emergence. However, $NHAc_{100}$ emerged significantly better (73 - 98%) when treated with higher concentrations of the chemicals than $NHAc_{33}$ and $NHAc_{30}$ that recorded between 41 - 89% and 36 - 89% seedling emergence respectively. The control of the cultivars also had poor rates (29 - 43%) of seedling emergence (Table 9b).

Soaking or dusting of infected seeds with appropriate chemicals as indicated earlier significantly controlled the seed-borne fungi hence seedling infection. During 1987, all the seedlings produced from treated seeds either by soaking or dusting were free from infection. All concentrations of the chemicals proved useful. The control seeds produced more than 70% infected seedlings in all cultivars (Table 10a). The fungi most frequently isolated from infected seedlings were *R. solani*, *C. cucurbitarum*, *P. aphanidermatum* and at times *T. terricola*.

Results of 1988 also show that chemical disinfection of seeds resulted in the production of healthy seedlings. The third concentration (10g a.i./kg seed) of all the chemicals especially by soaking was the most effective by

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Table 9 b: Comparative effectiveness of soaking and dusting with chemicals on seedling emergence of three *Amaranthus* cultivars during 1988

Chem	Rate (g) a.i./kg seed	Cv.	Mean* % seedling emergence: Soaking	Mean* % seedling emergence: Dusting
Iprodione	2.00	$NHAc_{100}$	78.00b	78.00b
	5.00		79.70b	85.70a
	10.00		82.70b	90.70a
	0.00		38.70d	38.30d
	2.00	$NHAc_{33}$	71.00b	75.70b
	5.00		75.00b	92.00a
	10.00		95.70a	96.30a
	0.00		34.30d	36.00d
	2.00	$NHAc_{30}$	62.00c	74.70bc
	5.00		77.70b	80.70b
	10.00		85.30b	86.00b
	0.00		39.30d	36.00d
Tecto	2.00	$NHAc_{100}$	68.00bc	87.30b
	5.00		71.70b	95.70a
	10.00		75.70b	98.00a
	0.00		30.70c	35.70c
	2.00	$NHAc_{33}$	50.30c	75.00b
	5.00		77.70b	84.00b
	10.00		86.30a	95.70a
	0.00		34.00c	34.30c
	2.00	$NHAc_{30}$	73.70b	77.30b
	5.00		83.30a	85.30b
	10.00		85.00a	99.30a
	0.00		34.00c	35.30c
Captafol	2.00	$NHAc_{100}$	74.70b	59.00c
	5.00		81.00a	87.00a
	10.00		88.30a	98.70a
	0.00		40.30d	38.30d
	2.00	$NHAc_{33}$	68.70c	74.70b
	5.00		74.70b	79.70b
	10.00		84.00a	86.30a
	0.00		37.00d	37.30d
	2.00	$NHAc_{30}$	60.30c	68.70c
	5.00		67.30c	78.70b
	10.00		75.30b	89.00a
	0.00		36.00d	35.30d

Chem	Rate (g) a.i./kg seed	Cv.	Mean* % seedling emergence: Soaking	Mean* % seedling emergence: Dusting
Benlate-Captafol	2.00	$NHAc_{100}$	80.70a	79.00c
	5.00		85.30a	83.30b
	10.00		90.70a	91.30a
	0.00		29.30c	44.30e
	2.00	$NHAc_{33}$	16.30c	42.00e
	5.00		20.00c	54.30d
	10.00		39.00b	78.30c
	0.00		36.00b	43.30e
	2.00	$NHAc_{30}$	13.30c	78.00c
	5.00		24.70c	92.70a
	10.00		41.70b	100.00a
	0.00		37.70b	43.00e
Iprodione-Tecto	2.00	$NHAc_{100}$	73.30a	72.30a
	5.00		77.70a	76.00a
	10.00		78.70a	79.00a
	0.00		35.00b	42.30d
	0.00	$NHAc_{33}$	13.30c	37.70e
	5.00		16.00c	41.00d
	10.00		35.00b	53.70c
	0.00		34.30b	43.30d
	2.00	$NHAc_{30}$	10.30c	65.00b
	5.00		19.30c	69.30ab
	10.00		35.70b	75.00a
	0.00		38.70b	40.70d

*Means of each treatment followed by a common letter are not significantly different at 5% probability level by Duncan's multiple range test. One hundred seedlings of 4 replicates were examined for seedling emergence.

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Table 10a: Comparative effectiveness of soaking and dusting with chemicals for the control of seedling infection** of three *Amaranthus* cultivars during 1987

Chem.	Rate (g) a.i./kg seed	Cv.	Mean* % seedling infection Soaking	Mean* % seedling infection Dusting
Iprodione	2.00	$NHAc_{33}$	0.0a	0.0a
	5.00		0.0a	0.0a
	10.00		0.0a	0.0a
	0.00		74.0b	72.0b
	2.00	$NHAc_{30}$	0.0a	0.0a
	5.00		0.0a	0.0a
	10.00		0.0a	0.0a
	0.00		75.0b	70.0b
	2.00	$NHAc_{100}$	0.0a	0.0a
	5.00		0.0a	0.0a
	10.00		0.0a	0.0a
	0.00		73.0b	70.0b
Tecto	2.00	$NHAc_{33}$	0.0a	0.0a
	5.00		0.0a	0.0a
	10.00		0.0a	0.0a
	0.00		76.0b	72.0b
	2.00	$NHAc_{30}$	0.0a	0.0a
	5.00		0.0a	0.0a
	10.00		0.0a	0.0a
	0.00		70.0b	70.0b
	2.00	$NHAc_{100}$	0.0a	0.0a
	5.00		0.0a	0.0a
	10.00		0.0a	0.0a
	0.00		74.0b	71.0b

Chem.	Rate (g) a.i./kg seed	Cv.	Mean* % seedling infection Soaking	Mean* % seedling infection Dusting
Captafol	2.0	$NHAc_{33}$	0.0a	0.0a
	5.0		0.0a	0.0a
	10.0		0.0a	0.0a
	0.0		75.0b	72.0b
	2.0	$NHAc_{30}$	0.0a	0.0a
	5.0		0.0a	0.0a
	10.0		0.0a	0.0a
	0.0		68.0b	66.0b
	2.00	$NHAc_{100}$	0.0a	0.0a
	5.0		0.0a	0.0a
	10.0		0.0a	0.0a
	0.0		75.0b	73.0b
Benlate-Captafol	2.0	$NHAc_{33}$	0.0a	0.0a
	5.0		0.0a	0.0a
	10.0		0.0a	0.0a
	0.0		78.0b	76.0b
	2.0	$NHAc_{30}$	0.0a	0.0a
	5.0		0.0a	0.0a
	10.0		0.0a	0.0a
	0.0		79.0b	75.0b
	2.0	$NHAc_{100}$	0.0a	0.0a
	5.0		0.0a	0.0a
	10.0		0.0a	0.0a
	0.0		77.0b	74.0b

*Means of each treatment followed by a common letter are not significantly different at 5% level by Duncan's multiple range test.

**The fungi most frequently isolated from the control seedlings either by soaking or dusting were *R. solani*, *C. cucurbitarum*, *P. aphanidermatum*, *T. terricola*.
One hundred seedlings each of 4 replicates were examined for seedling infection.

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producing a zero percent seedling infection (Table 10b). Non-treated seeds also produced between 64 - 75% infected seedlings. The fungi isolated from seedlings showing infection were the same as for those obtained in 1987 (Table 10b).

4.2.5. Control of inflorescence infection and field-acquired mycoflora of seeds by pre-harvest spray of chemicals

(i) Control of inflorescence infection

A typical inflorescence infection of *Amaranthus* is as shown in Plate 8. All the chemicals applied during the early season of 1987 were capable of controlling inflorescence infection by *C. cucurbitarum* to varying degrees. A mixture of Benlate-Captafol when sprayed at the rate of 1.25g a.i./plot had significantly least percentage inflorescence infection (5%). This was followed by Captafol and Iprodione with 10% and 20% inflorescence infection respectively (Fig. 3a). Plants sprayed with Tecto had 25% infection.

The second concentration of all the chemicals (2.5g a.i./plot) controlled inflorescence infection even better than the first. The inflorescence infection in plants sprayed with Benlate-Captafol was nil. Again Captafol, Iprodione and Tecto in order of increasing efficiency had

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Table 10 b: Comparative effectiveness of soaking and dusting with chemicals for control of seedling infection** of three *Amaranthus* cultivars during 1988

Chem.	Rate (g) a.i./kg seed	Cv.	Soaking	Dusting
		Mean % seedling infection		
Iprodione	2.00	NHAc100	0.1a	0.2a
	5.00		0.0a	0.1a
	10.00		0.0a	0.0a
	0.00		66.0c	68.0c
	2.00	NHAc33	0.1a	0.2a
	5.00		0.1a	0.2a
	10.00		0.0a	0.1a
	0.00		70.0c	71.0c
	2.00	NHAc30	0.1a	0.2a
	5.00		0.1a	0.0a
	10.00		0.0a	0.0a
	0.00		72.0c	69.5c
Tecto	2.00	NHAc100	0.1a	0.2a
	5.00		0.0a	0.0a
	10.00		0.0a	0.0a
	0.00		64.0c	65.0c
	2.00	NHAc33	0.1a	0.2a
	5.00		0.1a	0.1a
	10.00		0.0a	0.0a
	0.00		72.0c	73.5c
	2.00	NHAc30	0.8b	0.5b
	5.00		0.0a	0.1a
	10.00		0.0a	0.1a
	0.00		71.5c	73.0c
Captafol	2.00	NHAc100	0.2a	0.2a
	5.00		0.1a	0.0a
	10.00		0.0a	0.0a
	0.00		65.5c	66.0c
	2.00	NHAc33	0.2a	0.3a
	5.00		0.1a	0.1a
	10.00		0.0a	0.1a
	0.00		74.0c	75.0c
	2.00	NHAc30	0.2a	0.0a
	5.00		0.1a	0.1a
	10.00		0.0a	0.0a
	0.00		75.0c	74.0c

Chem.	Rate (g) a.i./kg seed	Cv.	Soaking	Dusting
		Mean* % seedling infection		
Benlate-Captafol	2.00	NHAc100	0.0a	0.1a
	5.00		0.0a	0.0a
	10.00		0.0a	0.0a
	0.00		63.5b	64.0b
	2.00	NHAc33	0.1a	0.2a
	5.00		0.1a	0.1a
	10.00		0.0a	0.0a
	0.00		73.0b	74.0b
	2.00	NHAc30	0.2a	0.2a
	5.00		0.1a	0.1a
	10.00		0.0a	0.0a
	0.00		75.0b	74.0b
Iprodione-Tecto	2.00	NHAc100	0.0a	0.0a
	5.00		0.0a	0.0a
	10.00		0.0a	0.0a
	0.00		60.0b	58.0b
	2.00	NHAc33	0.2a	0.2a
	5.00		0.1a	0.1a
	10.00		0.0a	0.1a
	0.00		59.5b	68.0b
	2.00	NHAc30	0.2a	0.2a
	5.00		0.2a	0.2a
	10.00		0.1a	0.1a
	0.00		66.1b	69.5b

*Means of each treatment followed by a common letter are not significantly different at 5% probability level by Duncan's multiple range test.

**The fungi most frequently isolated from seedlings of all cultivars either by soaking or dusting were *R. solani*, *C. cucurbitarum*, *P. aphanidermatum*, *T. terricola*.
One hundred seedlings each of 4 replicates were examined for seedling infection.

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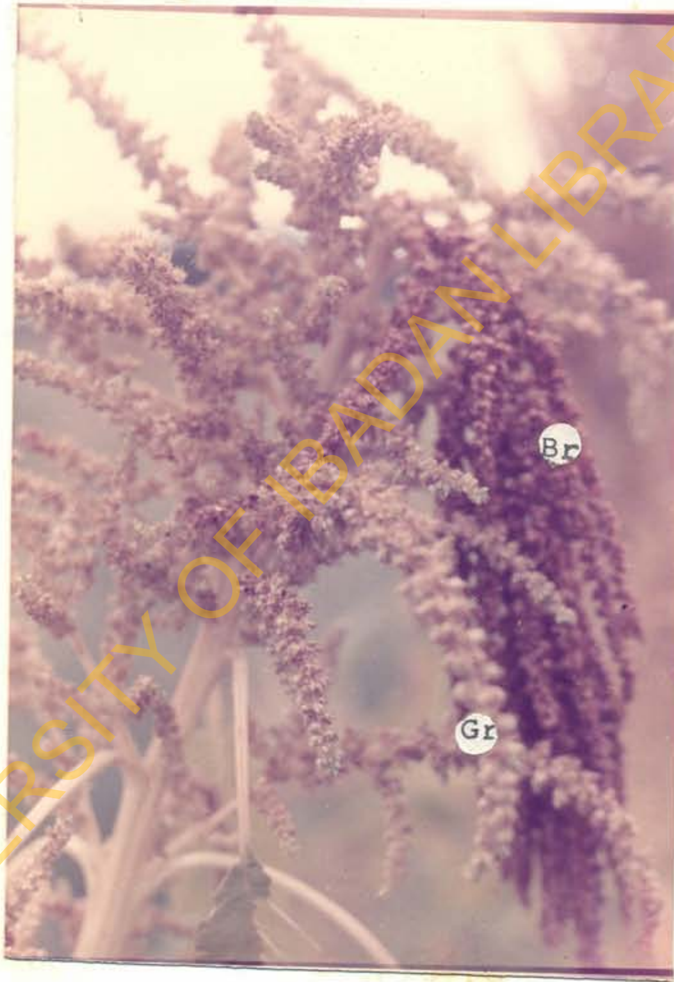

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Plate 8: A typical inforescence infection of Amaranthus sp. caused by Choanephora cucurbitarum. Note the characteristic browning and drooping of infected raceme (Br.) and green healthy inflorescence (Gr.).

