ETIOLOGY AND CHEMICAL CONTROL OF POWDERY MILDEW OF KARELA (*MOMORDICA CHARANTIA* L.) CAUSED BY *SPHAEROTHECA FULIGINEA* (Schlecht ex fr.)Poll

BY

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DECLARATION

DECLARATION BY CANDIDATE.

This thesis has not been submitted for a degree in any other University, and the contents of the thesis are my original work.

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To my beloved mother Mrs. Pauline W. Ndung'u and to my Brothers and Sisters.

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ABSTRACT

Powdery mildew of cucurbits is a devastating disease on cucurbits in Kenya. It is caused by either Sphaerotheca fuliginea (schlecht ex Fr.) Poll or Erysiphe cichoracearum Dc ex Mecat. Work was carried out on this disease to identify which of the two pathogens causes the disease on karela (Momordica Charantia L.) and to evaluate chemical fungicides suitable for its control.

Infected karela leaves were collected from three sites in Makueni district where the crop is widely grown. The pathogen was propagated on karela plants grown in a glasshouse. Inoculation was done by shaking the infected leaves over healthy plants. Fresh and dry infected leaves were collected from the glasshouse and taken to the laboratory for the fungus identification.

It was found that the fungus produced a cleistothecium with a single ascus and with no conspicous appendages. Based on this, the fungus was identified as Sphaerotheca fuliginea (Schlecht ex Fr.) Poll. Karela plants inoculated with powdery mildew spores from pumpkin (Cucurbita mixta Pang) did not develop the disease symptoms. Cleistothecium of Erysiphe cichoracearum Dc ex Mecat. contains two asci and it was observed to have conspicuous appendages.

Six fungicides folicur, bayleton, bayfidan baycor, cercobin and daconil were tested for their effectiveness in controlling the disease. Folicur, bayleton, bayfidan and cercobin were found to control powdery mildew. Folicur was superior to the other fungicides. Baycor and daconil showed no significant control of the mildew. Phytotoxicity was observed in folicur treated plants. However, the plants tended to recover from the phytotoxicity effects even after the subsequent sprays.

CHAPTER ONE

1. INTRODUCTION

One of the limiting factors to Kenya's development is the size of the foreign exchange gap. Historically, Kenya has depended largely on agricultural export earnings to meet the foreign exchange requirements. A major source of foreign exchange earnings has been horticultural crops which in recent years have not only been the fastest growing but also has become the fourth largest foreign exchange earner after tourism, coffee and tea. In 1987, production of horticultural crops was 40 thousand tonnes as compared to 105 thousand tonnes of coffee and 156 thousand tonnes of tea. The targets for 1993 productions were 70 thousand tonnes of horticultural crops, 150 thousand tonnes of coffee and 204 thousand tonnes of tea. Horticultural crops production growth rate has been 1.10% as compared to tea with 1.05% and coffee, 1.04% (Government of Kenya, 1988).

Although there is no clear cut subdivision of vegetables destined for local or export market, one can safely say that brassicas (cabbages and kale), tomatoes, onions, peas, carrots and indigenous vegetables are consumed locally while French beans, "Asian vegetables" (karela, dudhi, okra, brinjals, chillies, mooli, valore and guar) form the bulk of fresh vegetable exports (Madumadu et al., 1991). In 1983 and 1986

domestic consumption of fresh vegetables stood at 409,340 and 524,880 tonnes, respectively (Ministry of Agriculture 1986 Annual Report). From 1968 to 1989, fresh horticultural exports rose from 1,476 to 49,503 tonnes. Out of this, 38,158 tonnes were vegetables valued at Kshs.1.25 billion. In addition, processed horticultural produce earned Kshs.850 million in the same year. The recently introduced "Asian vegetables" consist mainly of plants in the family cucurbitaceae such as Karela (Momordica charantia L.). Karela is an important export vegetable crop in Kenya. However, the varieties currently grown are very susceptible to powdery mildew. Outbreaks of this disease coupled with lack of recommended control practices have resulted in farmers abandoning production of the crop especially in the coast province (Chweya and Buruchara, 1989).

Previous studies on powdery mildew of cucurbits have been focused mainly on more important commercial crops elsewhere in the world and karela being a less important crop world wide, studies on its powdery mildew are quite limited. Powdery mildew of cucurbits is caused by either Erysiphe cichoracearum or Sphaerotheca fuliginea. These fungi are taxonomically differentiated on the basis of the nature of their sexual fruiting bodies called cleistothecia. For both species cleistothecia are very rare in many regions of the world. Moreover, more than one species may occur in the same locality

and on the same plant (Spencer, 1978). This has led to difficulties in identifying the actual species causing the powdery mildew on a particular crop in a given region especially on tropical cucurbits.