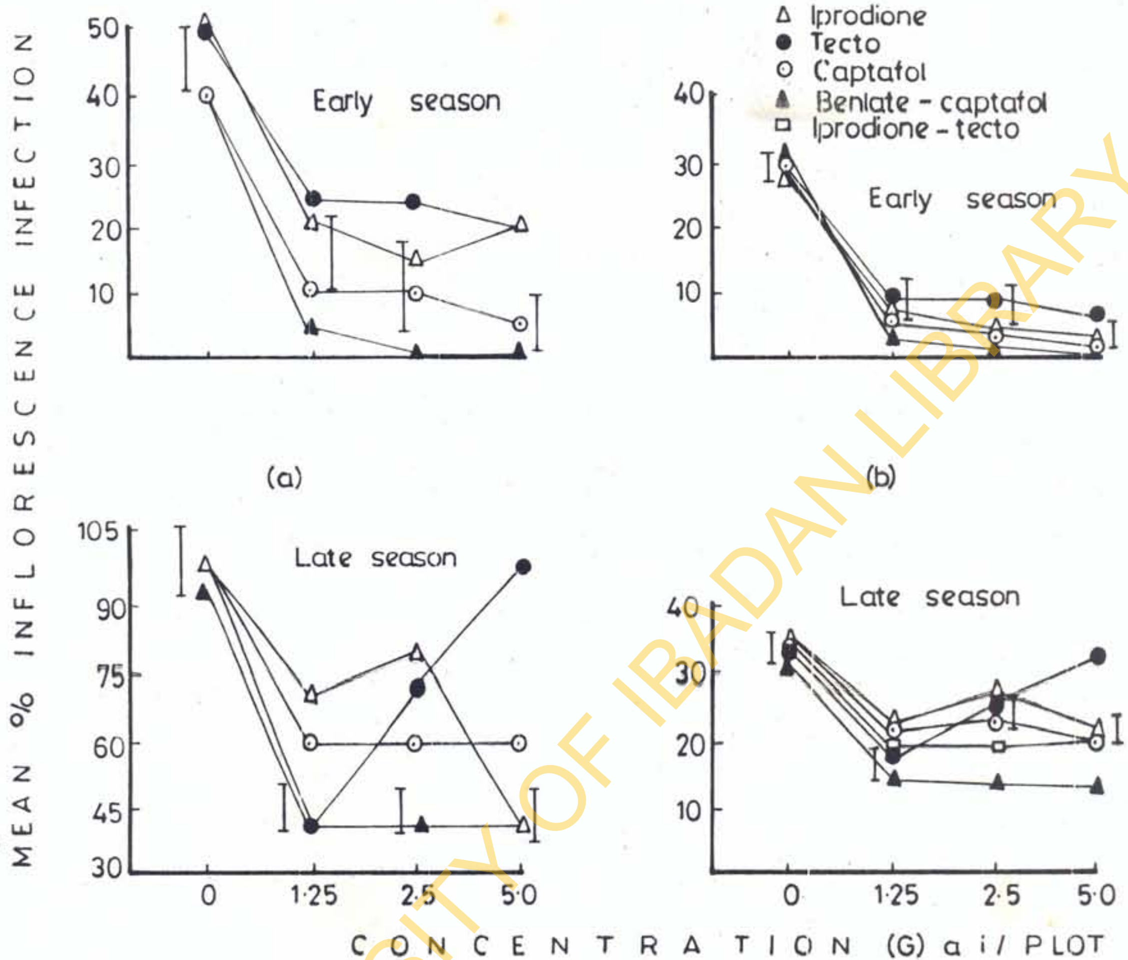

Fig. 3: The efficacy of different field - applied fungicides for the control of inflorescence infection on Amaranthus (NHAc 33) during the early and late seasons of 1987 (a) and 1988 (b) respectively I LSD at 0·05.

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10%, 15% and 25% inflorescence infections respectively (Fig. 3a).

The third concentration of all the chemicals (5g a.i./ plot) also effectively controlled inflorescence infection with the chemicals performing essentially like in 1.25 and 2.5g a.i./plot. Benlate-Captafol and Captafol alone recorded significantly least percentage inflorescence infection of 0.0% and 5.0% respectively. There were no significant differences in the percentage inflorescence infection in plots sprayed with Iprodione and Tecto at this concentration (Fig. 3a). All the control plots for each chemical in the early season of 1987 had significantly highest percentage (40 - 50%) inflorescence infection (Fig. 3a).

The behaviour of the chemicals at each concentration during the late season of 1987 were similar to that of the early season except that higher percentages inflorescence infections were recorded. Benlate-Captafol mixture recorded least percentage (40%) infection for all concentrations; followed by Captafol (60%) and Iprodione with 40 - 80% infection (Fig. 3b). Again, it is to be noted that the least percentage infections were observed from plants sprayed with the third concentration of all the chemicals this season. Significantly highest percentage (90 - 100%) inflorescence

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infection were recorded in the control plots (Fig. 3a).

During the second year, 1988, it was observed too that the chemicals also controlled inflorescence infection. When the individual chemical was considered in 1988, Benlate-Captafol at any of the concentrations had significantly least inflorescence infection. However, 2.5 and 5.0g a.i./plot had 1% and 0.7% infection respectively (Fig. 3b). Captafol, Iprodione-Tecto and Iprodione in order of effectiveness were next to Benlate-Captafol with minimum percentages of 2%, 4% and 4% infection respectively (Fig. 3b).

The percentage inflorescence infections during the late season of 1988 were again generally higher than those of the early season like was observed in 1987. Benlate-Captafol mixture at any of the three concentrations was again significantly superior to the other chemicals by having only 15% inflorescence infection (Fig. 3b). There were no significant difference in the percentage inflorescence infection in plots sprayed with Captafol and a mixture of Iprodione-Tecto this season. Captafol and Iprodione-Tecto had a minimum of 21% and 19% infection respectively (Fig. 3b). Single Iprodione application was next to these chemicals in effectiveness also with a minimum of 21% infection. The third concentration (5g. a.i./plot) of the chemicals was also generally better than the first and second concentrations in

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controlling inflorescence infection. The control plants for both the early and late seasons of this year had significantly highest rate of inflorescence infection ranging between 29 - 35% (Fig. 3b).

(ii) Effect of chemical application on seed yield

Application of appropriate chemicals on *Amaranthus* to control inflorescence infection affected seed yield differently depending on the type of chemical and rate of application. Out of all the chemicals applied in 1987 early season, Tecto at 5g a.i./plot produced significantly highest (70g) mean seed yield. The next best mean seed yield, 51g, was recorded when a mixture of Benlate-Captafol were applied at 1.25g a.i./plot. Iprodione applied at 5g a.i./plot produced the third highest yield of 43g. Tecto at 2.5g and 1.25g a.i./plot had 39g and 36g seed weight respectively. The highest seed yield for the control plots was 10g (Fig. 4a).

When all the chemicals applied during 1987 late season were considered, a mixture of Benlate-Captafol at 2.5g a.i./plot produced significantly highest seed yield (75g). This was followed by Captafol at 5g a.i./plot recording 58g seed yield (Fig. 4a). Iprodione when applied at 5g a.i./plot produced the third highest seed yield of 56g.

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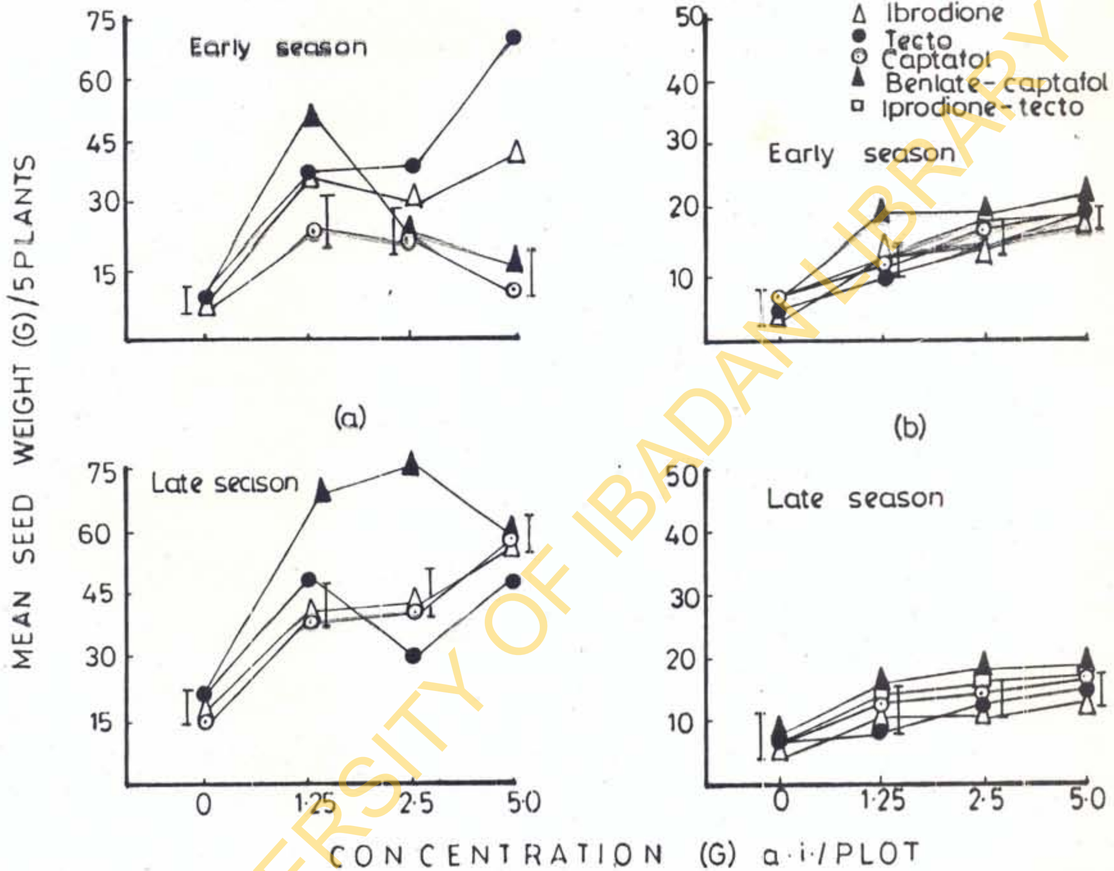

Fig. 4: The effect of fungicide application on Amaranthus. (NHAc 33) inflorescence for the evaluation of seed yield for the early and late season of 1987 (a) and 1988 (b) respectively.
I LSD at 0·05·

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The latter was not significantly different from 58g seed weight obtained from Captafol sprayed plots. The three best chemicals that recorded good yield this season in order of effectiveness were Benlate-Captafol mixture, Captafol and Iprodione. Tecto even at the highest rate (5g a.i./plot) produced 48g seed. The seed yield for the control plots ranged between 16 - 21g and were significantly least (Fig. 4a).

Considering individual chemicals during 1988 early season again Benlate-Captafol at 5g a.i./plot had the highest seed yield (22g). No significant difference in the yield obtained from Captafol (18g), Iprodione-Tecto (18g) and Tecto (19g). Again the third rate of all the chemicals was better than the first or second concentration. The control plots also produced significantly least seed yield of 3 -6g this early season (Fig. 4b).

In the late season of 1988, Benlate-Captafol mixture at 2.5 and 5.0g a.i./plot had highest yield of 19 and 18g respectively (Fig. 4b). Again, Captafol, Iprodione-Tecto and single Tecto application were next to Benlate-Captafol recording 13-14g seed yield. The second rate (2.5g a.i./plot) of these chemicals was next to the third in efficiency. The highest yield for the control plots this season was 7g and was significantly the least (Fig. 4b).

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(iii) Chemical control of seed-borne mycoflora and seedling emergence

During the early season of 1987, a mixture of Benlate-Captafol applied at 5g a.i./plot gave 90% seedling emergence (Table 11a). This was however, not significantly different from 88% and 84.6% emergence recorded for plots sprayed with 2.5 and 1.25g a.i. of the same chemical. Single Iprodione and Captafol sprayed at 5g a.i. each per plot were next best to Benlate-Captafol giving 85.7% and 75% seedling emergence respectively (Table 11a). No sigificant difference in the percentage seedling emergence between plants sprayed with 2.5g a.i./plot of Captafol (70.6%) and Iprodione (74%). These chemicals were also equally effective at 1.25g a.i./plot. The chemicals in order of their efficiency in improving seedling emergence were Benlate-Captafol, Iprodione, Captafol and Tecto at 5g, 2.5g and 1.25g per plot respectively. In the late season of 1987, Benlate-Captafol mixture at any of the rates applied increased seedling emergence up to 65%. Iprodione and Captafol at 5g a.i./plot each gave the next best (56.6% and 55%) seedling emergence respectively. The percentage seedling emergence recorded for the second rate (2.5g a.i./plot) of the chemicals was higher than the first (1.25g a.i./plot). Percentage seedling emergence from seeds in control plots for both seasons was significantly lower

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Table 11a: The effect of different field-applied fungicides on *Amaranthus* (NHAc33) inflorescence and percentage seedling emergence* from seeds of 1987 early and late season plantings.

1987 Early Season

Chem. x Conc.	Mean % emergence* at indicated field rates			
	0.0 (g) a.i./plot	1.25 (g) a.i./plot	2.5 (g) a.i./plot	5.0 (g) a.i./plot
Iprodione	40.00d	66.33b	74.00b	85.67a
Tecto	35.00d	57.00c	60.00b	68.33b
Captafol	45.00d	70.00b	70.67b	75.00a
Benlate-Captafol	42.00d	84.67a	88.00a	90.00a

1987 Late Season

Chem. x Conc.	0.0 (g) a.i./plot	1.25 (g) a.i./plot	2.5 (g) a.i./plot	5.0 (g) a.i./plot
Iprodione	8.00f	30.00d	34.67d	56.67b
Tecto	8.33f	27.33de	18.00e	18.33e
Captafol	10.33f	48.00c	49.67c	55.00b
Benlate-Captafol	9.00f	57.78a	65.00a	60.33a

*Values are means of four replications.

Mean values followed by a common alphabet(s) do not differ significantly at 5% probability level by Duncan's multiple range test.

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than the emergence from chemically treated plots (Table 11a).

The chemical treatments were similar during 1987 and 1988 - Benlate-Captafol being the best followed by Iprodione-Tecto, Captafol and Iprodione for both seasons (Table 11b). The chemicals at 5g a.i./plot was best followed by 2.5g a.i./plot.

The performance of the individual chemical during the early season of 1988 indicated that plots sprayed with 5g a.i. of Benlate-Captafol mixture had the highest seedling emergence rate (92%) (Table 11b). This was followed by the same chemical at 2.5g a.i./plot having 89.3% emergence. The next best chemical was a mixture of Iprodione-Tecto sprayed at any of the rates producing well over 81% seedling emergence. Iprodione and Captafol had 87.3% and 78% seedling emergence respectively. The highest seedling emergence for the non-sprayed plots this season was 34% (Table 11b).

In the late season of 1988, Benlate-Captafol mixture when applied on Amaranthus inflorescence at 2.5g and 5g a.i./plot had the highest seedling emergence (68.6% each). Captafol at 5g a.i./plot also produced high (64.3%) seedling emergence (Table 11b). Iprodione-Tecto mixture at any of the concentrations applied, Captafol at 2.5g a.i./plot and Iprodione at 5g a.i./plot produced the second highest seedling

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Table 11b: The effect of different field-applied fungicides on *Amaranthus* ($NHAc_{33}$) inflorescence and percentage seedling emergence* from seeds of 1988 early and late season plantings.

1988 Early season

Chem. x Conc.	Mean % emergence* at indicated field rates			
	0.0 (g) a.i./plot	1.25 (g) a.i./plot	2.5 (g) a.i./plot	5.0 (g) a.i./plot
Iprodione	30.33f	67.33d	76.67c	87.33ab
Tecto	30.67f	60.00e	62.67e	70.67d
Captafol	33.00f	74.00cd	73.00cd	78.00c
Benlate-Captafol	34.00f	86.67ab	89.33a	92.33a
Iprodione-Tecto	33.33f	83.33b	82.33b	84.67b

1988 Late season

Chem. x Conc.	Mean % emergence* at indicated field rates			
	0.0 (g) a.i./plot	1.25(g) a.i./plot	2.5 (g) a.i./plot	5.0 (g) a.i./plot
Iprodione	19.00f	33.33d	37.33c	53.67b
Tecto	18.00f	30.00d	24.00e	23.33e
Captafol	21.00f	42.33c	55.00b	64.33a
Benlate-Captafol	24.00f	60.67a	68.67a	68.67a
Iprodione-Tecto	25.33f	52.00b	53.00b	55.00b

*Values are means of three replicates.
Mean values followed by a common letter for each parameter are not significantly different at 5% probability level by Duncan's multiple range test.

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emergence of 55%, 55% and 53.6% respectively. The highest seedling emergence for the control plots was 25.3% (Table 11b).

(iv) Seed-borne mycoflora

The pre-harvest spray of *Amaranthus* inflorescence with fungicides in the field significantly reduced the incidence of seed-borne fungal flora. All the chemicals applied on the inflorescence significantly reduced the incidence of seed-borne *C. cucurbitarum* (Table 12a). The value of 0.4% seed infection each recorded for plots that received 5.0g a.i. of each of Benlate-Captafol, Captafol alone, Tecto and Iprodione were significantly less than the 6% seed infection for the control (Table 12a). The same trend goes for *A. amaranthi* and *F. moniliforme*. That is, these organisms invaded seeds of plants sprayed with these chemicals at a much lower rate compared with the non-sprayed (control) plants that were infected at a much higher rate. Also significantly higher seed infection were now recorded for the control plants for each of the chemical (Table 12a). It is noteworthy that as high as 17% seed infection were recorded for seeds from control (non-sprayed) plots. With the exception of *A. tamarii* and *A. fumigatus* which recorded between 2-5% infection, less than 4% seed infections were recorded for all the fungi

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Table 12a: The effect of fungicide application on Amaranthus ($NHAc_{33}$) inflorescence on percentage infection by seed-borne* fungi during 1987 early season.

Chemical	Rate/plot (g) a.i.	Mean percentage** seed infection by organisms***					
		1	2	3	4	5	6
Iprodione	1.25	0.4a	2.9b	3.5b	4.9b	5.3c	3.1c
	2.5	2.3b	2.5b	0.4a	4.5b	3.8b	1.6b
	5.0	0.4a	0.4a	0.4a	3.9b	3.2b	1.0a
Tecto	1.25	2.3b	3.2c	2.3b	3.9b	4.1b	1.6b
	2.5	1.6b	3.5c	1.6b	2.9ba	3.9b	1.0a
	5.0	0.4a	0.4a	1.0a	3.2b	3.5b	0.4a
Captafol	1.25	1.6b	1.6b	1.6b	4.1b	4.3bc	2.3b
	2.5	0.4a	0.4a	1.0a	3.5b	3.8b	1.0a
	5.0	0.4a	0.4a	0.4a	2.5a	3.2b	0.4a
Benlate-Captafol	1.25	0.4a	3.1b	2.9b	2.3a	3.2b	0.4a
	2.5	0.4a	2.3b	0.4a	1.0a	2.5a	0.4a
	5.0	0.4a	0.4a	0.4a	1.6a	1.6a	0.4a
Control	0.0	6.1c	14.0d	17.3c	24.1c	25.7d	24.9d

*Four plates each of 100 seeds were examined for each treatment.

**Values for each chemical/organism followed by same alphabet are mean comparison with control and do not significantly differ at 5% level by Duncan's multiple range test.

***1 - *Choanephora cucurbitarum*, 2 - *Alternaria amaranthi*, 3 - *Fusarium moniliforme*, 4 - *Aspergillus tamarii*, 5 - *Aspergillus fumigatus*, 6 - *Curvularia geniculata*

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isolated from all the sprayed plots. Conversely, significantly higher percentages (24% - 25%) were obtained for control plots where these two organisms were isolated (Table 12a). It was also observed that low incidence of <u>C. geniculata</u> as in <u>C. cucurbitarum</u> were recorded for all the plots sprayed with chemicals. The most effective concentration of all the chemicals was 5g a.i., followed by 2.5g a.i. and 1.25g a.i. in that order of effectiveness. All the control plots for each of the chemical produced highly significant percentages of seed-borne mycoflora (Table 12a).

Results of 1987 late season was basically similar to that of early season in that low incidence of seed-borne mycoflora were recorded in plots that were sprayed with chemicals compared to the very high seed infection rates recorded for non-sprayed plots (Table 12b). One prominent feature of the results of this season was that generally, for most of the treatments, higher percentage seed infection were obtained during this season compared to the early. For example, 0.4% seed infection by <u>C. cucurbitarum</u> were recorded for all the strengths of Benlate-Captafol mixture during the early season (Table 12a) compared to 1 - 4% infection by same organism and same chemical strengths during the late season (Table 12b). Again, the third rate (5.0g a.i.) of

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Table 12b: The effect of fungicide application on Amaranthus (NHAc33) inflorescence on percentage infection by seed-borne* fungi during 1987 late season.

Chemical	Rate/plot (g) a.i.	Mean percentage** seed infection by organisms***							
		1	2	3	4	5	6	7	8
Iprodione	1.25	2.3b	3.1bc	2.9bc	4.1d	4.1d	5.9c	6.9e	5.1de
	2.5	1.6a	2.8bc	2.5bc	4.7d	2.9c	5.8c	5.8d	4.5d
	5.0	1.0a	2.3b	0.4a	3.2c	2.3b	4.9b	7.0e	4.1d
Tecto	1.25	3.5c	3.9c	1.6b	3.1c	3.5c	4.9b	6.5e	4.9d
	2.5	2.8b	1.6b	1.0a	3.2c	3.2c	4.2ba	6.3e	4.3d
	5.0	2.3b	1.0a	1.0a	2.8c	1.6b	3.5a	5.8d	4.1d
Captafol	1.25	3.5c	1.6b	3.8d	3.1c	2.9c	4.9b	5.6d	3.1c
	2.5	3.1b	0.4a	3.5d	3.4c	1.6b	4.4ba	4.2c	2.5b
	5.0	2.9b	0.4a	1.0a	1.6b	0.4a	3.2a	1.9b	1.9b
Benlate-Captafol	1.25	3.5c	0.4a	1.0a	3.2c	2.3b	4.1ba	2.3b	2.3b
	2.5	2.8b	0.4a	1.0a	2.3b	2.3b	3.2a	0.4a	0.4a
	5.0	1.6a	0.4a	1.0a	0.4a	1.6b	3.9a	0.4a	0.4a
Control	0.0	9.5d	21.4d	25.9e	25.9e	25.3e	27.9d	27.5f	26.8f

*Four plates each of 100 seeds were examined for each treatment.

**Values for each chemical/organism followed by same alphabet(s) are mean comparison with control and do not significantly differ at 5% level by Duncan's multiple range test.

***1 - *Choanephora cucurbitarum*, 2 - *Rhizoctonia solani*, 3 - *Pythium aphanidermatum*, 4 - *Alternaria amaranthi*, 5 - *Fusarium moniliforme*, 6 - *Aspergillus tamarii*, 7 - *Aspergillus fumigatus*, 8 - *Curvularia geniculata*.

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the chemicals gave significant control of the seed-borne fungi than the rest of the concentrations used. Also during the late season of 1987, two additional fungi namely: R. solani and P. aphanidermatum recording significantly low percentage (1-3%) seed infection were isolated. Similarly, significantly low percentage seed-borne fungi were isolated from plots that received chemical sprays during this season compared with the control (Table 12b).

When all the chemicals sprayed on inflorescence to control field-acquired seed-borne fungi were considered in 1988 early season, 5g and 2.5g a.i. per plot of Benlate-Captafol mixture followed by either Captafol or Tecto significantly controlled the seed-borne mycoflora (Table 13a). The percentage seed infection by fungi when these rates of the chemicals were applied was between 0% - 0.2%. There were no significant differences in the ability of Iprodione-Tecto mixture and single Iprodione to control these seed-borne fungi that season. Generally, A. tamarii and A. fumigatus were not effectively controlled like the other fungi by these chemicals. Seeds obtained from the non-sprayed control plots had significantly higher rates of seed infection by the fungi (Table 13a).

Like in 1987, P. aphanidermatum and R. solani were also isolated from the seeds in addition to other fungi in the

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Table 13a: The effect of fungicide application on Amaranthus ($NHAc_{33}$) inflorescence on percentage infection by seed-borne* fungi during 1988 early season.

Chemical	Rate/plot (g) a.i.	Mean percentage** seed infection by organisms***				
		1	2	3	4	5
Iprodione	1.25	0.1b	0.1b	0.1b	0.1b	0.2b
	2.50	0.1b	0.1b	0.1b	0.1b	0.1a
	5.00	0.0a	0.1b	0.1b	0.0a	0.1a
Tecto	1.25	0.1b	0.1b	0.1b	0.1b	0.1a
	2.50	0.0a	0.1b	0.0a	0.1b	0.1a
	5.00	0.0a	0.1b	0.0a	0.0a	0.1a
Captafol	1.25	0.1b	0.1b	0.2c	0.1b	0.2b
	2.50	0.0a	0.0a	0.1b	0.1b	0.2b
	5.00	0.0a	0.0a	0.2c	0.0a	0.2b
Benlate-Captafol	1.25	0.1b	0.0a	0.1b	0.0a	0.1a
	2.50	0.0a	0.0a	0.1b	0.0a	0.1a
	5.00	0.0a	0.0a	0.1b	0.0a	0.1a
Iprodione-Tecto	1.25	0.1b	0.1b	0.1b	0.1b	0.2b
	2.50	0.1b	0.1b	0.1b	0.1b	0.1a
	5.00	0.0a	0.0a	0.1b	0.0a	0.1a
Control	0.00	10.5c	11.3c	10.0d	11.2c	11.6c

*Four plates each of 100 seeds were examined for each treatment.

**Values for each chemical/organism followed by same alphabet are mean comparison with control and do not significantly differ at 5% level by Duncan's multiple range test.

***1 - Choanephora cucurbitarum, 2 - Alternaria amaranthi, 3 - Aspergillus tamarii, 4 - Thielavia terricola, 5 - Aspergillus fumigatus.

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late season of 1988. Again, pre-harvest spray of a mixture of Benlate-Captafol, Captafol or Tecto singly, preferably at 5g or 2.5g a.i. per plot significantly reduced the incidence of seed-borne fungal flora (Table 13b). A mixture of Iprodione-Tecto and Iprodione singly applied on the inflorescence also controlled seed infection. *Aspergillus tamarii*, *A. fumigatus* and *A. flavus* were again not well controlled as the other fungi during this season by the chemicals. Majority of the seeds infected by these three fungi had between 0.1 - 0.3% infection compared to 0% or 0.1% obtained from seeds infected by other fungi. Seeds obtained from the non-sprayed (control) plots had significantly higher percentages (10 - 12%) seed infection Table 13b).

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Table 13b: The effect of fungicide application on Amaranthus (NHAc33) inflorescence on percentage infection by seed-borne* fungi during 1988 late season.

Chemical	Rate/plot (g) a.i.	Mean percentage** seed infection by organisms***							
		1	2	3	4	5	6	7	8
Iprodione	1.25	0.1b	0.1b	0.1a	0.2b	0.3c	0.2b	0.2b	0.1b
	2.50	0.1b	0.2c	0.1a	0.1a	0.2b	0.2b	0.2b	0.1b
	5.00	0.0a	0.1b	0.1a	0.1a	0.2b	0.2b	0.2b	0.0a
Tecto	1.25	0.1b	0.2c	0.1a	0.2b	0.2b	0.1a	0.2b	0.1b
	2.50	0.1b	0.2c	0.1a	0.2b	0.2b	0.1a	0.1a	0.1b
	5.00	0.1b	0.1b	0.1a	0.1a	0.2b	0.1a	0.1a	0.1b
Captafol	1.25	0.1b	0.1b	0.2b	0.1a	0.2b	0.2b	0.2b	0.1b
	2.50	0.1b	0.1b	0.2b	0.1a	0.2b	0.2b	0.2b	0.1b
	5.00	0.1b	0.1b	0.1a	0.1a	0.1a	0.2b	0.2b	0.0a
Benlate-Captafol	1.25	0.1b	0.1b	0.1a	0.1a	0.2b	0.1a	0.2b	0.1b
	2.50	0.1b	0.1b	0.1a	0.1a	0.2b	0.1a	0.2b	0.0a
	5.00	0.1b	0.0a	0.1a	0.1a	0.1a	0.1a	0.2b	0.0a
Iprodione-Tecto	1.25	0.2c	0.1b	0.2b	0.2b	0.2b	0.1a	0.2b	0.1b
	2.50	0.1b	0.1b	0.1a	0.2b	0.2b	0.1a	0.2b	0.1b
	5.00	0.1b	0.1b	0.1a	0.1a	0.2b	0.2b	0.2b	0.0a
Control	0.00	11.4d	12.3d	11.3c	10.4c	11.4d	11.4c	12.4c	11.5c

*Four plates each of 100 seeds were examined for each treatment.

**Values for each chemical/organism followed by same alphabet are mean comparison with control and do not significantly differ at 5% level by Duncan's multiple range test.

***1 – Choanephora cucurbitarum, 2 – Rhizoctonia solani, 3 – Pythium aphanidermatum, 4 – Thielavia terricola, 5 – Aspergillus tamarii, 6 – A. fumigatus, 7 – A. flavus, 8 – Fusarium moniliforme.

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CHAPTER 5

5. DISCUSSION AND CONCLUSION

5.1. Detection of mycofloral infection of seeds at different seasons of harvest

The dry inspection method afforded one the opportunity of determining how clean a seed was at each season of harvest. A clean or good quality seed should be free of mycelial growth on them, without dent or wrinkle and should be dark or brown in colour. The most outstanding clean cultivar among the ones used for the two years was $NHAc_{100}$. This again could be attributed to cultivar differences among them. Least healthy (infected) seeds were produced in all cultivars during the second season because of the optimum amount of rainfall, relative humidity and temperature that favoured high incidence of disease infestation of the seeds. Conversely, the dryness, low relative humidity and very high temperature that characterized the 3rd season naturally favoured the production of good quality (healthy) seeds. Healthy seeds were also produced from the first season planting because the seeds might have matured in the presence of low level inocula of the pathogens infecting them. Therefore, harvesting Amaranthus seeds, at the third season (December - February) of the year if irrigation

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facility is available, is desirable for the production of good quality seeds. George (1987) also agreed that high quality horticultural seeds can be produced by the use of environmental methods (temperature and relative humidity). If this however, is impossible due to lack of irrigation facilities, seed harvested from first season (April - June) planting should be preserved for planting purposes. Malformation of seeds could be due to infection of immature seeds.

Maximum number of fungi were recorded by the blotter method while the agar method was used for culturing fungi obtained by the blotter for identification and for despatch to the C.M.I. for confirmation.

The fungi found to be seed-borne in this study in all the cultivars and confirmed by the CMI were *Alternaria amaranthi* (PK) Van Hook, *Rhizoctonia solani* Kuhn, *Aspergillus niger* van Tieghen, *A. flavus* Link ex Fries, *A. fumigatus* Fres, *A. tamarii* Kita, *Fusarium moniliforme* Neish & Leggett, *F. pallidoroseum* (Cooke) Sacc., *Rhizopus oryzae* Went & Prinsen Geerlings, *Phytium aphanidermatum* (Ed.) Fitz, *Thielavia terricola* (Gilman & Abbot) Emmons, *Bipolaris zeae* Sivan, *Curvularia geniculata* (Trancy & Earle) Boedijn, *Phoma sorghina* (Sacc.) Boerema, and *Choanephora cucurbitarum* (Berk. and Rav.) Thaxt. Joaqium (1976) also found *A.*

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amaranthi, F. moniliforme, F. semitectum, Cyctopus bliti, A. flavus and Curvularia lunata to be seed-borne in Amaranthus hybridus. Fusarium semitectum, C. bliti were however not found to be seed-borne in this study as reported by Joaquim (1976) probably because she studied a different species of Amaranthus (A. cruentus). Besides, microflora change from year to year. Also, Sharma et al. (1980) similarly found many of the fungi reported to be seed-borne in this work also to be seed-borne in Amaranthus hybridus in India. They are A. amaranthi, A. flavus, Curvularia sp., F. moniliforme, Phoma amaranthi and R. solani. From the present study, the following fungi have not been associated with Amaranthus seed in Nigeria or elsewhere: F. pallidoroseum, P. aphanidermatum, T. terricola, B. zeae and P. sorghina. They can then be regarded as new records of seed-borne fungi of Amaranthus. Two of the fungi - P. sorghina and T. terricola are also new records at the CMI and have been dried and kept at their herbarium.

The presence of these seed-borne fungi on Amaranthus is a critical factor which should be given adequate attention. Their presence could lead to a reduced planting value of seeds; loss of germination; pre-and post-emergence mortality; diseases in the field and contamination of the soil. These

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seed-borne microorganisms usually become associated with the seeds while in the field. C. cucurbitarum, A. amaranthi, Fusarium sp. and R. solani have also been reported as field pathogens of growing Amaranthus plants (Palm and Jochems, 1924; Irvine, 1969; Maduewesi, 1970; Odebunmi-Osikanlu, 1977; Joaquim, 1976 and Bremer, 1952). Therefore, efforts to reduce the amount of inoculum available for seed infection before harvest will result in healthier and better quality seeds.

The highest number, types and percentage of seed-borne fungi were recorded for all the cultivars during the second season because of the optimum weather conditions earlier mentioned. The high level of P. aphanidermatum and R. solani recorded on seeds of the cultivars during the third season of both years is understandable because the plants were grown under irrigation. Since P. aphanidermatum and R. solani are soil borne (Anon., 1964), the irrigation water could splash soil containing these organisms onto maturing or mature inflorescences to infect the seeds. One of the cultivars, $NHAc_{100}$ had fewer number, various types and significantly low percentage seed infection. It thus appears to be more resistant than the other two cultivars.

Seedlings of all the cultivars used were highly

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susceptible to these seed-borne fungi: *C. cucurbitarum*, *R. solani*, *P. aphanidermatum* and *A. amaranthi*. This is in agreement with the work of Venkatarkishniah (1952) who reported a severe attack by *A. amaranthi* on stem and leaves of *Amaranthus paniculatus*. Deighton (1931) similarly recorded infection of the plant by *R. solani* while Sharples (1929) isolated *Pythium aphanidermatum* from seedlings of *Amaranthus*. Bialoskorski and Kimati (1982) also reported the damping off of *Amaranthus* spp. by *Pythium* sp.

The mild infection associated with *F. moniliforme* and *C. geniculata* in $NHAc_{33}$ and $NHAc_{30}$ and *B. zeae* in $NHAc_{33}$ seedlings is a new record. The third cultivar, $NHAc_{100}$ was however not infected by these three fungi (*F. moniliforme*, *C. geniculata* and *B. zeae*) indicating that it may be resistant to them. The pathogenic effect of some of these fungi on the seedlings was significant. Infection with *R. solani* and *P. aphanidermatum* resulted in damping off of seedlings while *C. cucurbitarum* caused severe dieback of shoot and death of seedlings. The death of many plants in a population could build up the inoculum in the soil environment. For the production of good quality *Amaranthus* shoot and fungi-free seeds, seedlings and plants must be free of

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these pathogenic fungi either by use of cultural, environmental or chemical means during growth.

The testa of all the three cultivars used harbour the highest amount and number of seed-borne fungi. The presence of *C. cucurbitarum* on the testa found in this study could be a carry-over of inflorescence infection by this pathogen (Adebanjo, 1989) into the testa. *Pythium aphanidermatum* on the other hand might have gotten into the seed either during harvesting, drying or processing. The presence of these organisms and many others on the testa indicates that they could easily be controlled by seed dressing. The cotyledon-borne storage fungi, *A. tamarii*, *A. fumigatus* found in this study have been reported as normal flora of many tropical seeds (Christensen and Kaufmann, 1974). However, since the inoculum are deep seated, their control by seed treatments might be a bit difficult. This probably explains why these organisms were consistently isolated (although at low levels) by seed treatment methods employed in this study.

The results that *P. aphanidermatum*, and *C. cucurbitarum* were seed transmitted in *Amaranthus* constitutes new information from this study. The fact that all the seedlings produced by seeds inoculated with *P. aphanidermatum* were

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completely damped-off has far reaching implications. Firstly, it could result in total crop failure as the establishment of Amaranthus will be made difficult. Secondly, since it was also established in this study that the pathogens are seed-borne their presence lead to seed contamination. They could also constitute sources of inoculum to seedlings raised from them apart from contaminating the soil. These go to confirm the observations and experience at NIHORT that direct establishment (direct seeding) of Amaranthus in the field could sometimes be made impossible due to presence of "contaminants on the seeds" (Denton, O.A. and Edema, A.O. Personal Communication).

Seed transmission of the pathogens was recorded at very low levels in $NHAc_{100}$ when compared to $NHAc_{30}$ and $NHAc_{33}$ because it was more tolerant.

The different reaction of the cultivars to be aerial shoot diseases over seasons of harvest could be attributed to their probable inherent genetic variability and variations in climatological factors during the seasons. The environmental factors such as amount of rainfall, relative humidity, temperature and wind will significantly affect the develop-

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ment, intensity, and spread of plant diseases (Van der Plank, 1963). If for example a susceptible plant variety is established, harvested, or stored during the wet humid periods of the year in the presence of high level inoculum of pathogens, a very high incidence of diseases would be recorded on the plant and/or produce. If however, the plant variety is resistant, little or no infection may be obtained on such plant or its produce. When the plant is harvested at a time that the above environmental parameters are at the barest minimum, the inoculum may be present at negligible level or may not even be able to survive. So, plants grown or harvested at this time may be free of pathogens or the pathogens may be present at significantly low levels that will require time to build up to sufficiently high levels that could result in an epidemic.

The fact that shoot dieback, stem and leaf blights were recorded in varying amounts all the three seasons of the year suggests that C. cucurbitarum is present on Amaranthus shoot throughout the year. Significantly highest incidence of the disease of all the above-ground parts of the plant were recorded during the second season

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because the initial inoculum that might be present in the soil and/or plant have started to build up during the first season to attain its maximum threshold during the second season hence the very high disease incidence of the above-ground parts of the plant. This can also be related to the high rainfall, relative humidity and temperature recorded during the second season (Appendix XX). Van der Plank (1963) also confirmed that diseases are built up from low levels to attain an epidemic (highest) level during favourable weather conditions. Plants harvested during the third season of both years obviously had the lowest incidence of the various phases of the disease because of low or no rainfall and relative humidity at this time which do not encourage much of the development of *Amaranthus* shoot diseases as in the second season (Appendix XX). Seasonal effects on the incidence of diseases have also been reported on other vegetable crops. Onesirosan (1982) observed a seasonal variation in the incidence of yeast rotters of tomato. Higher rots were recorded during the 2nd cropping season of the year. Cole *et al*. (1987) also demonstrated that carrots were progressively predisposed to even greater root damage by flies in

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the latter part of the season than the early.

Stem blight and shoot dieback were recorded in higher percentages for most of the cultivars and for most of the seasons. The apical shoot is the youngest portion of the plant. Since it has been reported that C. cucurbitarum is soil-borne (Adesunloye, 1983), rainsplashes could get the inoculum of the pathogen from the soil onto the young easily susceptible shoot resulting in dieback. In fact, field observations show that dieback is extremely rampant at the seedling stage when plants were between 12-36 cm in height. At this height, soil can easily be splashed on the plant causing stem and/or shoot tip infection.

C. cucurbitarum was readily isolated from the infected parts of Amaranthus. Irvine (1969), Maduewesi (1970) and Ikediugwu (1981) also isolated the fungus from the different above-ground parts of Amaranthus. Observations from these studies suggest that the petiole, stem, lamina, mid-rib and the veins of Amaranthus were all susceptible to C. cucurbitarum. The finding that the young apical growing region of the plants were the most susceptible is expected since these organs are not as hardened as the older parts hence were easily invaded and colonized by the

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fungus. Water soaking of infected Amaranthus parts preceeding necrosis of tissues could be an enzymatic activity of the fungus that is usually associated with some fungal infection and colonization of intact tissue.

The differences recorded in the incidence of dieback, stem and leaf blights for the cultivars could be attributed to the differences in their genetic make-up. The vigorous and luxuriant growth habit of $NHAc_{100}$ in terms of leaf size, stem size and plant height compared to $NHAc_{33}$ and $NHAc_{30}$ were all on indication of genetic differences among them.

Thermotherapy of infected Amaranthus seeds at appropriate temperature(s) and duration reduced the incidence of seed-borne fungi and improved emergence ability depending on the cultivar. Results indicated that treatment at very low temperature regardless of duration resulted in significantly ($P=0.05$) high seed infection and a reduction in seedling emergence. The low temperature will have no lethal effect on the pathogens hence they will still be alive to infect seeds and reduce germinability. This could then explain why the highest number of fungi were isolated from seeds treated at 28°C and 40°C for shorter durations e.g. 2 or 4 minutes. Treatment at 40°C for

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longer durations like 8 to 10 minutes resulted in a significant reduction of the seed-borne fungi ($<$ 19%) and the emergence of the seedlings increased ($>$ 69%) accordingly. Although, these temperatures are not too high it seems that the high temperature required to kill the pathogens was being compensated for by the longer stay of the seeds in hot water. During this time, the thermal effect of the hot water would be experienced by the soaked seeds much longer and so inactivates the seed-borne fungi. Since it has been found from these studies that the hard testa of the seed harbour some of the pathogens, their longer stay in hot water might not exclude the possibility of the seeds imbibing a little hot water that could inactivate the pathogens.

The findings that no seed-borne fungi were isolated at higher temperatures (50 and 60°C) for longer duration of seed treatment (8 and 10 mins.) means that the pathogens were killed within that time. There have also been reports of hot water inactivation of seed-borne pathogens in other vegetables including spinach (Shurtleff and Linn, 1971). Joaquim (1976) also reported the inactivation of seed-borne fungi of *Corchorus olitorius* by hot water treatment. Since the seed-borne pathogens have been killed, post-emergence

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infection would be reduced or absent hence, the very high seedling emergence observed for seeds treated at these higher temperatures. The few pathogens isolated from seeds treated at higher temperatures were the thermotolerant ones like the <u>Aspergillus</u> group. The rapid decline in seedling emergence at higher temperatures (70° and $80^{o}C$) for longer durations is an indication of the damaging effects of hot water on the embryo.

The variations observed in the percentage infection of treated seeds even at the same temperature for the cultivars could be genetic. This is in line with the work of Hodgkin and Hegarty (1978) who implicated percentage genetic purity as an important factor in the variation in performance of seedlots. Again, the cultivars were found to harbour the pathogens at different levels (even when treated) and possessed different emergence ability whether treated in hot water or not. These results could support the findings in the review of Perry (1972) that within types of horticultural crops, cultivars may be identified with high emergence potential in the presence of pathogens. The rapid emergence of seeds treated in hot water could be due to breaking or weakening or softening of the testa by hot water. The non-treated control seeds of all cultivars had

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high infection rate and low emergence because of their high level of seed-borne pathogens. Even if the seeds germinate, seedling emergence may be poor due to pre-emergence damping-off. If infected seeds of Amaranthus are treated in hot water at 50°C or 60°C for 6, 8 or maximum of 10 minutes, they would be free of seed-borne pathogens and emergence ability of such seeds will be significantly enhanced.

Information gathered from studies on chemical treatment of seed-borne fungi shows that there were no significant effects of application methods. That is soaking and dusting with appropriate chemicals are good methods of controlling seed-borne fungi of Amaranthus leading to the production of healthy seedlings. The percentage seedling emergence observed for various chemicals and application methods were equally improved in treated seeds over the non-treated seeds. Sharma et al. (1980) also reported the control of seed-borne fungi of Amaranthus caudatus by treatment with chemicals. Although no pathogen was isolated from treated seeds of all varieties regardless of application methods and seedlings produced were healthy but the highest (numerical) percentage seedling emergence was obtained following soaking of seeds in aqueous fungicide solution.

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This may be a better method over dusting since it has been established that the seed-borne fungi did infest the testa. Soaking in aqueous solution provided the opportunity for the seeds to imbibe the fungicides. The latter could arrest the growth or kill the seed-borne inoculum present in the outer or inner parts of the testa. Dusting the seeds on the surface does not make room for seed penetration by the chemicals and subsequent unseating of the outer or inner inoculum. This probably explains why soaking resulted in higher seedling emergence than dusting. Williams and Singh (1981) also reported greater degree of control of millet downy mildew with a fungicide following soaking of the seeds than dusting. Benlate-Captafol mixture enhanced seedling emergence than any of the other chemicals used and $NHAc_{100}$ emerged best. In these circumstances, preferably soaking of infected seeds with fungicides effectively controlled the primary seed-borne inoculum, increased seedling emergence thus protecting them from primary infection. Since according to Van der Plank (1963) estimate or amount of disease is related to the amount of initial inoculum, it then means that seeds without inoculum on them will record no disease and if recorded at all, it will take a long time. The latter is required to build up

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the inoculum up to the required level that could initiate infection. Soaking or dusting of infected *Amaranthus* in a mixture of Benomyl-Captafol or Captafol at 10g a.i./kg seed effectively controlled the seed-borne fungi and significantly increased seedling emergence to between 90 - 98%.

The study for the two year's fungicide spray trials showed clearly the need for protecting the inflorescence of *Amaranthus* during the growing seasons. This is because the primary inflorescence infection incited by *C. cucurbitarum* and later by other pathogens could cause seed infection or even complete "cut-off" of the inflorescence (Adebanjo, 1989). This will ultimately result in the production of infected seeds apart from the significant reduction in quantity. The non-sprayed plots had the highest percentage inflorescence infection. Agunloye and Osisanya (1985) recommended chemical protection of *Amaranthus* against pests and diseases for its successful cultivation. A mixture of Benlate-Captafol was the best of all the chemicals used. This might be due to the fact that this mixture contained both a systemic (Benlate) and a protectant (Captafol) that conferred high efficiency on it over others. The protectant will take care of surface - borne pathogens on the

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inflorescence while the systemic fungicide took care of the internally-borne inoculum.

The findings that high percentage inflorescence infections were recorded for the late seasons of both years could be due to the high rainfall and relative humidity that encourages disease development (Appendix XX). Besides, the inoculum that might be present at low levels in the soil environment in the early part of the season will build up as the season advanced. This means that one had to control a large amount of inoculum in the second season. All these factors might contribute to recording of higher inflorescence infections in sprayed plants in the second season than the first.

Application of fungicides on Amaranthus inflorescence to control its infection significantly increased seed yield. A similar increase in yield for rice, wheat and soybeans resulting from pre-harvest chemical sprays against seed-borne fungi have also been recorded by Sinclair (1981). No such work have been done for Amaranthus before this study. Also, average grain weight has been reportedly increased for barley by fungicide treatment either of the seed or foliar fungicide application (Wright and Hughes, 1987). The decreases in seed yield for the non-sprayed

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plots should be expected because infection of the inflorescence would ultimately cause seed infection. Seed infection means the seed-borne pathogen(s) would live in/on the seed, thrive and draw their food from such seeds. This would lead to a depletion in the food contents of such invaded seed hence the loss in seed weight. Preventing or controlling inflorescence infection by pre-harvest chemical sprays would control seed infection thereby resulting in healthier (non-infected) seeds that will increase grain yield.

It is advantageous to spray *Amaranthus* inflorescence (seeds) prior to harvest in the field in order to reduce very considerably the amount of seed-borne inoculum. Very low incidence of seed-borne mycoflora were recorded in plots that were sprayed with chemicals compared to the very high seed invasion by fungi recorded for the non-sprayed plots. A similar significant reduction in seed-borne *Phomopsis* of soybean was reported in plants sprayed with fungicides in Brazil than from unsprayed plants (Bolkan and Cupertino, 1977). Also, Vidhyasekaran and Kandaswamy (1980) observed a reduction in the incidence of seed-borne pathogens such as *Fusarium* spp. and *Aspergillus* flavus, in another vegetable, okra, by pre-

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harvest spray of chemicals. Sinclair (1981) also confirmed that many seed-borne microorganisms of rice, soybeans and wheat were controlled by fungicide sprays. This study show further that the third rate (5g a.i./plot) of each of the chemicals were more effective than the other rates. This means that the efficiency of the chemicals increased with strength. Generally, higher percentage seed infection were recorded for most of the treatments during the late seasons of both years because of the favourable environmental conditions earlier mentioned.

When all the chemicals evaluated during the early and late seasons of the years were considered for their effectiveness in controlling all the organisms isolated, the mixture of Benlate-Captafol at 5g a.i./plot was the most effective. _A tamarii_ and _A. fumigatus_ were not as effectively controlled as the other fungi because there have been reported cases of _Aspergillus_ spp. resistance to chemicals (Shurtleff and Linn, 1971). Application of Benlate-Captafol mixture or Captafol onto _Amaranthus_ inflorescence at 5.0g a.i./plot would result in the production of healthy seeds having little amount or no seed-borne mycoflora.

Just as recorded for the other studies involving the

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use of fungicides, application of the chemicals in the field on Amaranthus inflorescence prior to harvest is highly beneficial because it significantly increased seedling emergence. All the chemicals applied at all concentrations improved seedling emergence. As earlier reported under seed yield, a mixture of Benlate-Captafol at 5g a.i./plot stood out as the best of all the chemicals tested for 4 seasons in two years. Chemical protection of infected seed not only reduced seed-borne pathogens but it also prevented post-emergence damping-off. This would result in good seed germination and subsequent seedling emergence. If however, Benlate-Captafol is not available, either single Captafol or Iprodione at 5g a.i./plot could be used because they are almost as effective. Although the second rate of these chemicals were equally effective, the third is preferred. No reason could be advanced why Tecto at the least concentration performed best during the late season of 1987 alone.

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CHAPTER 6

6.1. SUMMARY

The seed-borne fungi isolated from three *Amaranthus* cultivars during the three seasons of the two years were *Alternaria amaranthi*, *Rhizoctonia solani*, *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *Fusarium moniliforme* var. *intermedium*, *F. pallidoroseum*, *Rhizopus oryzae*, *Pythium aphanidermatum*, *Thielavia terricola*, *Bipolaris zeae*, *Curvularia geniculata*, *Aspergillus tamarii*, *Phoma sorghina* and *Choanephora cucurbitarum*. However, the highest number, types and percentage mycofloral infection of the seeds were recorded for all cultivars during the 2nd and 1st seasons in order of increasing abundance each year. Fewer number, types and least mean percentage seed infection by fungi were obtained for all the cultivars during the 3rd season of each year. Cultivar $NHAc_{100}$ was found to harbour significantly fewer number of seed-borne fungi each year compared to $NHAc_{33}$ or $NHAc_{30}$. The seed testa harboured the highest number and percentage seed mycoflora for all cultivars.

The following seed-borne fungi - *C. cucurbitarum*, *A. amaranthi*, *R. solani* and *P. aphanidermatum* were highly pathogenic on the seedlings of $NHAc_{33}$ and $NHAc_{30}$ while the seedlings of $NHAc_{100}$ were tolerant to these fungi. *Fusarium moniliforme* and *B. zeae* had mild infection on seedlings of $NHAc_{33}$ and $NHAc_{30}$. Out of all these seed-borne fungi, *P.*

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aphanidermatum, and C. cucurbitarum were found to be seed transmissible at higher percentage in $NHAc_{30}$ and $NHAc_{33}$ and to a lesser degree in $NHAc_{100}$.

Studies were also carried out in the field for three seasons each in two years to investigate the effect of season of harvest on the incidence of seed-borne mycoflora and shoot diseases of three Amaranthus cultivars. The season of harvest and the cultivars planted significantly (P=0.05) affected the incidence of stem blight, leaf blight and shoot dieback. Significantly (P=0.05) least percentage dieback and leaf blight were recorded during the 3rd season (December - February) of both years. Least incidence of stem blight was observed during the 1st season (April - June). The 2nd season (August - October) recorded the highest incidence of shoot tip dieback, stem and leaf blights during the two years. The cultivars reacted differentially to the shoot diseases. For the two years significantly (P=0.05) least percentage dieback (14%), stem (17%) and leaf (19%) blights were observed from cultivar $NHAc_{33}$, while $NHAc_{100}$ had 30% leaf blight. The mean percentage dieback was not significantly different for $NHAc_{30}$ and $NHAc_{100}$ while the mean, percentage stem blight was significantly lower (15%, 25%) in former cultivar during the two years.

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The season of harvest of the seeds also had an effect on their health (quality). The seeds of the cultivars were either discolored, had mycelial growth on them or malformed depending on the season of harvest and cultivars. The mean percentage mycelial growth on seeds were significantly ($P=0.05$) higher during the 2nd and 1st seasons than in the 3rd regardless of the cultivars. Significantly ($P=0.05$) low mean percentage mycelial growth were recorded for seeds of $NHAc_{100}$ while seeds of $NHAc_{33}$ had the highest and was closely followed by $NHAc_{30}$.

All the cultivars had malformed seeds in all the seasons of both years. With the exception of $NHAc_{100}$, the cultivars produced significantly ($P=0.05$) higher percentages of discoloured seeds during the 3rd and 2nd seasons respectively and the least mean percentage in the 1st. The healthiest seeds of all cultivars were produced in the 3rd and 1st seasons of each year. Seeds of all cultivars produced during the 2nd season were least healthy than the 1st or 3rd. Again high quality (clean) seeds were obtained from $NHAc_{100}$ followed by $NHAc_{30}$ and lastly by $NHAc_{33}$.

Attempts to control these seed-borne fungi by thermotherapy showed that dipping of infected seeds of all Amaranthus cultivars used ($NHAc_{100}$, $NHAc_{33}$ and $NHAc_{30}$) for 4, 6 and 8 minutes at $50^{o}C$ or $60^{o}C$ resulted in a

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considerable or total reduction of the seed-borne fungi. This led to a corresponding increase of the mean percentage seedling emergence of all cultivars. Treatment of seeds at 28°C irrespective of treatment duration were not particularly effective in the control of seed-borne pathogens.

Studies were also carried out to control these seed-borne fungi either by seed treatment with chemicals or pre-harvest spray of infected inflorescence with chemicals. It was established that soaking or dusting of infected *Amaranthus* seeds with 10g a.i./kg seed of any of the chemicals significantly (P=0.05) controlled the seed-borne fungi resulting in the production of healthy seedlings. However, a mixture of Benlate-Captafol, Captafol singly or Tecto at 10g a.i./kg in order of preference was significantly (P=0.05) superior to the rest of the chemicals.

Similarly, pre-harvest sprays of a mixture of Benlate-Captafol, single Captafol application, Iprodione-Tecto mixture and single Iprodione in order of efficacy at 5g a.i./plot of infected *Amaranthus* inflorescence significantly (P=0.05) controlled inflorescence infection. Application of these chemicals at same rate on *Amaranthus* inflorescence also resulted in increased fresh seed weight, significant control of seed-borne fungi and considerable increase in seedling emergence over the non-treated control.

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6.2. RECOMMENDATIONS AND SUGGESTIONS

The research work carried out here considered the fungal problems of *Amaranthus* plant (shoots), inflorescence and seeds. Control measures involving environmental, chemical and non-chemical means were also investigated. From the results of these studies, the following recommendations and suggestions for further work can be made:

(i) since it was established that *Amaranthus* has the problem of shoot dieback, stem and leaf blights caused primarily by *C. cucurbitarum* and that the incidence of these diseases were least in the 3rd and 1st seasons, efforts should be made to grow the crop in large quantity at these seasons of the year. Studies should be carried out on the combined effect of chemical application and seasons effect for the control of these major shoot diseases of *Amaranthus*. Since $NHAc_{100}$ was found to be tolerant to these shoot disease, it is an indication that it is possible and desirable to screen and breed for resistance to this important fungus.

(ii) the healthiest (cleanest) seeds of all cultivars were produced in the 3rd and 1st seasons in order of superiority. Therefore, for commercial production of good quality (clean)

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planting seeds, should be embarked upon in the 3rd season. Where such seeds will be kept for some time before planting or for exportation, they can be dusted with Benlate-Captafol mixture at 2.5 or 5g a.i./kg seed. This will protect the seeds against invasion by any of the storage fungi in addition to protecting them against any possible field-acquired seed-borne mycoflora.

(iii) soaking and dusting with chemicals to control the seed-borne fungi should be carried beyond the seedling or blotter method stage to monitor the effectiveness of each method. Such treated seeds should be planted out in the field to monitor seedling/plant infection if any. The ability of plants from such chemically treated seeds to tolerate or withstand infection should be closely observed under the vagaries of field conditions.

(iv) further work needs be done on whether the hot water treatment of seeds that facilitated emergence is due to the effect of hot water on seed testa alone or that it has some stimulatory effects on the hormonal system of such treated seeds or both. Treatment of <u>Amaranthus</u> seeds with chemicals before planting and foliar spray of the inflorescence is recommended for those who may want to produce seeds during the 2nd season of the year when the highest seed-borne

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pathogens will be acquired by the seeds.

(v) finally, there is the need to carry out biochemical studies on seeds that were invaded by these seed-borne pathogens with the aim of determining possible production of carcinogens and/or any other toxic metabolites by the fungi. This is highly desirable since *Amaranthus* seeds as grains are consumed by man in various forms as confectioneries etc. apart from feeding the seeds unto livestocks which are of economic and food value to man.

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REFERENCES

Adebanjo, A. (1989). An inflorescence disease of Amaranthus associated with Choanephora cucurbitarum J. of Phytopathology (In Press).

Adesunloye, M.O. (1983). Persistence of okra pathogen in the soil. H.N.D. Project Report, University of Ife, Nigeria. 50p.

Agunloye, O. and Osisanya, E.O. (1985). Pirimiphosmethyl in the control of Psara bipunctualis F. on Amaranthus cruentus in southern Nigeria. Tropical Pest Management 28(2): 115-121.

Aluko, M.O. (1969). Relative prevalence of Blast and Brown leaf spot on upland rice in Nigeria. Pl. Dis. Reptr. 53(11); 875-877.

Anon. (1933). Botany and Plant Pathology Section. Ann. Rept. IOWA Agric: Exper. Stat. 43-45.

Anon. (1964). Commonwealth Mycological Institute (CMI). Description of Pathogenic Fungi and Bacteria. No. 36. 26pp.

Anon. (1983). Annual Report: National Horticultural Research Institute, (NIHORT), Ibadan, Nigeria.

Anon. (1984). Amaranth: Modern Prospects for an Ancient Crop. National Academy Press. Washington, D.C. 81p.

Anon. (1985). Annual Report: National Horticultural Research Institute, (NIHORT), Ibadan, Nigeria.

Bailey, A.G. (1966). A check-list of Plant Diseases in Nigeria. Federal Department of Agric. Research, Ibadan, Nigeria. Memo, 969.

Baker, K.F. (1962). Thermotherapy of planting material Phytopathology 52: 1244-1255.

UNIVERSITY OF IBADAN LIBRARY

Baker, K.F. (1972). Seed pathology in Seed Biology. Academic Press, New York. 317-416.

Barnett, H.L. and Hunter, B. (1972). Illustrated Genera of Imperfect Fungi: Third Edition, Burgess Pub. Comp., Minneapolis, U.S.A.

Berhaut. J. (1971). Flore illusfree du Senegal. Tome I, Dakar.

Bialoskorski, N. and Kimati, H. (1982). Damping off due to Pythium sp. in Amaranthus spp. seed beds. Revista de Agricultura Brazil 57(3): 145-146.

Bisht, N.S. and Mathur, R.S. (1964). Occurrence of two strains of Jute mosaic virus in Ulter Pradesh. Curr. Sci. 33(14): 434-435.

Bolkan, R.A. and Cupertino, F.P. (1977). Control of seed-borne Phomopsis sojae with foliar applications of fungicides. Fungicides and Nematicide Tests 32, 121.

Bremer, H. (1954). Observations on root rot in a dry climate. Z. Pflkrankh. 61: 575-587.

Christensen, C.M. and Kaufmann, H.H. (1974). Microflora in storage of cereal grain and their products: Publication of the Association of Cereal Growers Inc., St. Paul, Minnesota.

Cole, R.A.K.; Phelps, P.R.; Ellis and Hardman, J.A. (1987). The effect of time of sowing and harvest on carrot biochemistry and the resistance of carrots to carrot fly. Ann. appl. Biol. 110, 135-143.

UNIVERSITY OF IBADAN LIBRARY

Cook, R.J. (1977). The effect of timed fungicide sprays on yields of winter wheat in relation to Septoria infection period. Plant Pathl. 26: 30-34.

Crittenden, B.H.E. and Yalen, F.A. (1967). Pod and stem blight of the soybean in Delaware. University of Delaware Agricultural Experimental Station, Circular 4.

Deighton, F.C. (1931). Mycological work. Ann. Rept. Agric. Dept. Sierra-Leone (1930): 28-31.

Eberle, O. and Mayr, A. (1979). Practical experience with the combination thiabendazole/captafol against cereal foot rot and ear diseases. Proceedings of the English British Crop Protection Conference: Pest and Disease, 5: 31-36.

Evans, G. (1968). Some new host for Verticillium nigrescens. Pl. Dis. Reptr. 52 (11); 904.

FAO (1988). Traditional Food Plants: Food and Nutrition Paper No. 42. Rome.

George, R.A.T. (1987). Review of the factors affecting seed yield and quality. Acta Horticulturae: 215, 15-16.

Grubben, G.J.H. (1976). The cultivation of amaranth as a Tropical Leaf Vegetable (with special reference to South Dahomey). Comm. 67. Department of Agricultural Research, Amsterdam. 207p.

UNIVERSITY OF IBADAN LIBRARY

Harwood, R.R. (1980). The present and future status of Amaranth: Proc. 2nd Amaranth. Rodale Press. Emmas. 9pp.

Herklots, G.A.C. (1972). Vegetables in South-East Asia. London.

Heyne, K. (1950). De nuttige Planten van Nederlandsch. Indie I. The Hague.

Hodgkin, T. and Hegarty, T.W. (1978). Genetically determined variation is seed germination and field emergence of Brassica oleracea. Ann. appl. Biol. 88: 407-413.

Ikediugwu, F.E.O. (1981). A shoot disease of Amaranthus spp. in Nigeria associated with Choanephora cucurbitarum. Journal of Horticultural Science, 56: 289-293.

Irvine, F.R. (1969). West African Crops. 3rd Edition Oxford University Press, London. 272p.

ISTA (1966). International Seed Testing Association: Proceedings of the International Seed Testing Association. 31(1): 107-114.

Joaquim, M. (1976). Seed-borne mycoflora of Nigerian local vegetables and their importance in disease development. Ph.D. Thesis, University of Ibadan, Ibadan, Nigeria.

Kilpatrick, R.A. (1957). Fungi associated with flowers, pods and seeds of soybean. Phytopathology: 47: 131-135.

UNIVERSITY OF IBADAN LIBRARY

Kusakari, S.; Tsuji, H.; Yamada, K. and Tanaka, Y. (1979). Damping-off of spinach seedling caused by Pythium sp. Annals of the Phytopathological Society of Japan. 45(2): 268-271.

Leach, C.M. (1979). A theoretical consideration or epidemiology of seed-borne pathogens. In seed pathology-progress and problems. (Eds. J.J. Yorinori, J.B. Sinclair, Y.R. Mehta and S.K. Mohen), Fundacao Institute Agronomico do Parana, Brazil. pp. 227-233.

Mac-Gillivary, J.H. (1961). Vegetable production: With special reference to Western Crops. McGrew-Hill Book Company, N.Y., London. 65-75pp.

Maduewesi, J.N.C. (1970). Role of microbiology and microbiologists in plant protection, Symposium. Proc. Nigerian Society for Microbiology, 1: 45-72.

Martinson, C.A. (1964). Active survival of Verticillium dahliae in soil. Dis. Abstr. 25(1): 18p

Matthews, R.E.F. (1970). Plant Virology. Academic Press, New York. 778p.

Melhus, I.E. (1931). The presence of mycelium and oospores of certain mildews in the seeds of their hosts: IOWA State Coll. Jour. of Sci. Vol. 4: 185-188.

Mishra, M.D. and B.L. Chona (1963). Factors affecting sporangial germination and epidemiology of white rust of Amaranthus (Albugo bliti) (Bivi) Kze. India Phytopath. 16(4): 333-343.

UNIVERSITY OF IBADAN LIBRARY

Nakamura, S.T.; Sato and Mine, T. (1972). Storage of vegetable seeds dressed with fungicidal dusts. Proc. ISTA. 37(3): 961-968.

Nangju, D. (1977). Effect of date of harvest on seed quality and viability of soyabeans. J. Agric. Sci. 89, 107-112.

Neergaard, P. (1977). Seed pathology. Vols. 1 and 2 Macmillan Press Ltd., London. 1187p.

Nicholas, G. and B. Aggery (1952). On a very special mode of action of Cyctopus bliti (Biv-Bern) Lev. Bull. Soc. Mycol. Fr. 67(4): 419-421.

Nwoboshi, L.C. (1982). Tropical Silviculture: Principles and Techniques. Ibadan University Press, 333p.

Odebunmi-Osikanlu, Y.O.K. (1977). The more important diseases of selected local vegetables in Nigeria, Nigerian Journal of Plant Protection. 3: 79-83.

Okoro, O.O. and N. Jones (1977). Variation in the fruits of Triplochiton scleroxylon colled in 1972. Research Paper: Forestry Research Institute of Nigeria, Ibadan. Forest Series No. 36.

Onesirosan, P.T. (1978). Mouldiness of early season cowpea seed. Niger. J. Plant Protect. 4: 30-37.

Onesirosan, P.T. (1982). Season variation in the incidence of yeast rotters of tomato fruit in soil and on various parts of tomato. Ife Journal of Agric. 1: 162-171.

UNIVERSITY OF IBADAN LIBRARY

Onesirosan, P.T. (1983). Effect of season of harvest on the level of microbial infection, quality and viability of the seed of cowpea (Vigna unguiculata). Trop. Agric. 60(2): 144-147.

Oshinma, N.; C.H. Livinston and M.D. Harrison (1963). Weeds as carriers of two potato pathogens in Colorado. Pl. Dis. Reptr. 47(6): 466-469.

Oyenuga, V.A. (1968). Nigeria Foods and Feedling Stuffs. 3rd Edition, Ibadan University Press. 15pp.

Oyenuga, V.A. and B.L. Fetuga (1975). Dietary importance of fruits and vegetables. Paper presented at the National Seminar on fruits and vegetables, Ibadan.

Palm, B.T. and Jochems, S.C.J. (1924). A disease of Amaranthus caused by Choanephora cucurbitarum (B. & Rew.) Thax. Phytopathology. 14: 490-494.

Paschal, E.H. and M.A. Ellis (1978). Variation in seed quality characteristics of tropically grown soybeans. Crop Sci. 18: 837-840.

Perry, D.A. (1972). Seed vigour and field establishment. Horticultural Abstract 42: 334-342.

Prasad, S.S. and Sinha, B.D. (1963). Occurrence of Curvularia specifera (Bainier) Boedijn, Helminthosporium speciferum (Bainier) Nicot on Celosia cristata L. (Curr. Sci.) 32(11): 517-518.

Roy, K.W. and J.S. Abney (1977). Antagonism between Cercospora kikuchii and other seed-borne fungi soybeans. Phytopathology, 67: 1062-1066.

UNIVERSITY OF IBADAN LIBRARY

Sauer, J.D. (1967). The grain amaranths and their relatives. A revised taxonomic and geographic survey. Ann. Missouri Bot. Car. 54: 104-137.

Schneider, W.R.; Sinclair, J.B. and Williams, R.J. (1973). Induced conidial production of Cercospora spp. 2nd International Congress of Plant Pathology, 156.

Sealy, R.L. (1988). Evaluation of Amaranthus accessions for resistance to damping-off by Pythium myriotylum. Plant Dis. 985-989.

Sharma, B.D. and Mukherji, S.K. (1970). A bacterial wilt of jute (Corchorus capsularis and Corchorus olitorius L.) caused by Pseudomonces solanacearum. 2nd International Congress of Plant Pathology. 156.

Sharma, A.D.; Tnakur, V. and Munjal, R.L. (1980). Seed mycoflora of Amaranthus caudatus, its pathology and control. Indian Phytopath. 33(2): 242-244.

Sharples, A. (1929). Division of mycology Annual Report for 1928. Malayan Agric. Jour. XVII(8): 294-303.

Shurtleff, M.C. and Linn, M.B. (1971). Reports on Plant Diseases. Dept. of Plant Pathology, University of Illinois at Urbana - Champaign. Ext. Paper No. 915, 6pp.

Sinclair, J.B. (1981). Fungicide sprays for the control of seed-borne pathogens of rice, soybeans and wheat. Seed Sci. and Technol. 9: 697-705.

Sinclair, J.B. (1983). Fungicidal sprays for control of internally seed-borne fungi. Seed Sci. and Technol. 14: 959-968.

UNIVERSITY OF IBADAN LIBRARY

Solheim, W.G. and Stevens, F.L. (1931). Cercospora studies II. Some tropical Cercosporae. Mycologia XXIII (5): 365-405.

Taubenhaus, J.S. and Ezekiel, W.N. (1931). A sclerotinia limb blight of figs. Phytopathology, XII 12: 1195-1197.

Tuite, J. (1969). Plant Pathological Methods: Minneapolis. Burgess Publishing Company, New York.

Van der Plank, J.E. (1963). Plant Diseases: Epidemics and control. Academic Press, New York and London. 345p.

Vavilov, N.I. (1951). The origin, variation, immunity and breeding of cultivated plants. Chron. Bot. 13 (1-6): 1949-1900.

Venkatakrishniah, N.S. (1952). Blight of Amaranthus paniculatus L. caused by Alternaria. Phytopathology, 42(12): 668-669.

Verona, O. and Ceccarelli, A. (1935). On a tracheomycosis of the Amaranth (Amaranthus tricolor, L.) produced by a species of Fusarium and Verticillium amaranthi sp. and on the biology of some pathogenic species of Verticillium in general. Phytopathology z. VIII (4): 373-400.

Vidhyasekaran, P. and Kandaswamy, T.K. (1980). Control of seed-borne pathogens in okra by pre-harvest sprays. Indian Phytopathology, 33(2): 239-241.

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Vir, D. and Gaur, A. (1970). Efficacy of fungicides XI. Seed disinfection in relation to *Rhizoctonia bataticola* on okra seeds. *Pesticides* 4(7): 25-26.

Vir, D. (1983). Chemical seed treatment for controlling seed-borne diseases in India. *Seed Sci. and Techl.* 11: 935-946.

Watson, R.D.; Coltrin, L. and Robinsion, R. (1951). The evaluation of materials for heat treatment of peas and beans. *Plant Dis. Reptr.* 35: 542-544.

Wilcox, J.R.; Laviolletle, F.A. and Anthow, K.L. (1974). Deterioration of soybean seed quality associated with delayed harvest. *Plant Dis. Reptr.* 58(2): 130-133.

Williams, R.J. and Singh, S.D. (1981). Control of pearl millet downy mildew by seed treatment with metalaxyl. *Ann. appl. Biol.*, 97: 263-268.

Wit, H.C.D. (1963). De wereld der planten 1: The Hague.

Wright, D. and Hughes, LI.G. (1987). Effects of fungicide treatments and variety on development, grain growth and yield of spring barley. *Ann. appl. Biol.* 111: 89-102.

Zinneen, T.M. and Sinclair, J.B. (1982). Thermotheraphy of soybeans seeds to control seed-borne fungi. *Phytopathology* 72(7): 831-834.

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APPENDIX I: ANOVA Table for effect of season of harvest during 1987 on:

(a) % Dieback

SOURCE	DF	SS	MS	F
Rep.	3	12.8288	4.2762	0.541
Season	2	231.7705	115.8852	14.680
Cv.	2	299.0905	149.5452	18.945
Season x Cv.	4	180.4927	45.1231	5.716
Error	24	189.4461	7.8935	
Total	35	931.6288	26.1036	

(b) % Leaf Blight

SOURCE	DF	SS	MS	F
Rep.	3	258.333	86.1111	2.224
Season	2	5651.3888	2825.6944	72.986
Cv.	2	1284.7222	642.3611	16.591
Season x Cv.	4	706.9444	176.7361	4.565
Error	24	929.1666	38.7152	
Total	35	8830.5555	252.3015	

(c) % Stem Blight

SOURCE	DF	SS	MS	F
Rep.	3	3220.5763	1073.5259	42.091
Season	2	389.1805	194.5902	7.629
Cv.	2	1275.0138	637.5069	24.995
Season x Cv.	4	281.6944	70.4236	2.761
Error	24	612.1111	25.5046	
Total	35	5778.5763	165.1021	

(d) Fresh Shoot Wt. (g)

SOURCE	DF	SS	MS	F
Rep.	3	408.6875	136.2291	0.983
Season	2	1466.8472	733.4236	5.293
Cv.	2	13147.3888	6573.6944	47.443
Season x Cv.	4	351.6111	87.9027	0.634
Error	24	3325.3750	138.5572	
Total	35	18699.9097	534.2831	

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APPENDIX II: ANOVA Table for effect of season of planting on % Dieback - 1988 Data

(a)

SOURCE	DF	SS	MS	F
Rep.	3	655.1075	218.3691	4.136
Season	2	2340.5259	1170.2629	22.169
Var.	2	1488.8445	744.4222	14.102
Season x Var.	4	326.174	81.5435	1.544
Error	24	1266.871	52.7863	
Total	35	6077.5234		

(b) % Stem Blight

SOURCE	DF	SS	MS	F
Rep.	3	2617.5160	872.5053	18.396
Season	2	1491.2642	745.6321	15.721
Var.	2	817.2827	408.6413	8.615
Season x Var.	4	834.6098	108.6524	2.290
Error	24	1138.2952	47.4289	
Total	35	6498.9681		

(c) % Leaf Blight

SOURCE	DF	SS	MS	F
Rep.	3	140.3127	46.7709	0.611
Season	2	11383.8960	5691.9480	74.388
Var.	2	1350.5069	675.2534	8.824
Season x Var.	4	156.6714	39.1678	0.511
Error	24	1836.3859	76.5160	
Total	35	14867.7730		

(d) Fresh Shoot Wt. (g)

SOURCE	DF	SS	MS	F
Rep.	3	1717.0319	572.3439	1.562
Season	2	1028.6316	514.3158	1.403
Var.	2	25763.251	12881.6258	35.157
Season x Var.	4	923.7716	230.9429	0.630
Error	24	8793.6605	366.4025	
Total	35	38226.3475		

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APPENDIX III: ANOVA Tables for the effect of season of harvest on the health of three cultivars of *Amaranthus* seeds - 1987 seasons.

(a) Mycelial growth on seeds

SOURCE	DF	SS	MS	F
Treatments	8	715.0	89.375	25.98*
Season	2	282.17	141.085	41.01*
Variety	2	429.50	214.75	62.43*
Season x Variety	4	3.33	0.83	0.24ns
Error	27	93.0	3.44	
Total	35			

(b) Malformed seeds

SOURCE	DF	SS	MS	F
Treatments	8	219.06	27.3825	6.37
Season	2	100.06	50.03	11.645
Variety	2	107.73	53.865	12.54
Season x Variety	4	11.27	2.8175	0.65ns
Error	27	116	4.296	
Total	35			

(c) Discoloured seeds

SOURCE	DF	SS	MS	F
Treatments	8	257.56	32.20	14.97
Season	2	110.89	55.45	25.79
Variety	2	117.72	58.86	27.38
Season x Var.	4	28.99	7.25	3.37
Error	27	58.13	2.15	
Total	35	315.69		

(d) Healthy seeds

SOURCE	DF	SS	MS	F
Treatments	8	2381.56	297.695	535.85
Season	2	644 .389	322.195	579.95
Variety	2	1718.389	859.195	1546.55
Season x Var.	4	18.782	4.696	8.45
Error	27	15.0	0.56	
Total	35			

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APPENDIX IV: ANOVA Tables for the effect of seasons of harvest on the health of three cultivars of *Amaranthus* seeds - 1988 seasons.

(a) Mycelial growth on seeds

SOURCE	DF	SS	MS	F
Treatments	8	635.89	79.486	23.15*
Season	2	244.80	122.40	35.65*
Variety	2	381.50	190.75	55.56*
Season x Var.	4	9.59	2.398	0.70ns
Error	27	92.70	3.433	
Total	35			

(b) Malformed seeds

SOURCE	DF	SS	MS	F
Treatments	8	284.76	35.60	28.10*
Season	2	146.70	73.35	57.89*
Variety	2	125.56	62.78	49.55*
Season x Var.	4	12.50	3.125	2.47ns
Error	27	34.21	1.267	
Total	35			

(c) Discoloured seeds

SOURCE	DF	SS	MS	F
Treatments	8	354.19	44.27	17.68
Season	2	97.90	48.95	19.55
Variety	2	251.13	125.57	50.15
Season x Var.	4	5.16	1.29	
Error	27	67.60	2.50	

(d) Healthy seeds

SOURCE	DF	SS	MS	F
Treatments	8	1916.056	239.51	58.69*
Season	2	841.514	420.76	103.10*
Variety	2	969.681	484.84	118.80*
Season x Var.	4	104.861	26.22	
Error	27	110.187	4.081	
Total	35			

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APPENDIX V: Analysis of variance table for % recovery of fungi by hot water treatment 1988 and 1989

SOURCE	DF	SS	MS	F.VALUE
Rep.	3	18.6799	6.2266	1.949
Dur.	4	2701.4000	675.3500	211.487
Error A	12	38.3200	3.1933	
Temp.	4	11581.8000	2895.4500	1258.891
Dur. x Temp.	16	599.7999	37.4874	16.298
Error B	60	138.0000	2.3000	
Var.	1	2073.6800	2073.6800	744.143
Dur. x Var.	4	581.7200	145.4300	52.187
Temp. x Var.	4	553.3200	138.3300	49.639
Var. x Temp. x Dur.	16	356.2800	22.2675	7.990
Error C	75	209.0000	2.7866	
Total	199	18852.0000	94.7336	

APPENDIX VI: Analysis of variance table for % seedling emergence by hot water treatment 1988 and 1989

SOURCE	DF	SS	MS	F.VALUE
Rep.	3	45.1599	15.0533	3.273
Dur.	4	1116.9700	279.2425	60.715
Error A	12	55.1900	4.5991	
Temp.	4	36141.8700	9035.4674	1335.619
Dur. x Temp.	16	312.0299	19.5018	2.882
Error B	60	405.9000	6.7650	
Var.	1	1579.2200	1579.2200	188.526
Dur. x Var.	4	12.6299	3.1574	0.376
Temp. x Var.	4	567.1300	141.7825	16.925
Var. x Temp. x Dur.	16	116.7700	7.2981	.871
Error C	75	628.2500	8.3766	
Total	199	40981.1200	205.9352	

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APPENDIX VII: ANOVA Table for chemical seed treatment (Dusting Vs Soaking) on three cultivars of *Amaranthus* during 1987

SOURCE	DF	SS	MS	F.
Rep.	2	13.73	6.87	0.0006
Conc.	2	2059.28	1029.64	0.008
Trt.	1	14933.40	14933.40	0.12
Chem.	3	16407.22	5469.07	0.04
Chem. x Conc.	6	1753.09	292.18	0.002
Chem. x Trt.	3	574947.08	191649.03	1.50
Var.	2	8634.95	4317.48	0.03
Var. x Chem.	6	38004.03	6334.01	0.05
Error	190	24346594.81	128139.97	
Total	215	25003347.59		

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APPENDIX VIII: ANOVA Table for chemical seed treatment (Dusting Vs Soaking) on *Amaranthus* seed: seedling emergence - 1988

SOURCE	DF.	SS	MS	F.
Rep.	2	27.7389	13.8695	0.14 ns
Var.	2	13281.0722	6640.5361	66.48
Trt.	1	16673.6113	16673.6113	166.93
Var. x Trt.	2	3737.3387	1868.6694	18.71
Chem.	4	46582.1000	6645.5250	66.53
Var. x Chem.	8	1409.2334	1758.6542	17.61
Conc.	3	85661.7889	28553.9296	285.87
Chem. x Conc.	12	11803.7667	983.6472	9.85
Error	325	32462.6721	99.8851	
Total	359	211639.3222		

APPENDIX IX: ANOVA Table for chemical seed treatment (Dusting Vs Soaking) on *Amaranthus* seed infection by fungi: 1988

SOURCE	DF	SS	MS	F.
Rep.	2	0.0394	0.0197	8.9545
Var.	2	0.0337	0.0169	7.6818
Trt.	1	0.0479	0.0479	21.7727
Var. x Trt.	2	0.0069	0.0035	1.5909ns
Chem.	4	0.1723	0.0431	19.5909
Var. x Chem.	8	0.0525	0.0066	3.0000
Conc.	3	68.6353	22.8784	10399.2727
Chem. x Conc.	12	0.3509	0.0292	13.2917
Error	325	0.7297	0.0022	
Total	359	70.0686		

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APPENDIX X: ANOVA Table for chemical seed treatment (Dusting Vs Soaking) on *Amaranthus* seeds: Seedling infection: 1988

SOURCE	DF	SS	MS	F.
Rep.	2	0.0207	0.0104	1.86ns
Var.	2	0.3535	0.1768	31.57
Trt.	1	0.0057	0.0057	1.02ns
Var. x Trt.	2	0.0128	0.0064	1.14ns
Chem.	4	0.1230	0.0308	5.50
Var. x Chem.	8	0.0665	0.0083	1.48ns
Chem.	3	43.1156	14.3719	2566.41
Chem. x Conc.	12	0.1796	0.0150	2.68
Error	325	1.8196	0.0056	
Total	359	45.6970		

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APPENDIX XI: ANOVA Table for field-applied fungicides on % *Amaranthus* inflorescence infection - 1987 early season

SOURCE	DF	SS	MS	F.
Rep.	2	4.667	2.3335	1.810
Chem.	3	372.3959	124.1319	96.30
Conc.	3	1109.8959	369.9653	287.04
Chem. x Conc.	9	58.8543	6.5394	5.0736
Error	30	138.6666	1.2889	
Total	47	1684.4792		

(b) Late Season - 1987

SOURCE	DF	SS	MS	F.
Rep.	2	15.3506	7.6753	0.446
Chem.	3	597.3058	199.1019	11.57
Conc.	3	1397.1392	465.7130	27.059
Chem. x Conc.	9	819.4162	91.0463	5.29
Error	30	516.3227	17.211	
Total	47	3345.535		

(c) Seed Yield - 1987 Early Season

SOURCE	DF	SS	MS	F.
Rep.	2	5.8631	2.9315	0.866
Chem.	3	481.2375	160.4125	47.361
Conc.	3	493.0675	164.3560	48.525
Chem. x Conc.	9	492.2248	54.6916	16.148
Error	30	101.6100	3.387	
Total	47	1574.000		

(d) Seed Yield - 1987 Late Season

SOURCE	DF	SS	MS	F.
Rep.	2	10.8769	5.4385	2.1512
Chem.	3	319.6284	106.5428	42.145
Conc.	3	1120.8576	377.6192	147.792
Chem. x Conc.	9	260.6230	28.9581	11.455
Error	30	75.8431	2.528	
Total	47	1701.1099		

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APPENDIX XII: ANOVA Table for field applied fungicides on % *Amaranthus* (NHA_{33}) inflorescence infection and seed yield - 1988 early season.

(A)

SOURCE	DF	SS	MS	F.
Rep.	2	15.6999	7.8499	2.915
Chemical	4	122.2333	30.5583	11.351
Conc.	3	6823.6500	2274.5500	844.896
Chem. x Conc.	12	169.7600	14.1472	5.255
Error	38	102.3000	2.6921	
Total	59	7233.6500	122.6042	

(b) Seed Yield

SOURCE	DF	SS	MS	F.
Rep.	2	7.2120	3.6060	1.117
Chemical	4	171.4123	42.8530	13.282
Conc.	3	1589.2005	529.7335	164.198
Chem. x Conc.	12	72.7769	6.0647	1.879
Error	38	122.5946	3.2261	
Total	59	1963.1965	33.2745	

(c) % inflorescence infection 1988 Late season

SOURCE	DF	SS	MS	F.
Rep.	2	25.4333	12.7166	1.644
Chemical	4	556.2333	139.0583	17.979
Conc.	3	1912.5833	637.5277	82.429
Chem. x Conc.	12	512.8333	42.7361	5.525
Error	38	293.8999	7.7342	
Total	59	3300.9833	55.9488	

(d) Seed Yield - 1988 Late season

SOURCE	DF	SS	MS	F.
Rep.	2	2.7250	1.3625	0.771
Chemical	4	215.4416	53.8604	30.498
Conc.	3	648.9000	216.3000	122.479
Chem. x Conc.	12	71.7250	5.9770	3.384
Error	38	67.1083	1.7660	
Total	59	1005.9000	17.0491	

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APPENDIX XIII: ANOVA Table for field-applied fungicides on Amaranthus inflorescence on seedling emergence in:

1987 Early Season

SOURCE	DF	SS	MS	F.
Rep.	2	110.04	55.02	5.14ns
Chemical	3	2678.23	892.74	83.36
Conc.	3	1083.56	3610.52	337.82
Chem. x Conc.	9	750.36	83.37	7.78
Error	30	321.29	10.71	
Total	47	14691.48		

1987 Late Season

SOURCE	DF	SS	MS	F.
Rep.	2	102.53	24.855	3.425
Chemical	3	3241.5211	458.2110	58.302
Conc.	3	6201.3420	2056.4221	52.031
Chem. x Conc.	9	852.2002	72.34	6.521
Error	30	288.3004	7.134	
Total	47	13519.5210		

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APPENDIX XIV: ANOVA Table for field-applied fungicides on *Amaranthus* inflorescence on seedling emergence in:

1988 Early Season

SOURCE	DF	SS	MS	F.
Rep.	2	227.6333	113.8166	7.471
Chemical	4	2616.2333	654.0583	42.935
Error A	8	121.8666	15.2333	
Conc.	3	24168.8666	8056.2888	1300.566
Chem. x Conc.	12	954.3000	79.5250	12.838
Error B	30	185.8333	6.1944	
Total	59	28274.7333	479.2327	

1988 Late Season

SOURCE	DF	SS	MS	F.
Rep.	2	45.7333	22.8666	1.435
Chemical	4	7244.6666	1811.1666	113.701
Error A	8	127.4333	15.9291	
Conc.	3	7702.4666	2567.4888	83.045
Chem. x Conc.	12	2520.5333	210.0444	6.793
Error B	30	927.5000	30.9166	
Total	59	18568.3333	314.7175	

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APPENDIX XV: ANOVA Table for effect of fungicide application on Amaranthus inflorescence on % infection by seed-borne fungi during 1987 early season.

	Source	DF	SS	MS	F
1. C. cucurbitarum	Rep.	3	2.65	0.88	1.52ns
	Chem.	3	4.7	1.57	2.71
	Conc.	3	315.61	105.20	181.38
	Chem. x Conc.	9	24.45	2.72	4.69
	Error	45	26.09	0.58	
	Total	63			
2. A. amaranthi	Rep.	3	3.33	1.11	1.46ns
	Chem.	3	21.45	7.15	9.41
	Conc.	3	2006.78	668.93	880.17
	Chem. x Conc.	9	27.09	3.01	3.96
	Error	45	34.18	0.76	
	Total	63	2092.83		
3. F. moniliforme	Rep.	3	7.21	2.4	1.85ns
	Chem.	3	3.3	1.1	0.85ns
	Conc.	3	3094.51	1031.5	793.46
	Chem. x Conc.	9	30.7	3.4	2.61ns
	Error	45	58.55	1.3	
	Total	63	3194.27		
4. A. tamarii	Rep.	3	5.33	1.78	2.62ns
	Chem.	3	82.27	27.76	40.82
	Conc.	3	5206.69	1735.56	578.52
	Chem. x Conc.	9	19.61	2.18	3.21
	Error	45	30.51	0.68	
	Total	63	5345.41		
5. A. fumigatus	Rep.	3	4.62	1.54	1.52ns
	Chem.	3	52.72	17.57	17.40
	Conc.	3	5934.23	1978.08	1958.50
	Chem. x Conc.	9	22.7	2.52	2.50ns
	Error	45	45.32	1.01	
	Total	63	6059.59		
6. C. geniculata	Rep.	3	6.62	2.21	1.26ns
	Chem.	3	20.46	6.82	3.9ns
	Conc.	3	6824.59	2274.86	1299.92
	Chem. x Conc.	9	43.20	4.8	2.74ns
	Error	45	78.77	1.75	
	Total	63	6973.64		

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APPENDIX XVI: ANOVA Table for effect of fungicide application on *Amaranthus* (NHA_{33}) inflorescence on % infection by seed-borne fungi during 1987 late season.

	Source	DF	SS	MS	F.
1. *C. cucurbitarum*	Rep.	3	6.56	2.19	1.33ns
	Chem.	3	39.39	13.13	8.01ns
	Conc.	3	435.24	145.08	88.46
	Chem. x Conc.	9	47.84	5.32	3.24
	Error	45	73.83	1.64	
	Total	63	602.86		
2. *R. solani*	Rep.	3	7.26	2.42	0.49ns
	Chem.	3	13.73	4.58	0.92ns
	Conc.	3	4769.09	1589.70	319.86
	Chem. x Conc.	9	120.34	13.37	2.69ns
	Error	45	223.69	4.97	
	Total	63	5134.11		
3. *P. aphanidermatum*	Rep.	3	5.14	1.71	0.36ns
	Chem.	3	20.94	6.98	1.48ns
	Conc.	3	7066.64	2355.55	500.12
	Chem. x Conc.	9	21.63	2.40	0.51ns
	Error	45	211.8	4.71	
	Total	63	7326.15		
4. *A. amaranthi*	Rep.	3	12.46	4.15	3.07ns
	Chem.	3	15.1	5.03	1.68ns
	Conc.	3	6376.6	2125.53	1574.47
	Chem. x Conc.	9	208.77	23.20	17.19
	Error	45	60.72	1.35	
	Total	63	6673.65		
5. *F. moniliforme*	Rep.	3	8.13	2.71	1.78ns
	Chem.	3	21.69	7.23	4.76ns
	Conc.	3	6335.04	2111.68	1389.27
	Chem. x Conc.	9	130.46	14.5	9.54
	Error	45	68.2	1.52	
	Total	63	6563.57		

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APPENDIX XVI (Cont'd)

	Source	DF	SS	MS	F.
6. *A. tamarii*	Rep.	3	3.19	1.06	0.81
	Chem.	3	20.28	6.76	5.2ns
	Conc.	3	6611.18	2203.73	1682.23
	Chem. x Conc.	9	9.32	1.04	0.79ns
	Error	45	59.11	1.31	
	Total	63	6703.08		
7. *A. fumigatus*	Rep.	3	12.69	4.23	0.51ns
	Chem.	3	284.06	94.69	11.42
	Conc.	3	6441.01	2147.00	258.99
	Chem. x Conc.	9	30.52	3.39	0.41
	Error	45	372.99	8.29	
	Total	63	7141.27		
8. *C. geniculata*	Rep.	3	9.44	3.15	0.37ns
	Chem.	3	128.34	42.78	5.09ns
	Conc.	3	6730.37	2243.46	266.76
	Chem. x Conc.	9	36.65	4.07	0.48ns
	Error	45	378.35		
	Total	63	7283.15		

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APPENDIX XVII: ANOVA Table for effect of fungicide application on *Amaranthus* inflorescence on % infection by seed-borne fungi during 1988 early season.

	Source	DF	SS	MS	F.
1. *C. cucurbitarum*	Rep.	3	0.0024	0.008	0.4211ns
	Chem.	4	0.0065	0.0016	0.8421ns
	Conc.	3	12.2167	4.0722	2143.2632*
	Chem. x Conc.	12	5.7080	0.4757	250.3684*
	Error	57	0.1094	0.0019	
	Total	79	18.0430		
2. *A. amaranthi*	Rep.	3	0.007	0.0023	1.0606ns
	Chem.	4	0.0638	0.016	7.25*
	Conc.	3	7.8471	0.0096	4.3788*
	Chem. x Conc.	12	0.0289	0.0024	1.0909ns
	Error	57	0.1278	0.0022	
	Total	79	8.0746		
3. *A. niger*	Rep.	3	0.0288	0.0096	3.0*
	Chem.	4	0.0503	0.0126	3.9375*
	Conc.	3	11.4154	3.8051	1189.0938*
	Chem. x Conc.	12	0.0762	0.0064	2.00*
	Error	57	0.1805	0.0032	
	Total	79	11.7512		
4. *T. terricola*	Rep.	3	0.0241	0.008	4.2281*
	Chem.	4	0.0191	0.0048	2.5263ns
	Conc.	3	0.1845	0.0615	32.3684*
	Chem. x Conc.	12	11.1159	0.9263	487.5263*
	Error	57	0.109	0.0019	
	Total	79	11.4526		
5. *A. fumigatus*	Rep.	3	0.0241	0.0080	3.6515*
	Chem.	4	0.0131	0.0033	1.5ns
	Conc.	3	10.1359	3.3786	1535.727*
	Chem. x Conc.	12	0.0614	0.0051	2.3182*
	Error	57	0.1276	0.0022	
	Total	79	10.3621		

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APPENDIX XVIII: ANOVA Table for effect of fungicide application on *Amaranthus* inflorescence on % infection by seed-borne fungi during 1988 late season.

	Source	DF	SS	MS	F.
1. *C. cucurbitarum*	Rep.	3	0.0096	0.0032	2.29ns
	Chem.	4	0.0340	0.0085	6.07*
	Conc.	3	10.8335	3.6112	2579.43*
	Chem. x Conc.	12	0.0266	0.0022	1.57ns
	Error	57	0.0777	0.0014	
	Total	79	10.9814		
2. *R. solani*	Rep.	3	0.0104	0.0035	1.09ns
	Chem.	4	0.0637	0.0159	5.89*
	Conc.	3	9.1722	3.0574	1132.37*
	Chem. x Conc.	12	0.0864	0.0072	2.67*
	Error	57	0.1557	0.0027	
	Total	79	9.4884		
3. *P. aphanidermatum*	Rep.	3	0.0310	0.0103	0.62ns
	Chem.	4	0.0865	0.0216	1.30ns
	Conc.	3	21.0805	7.0268	423.30*
	Chem. x Conc.	12	0.2565	0.0214	1.29ns
	Error	57	0.9466	0.0166	
	Total	79	22.4011		
4. *T. terricola*	Rep.	3	0.0267	0.0089	1.07ns
	Chem.	4	0.0396	0.0099	1.19ns
	Conc.	3	24.0566	8.0189	901*
	Chem. x Conc.	12	0.0182	0.0015	0.18ns
	Error	57	0.4715	0.0083	
	Total	59	24.6126		
5. *A. tamarii*	Rep	3	0.0228	0.0076	1.31ns
	Chem.	4	0.1042	0.0261	4.5*
	Conc.	3	23.0191	7.6730	1322.93*
	Chem. x Conc.	12	0.0473	0.0158	2.72*
	Error	57	0.3283	0.0058	
	Total	79	23.5217		

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APPENDIX XVIII: (Cont'd)

	Source	Df	SS	MS	F.
6. *A. fumigatus*	Rep.	3	0.0014	0.0005	0.08ns
	Chem.	4	0.0462	0.0116	1.93ns
	Conc.	3	24.7491	8.2497	1374.95*
	Chem. x Conc.	12	0.0436	0.0036	0.6ns
	Error	57	0.3434	0.0060	
	Total	79	25.1837		
7. *A. flavus*	Rep.	3	0.0340	0.0113	1.61ns
	Chem.	4	0.0412	0.0103	1.47ns
	Conc.	3	22.4506	7.4835	1069.07*
	Chem. x Conc.	12	0.0457	0.0038	0.54ns
	Error	57	0.4012	0.0070	
	Total	79	22.9727		
8. *F. moniliforme*	Rep.	3	0.0120	0.0040	0.44ns
	Chem.	4	0.0280	0.0070	0.78ns
	Conc.	3	29.4684	9.8228	1091.42*
	Chem. x Conc.	12	0.0359	0.0030	0.33ns
	Error	57	0.5157	0.0090	
	Total	79	30.06		

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APPENDIX XIX: Information* LD 50 and Toxicity levels of the chemicals used for this study

Chemical	LD 50 mg/kg body wt.		Toxicity on fish
	Oral	Dermal	
Benlate	5000	1000	No information
Captafol	4200	15400	Highly toxic
Iprodione	3500	—	Highly toxic
Thiabendazole	3330	—	Low toxicity

Pre-harvest spray interval: Captafol >14 days, Iprodione >7 days, Benlate >10 days. No information about Thiabendazole.

*Source: Agricultural Chemicals Hazard Response Handbook by Euan Wallace, Agro-Research Limited, New Zealand. 96pp.

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APPENDIX XX: Climatological data (Rainfall, Relative humidity and Temperature) during the period of experimentation - 1987 and 1988 at NIHORT, Ibadan.

Year	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
	Mean monthly maximum temperature °C											
1987	34	36	36	35	34	35	33	40	30	32	33	33
1988	33	36	34	33	32	30	28	28	30	32	29	
	Mean monthly minimum temperature °C											
1987	22	18	24	24	27	23	24	22	23	25	23	20
1988	22	29	24	24	23	22	26	22	22	23	23	32
	Mean monthly rainfall (mm)											
1987	0.18	0.28	1.4	0.4	3.8	7.5	8.2	9.9	6.1	6.4	0.1	0.9
1988	0.01	1.0	4.6	4.8	4.5	7.2	8.6	8.9	7.1	7.1	0.6	0.0
	Mean monthly relative humidity %											
1987	22	17.7	23.6	24.2	26.6	23.4	23.5	22.0	22.7	25.3	22.5	19.8
1988	21.5	29.0	24.4	23.5	23.3	22.3	25.5	22.7	22.7	22.8	23.1	32.2

Source: Agro-metereological Department, National Horticultural Research Institute (NIHORT), Ibadan, Nigeria.

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