On the other hand, although several fungicides are recommended for control of powdery mildew on cucurbits in general no fungicide has specifically been recommended for use against powdery mildew of karela. Chemical control of this disease is economically feasible because the crop is principally grown for export.

In light of the above, work was carried out with the following objectives.

1. To isolate and characterize the fungal pathogen causing the powdery mildew of karela.
2. To study disease development with respect to symptoms, signs and tissue colonization.
3. To study the environmental factors that influence spore germination.
4. To determine relative protectant and systemic activity of several fungicides against the fungal pathogen.
5. To determine efficacy and suitability of several fungicides for the control of powdery mildew of karela.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Karela (*Momordica charantia* L.)

Momordica charantia L. has several common names depending on locality and these include bitter gourd, bitter cucumber, bitter melon, balsam pear, margose, cundiamer, karela, tita karela, peria, ampalaya, amrgoao, pavakai, fur kwa and kiuri (Tindall, 1983). It's origin is probably in India but secondary diversity is seen in China and South East Asia (Grubben, 1977). It was introduced into Brazil in the 17th and 18th centuries. Now it is widely spread throughout the tropics (Tindall, 1983).

The status and uses of karela vary from one region to another. In temperate climate it is grown in gardens and greenhouses primarily as an ornamental annual. In West Indies and tropical areas of Mexico, Central and South America, it is one of the most widely used medicinal herb. In India it is commercially cultivated both as a vegetable and for remedial purposes. In China the fruits are used as a vegetable when small and immature, as they are less bitter (Herklots, 1972). In Indonesia, Philippines and Hawaii, the importance of vine in the diet greatly exceeds that of the fruit (Tindall, 1983). In Kenya the crop is grown mainly for export to European

countries although there is limited local consumption. The yields in Kenya are 10-12 tonnes/ha and the price ranges between Ksh 30-70 per 5 Kg carton (Ministry of Agriculture and Livestock Development, Yatta division, 1986).

The common pests of Karela are stainers, mites, white flies and leaf miners. They suck the sap especially from flowers, fruits and young shoots. Ambush, simicidin and other systemic insecticides are used for their control. Other pests include nematodes and caterpillars. Powdery mildew is the commonest disease and it has been the major production constraint (Ministry of Agriculture and Livestock Development, 1986).

2.2. Karela powdery mildew.

Powdery mildew has been observed on cucurbits since 1800 but it became destructive during the recent years (Chupp, 1960). The disease occurs throughout the world on cucurbits both in glasshouse and in the field. Powdery mildews are generally of minor importance on field cucurbits, except in limited geographical areas with cool, dry seasons. Much of the world's cucurbit production occurs in such areas and powdery mildews are quite important there. This is particularly true in the tropics where it is sometimes so common that there is a tendency to accept it as a normal part

of the plant foliage. The most striking aspect of the losses caused by the disease is reduction of quality rather than yield (Dixon, 1981).

2.2.1 Etiology

There have been three genera and six species of the casual organism of powdery mildew recorded on the major species of the family cucurbitaceae. These include Erysiphe cichoracearum Dc ex Mecat, Erysiphe communis (Wallr) link, Erysiphe polygoni (DC) St-Amm., Erysiphe polyphaga Hammarlund, Leveillula taurica(Lev) Arnaud and Sphaerotheca fuliginea (Schlecht ex Fr) Poll. The two commonly recorded species are Erysiphe Cichoracearum and Sphaerotheca fuliginea. More than one species may occur in the same locality and on the same plant (Spencer, 1978).

The cucurbit powdery mildew pathogens are classified as being in the class ascomycetes, order erysiphales, family erysiphaceae and genus and species as either Erysiphe cichoracearum or Sphaerotheca fuliginea. Classification to genus has been based on the perfect stage, characteristics of mycelium location, the type of appendages on cleistothecium and the number of asci in the cleistothecium (Ainsworth, Bisby, 1950).

The mycelium grows closely appressed to the host, tending to follow the depressions at the contact of two epidermal cells. Hyphal cells are thin walled, uninucleate and vacuolate with large nuclei. Along the hyphae are lateral swellings or appressoria, one on each alternate hyphal cell. Appressoria are lobed. Haustoria arise from the centre of attachment and penetrate host cell by a very narrow penetration tube. Most haustoria are uninucleate and globular (Yarwood, 1975). Cleistothecia of Erysiphe cichoracearum which occur very infrequently are 80-140 μm in size and contain 10-50 asci. Each ascus is narrowly to broadly ovate, more or less stalked and 30-35 x 55-90 μm in size (Spencer, 1978). Asci of Sphaerotheca fuliginea are elliptical to nearly globular, measure 50-80 μm and contain eight ascospore which are elliptical to spherical and are 17-22 μm x 12-20 μm in size (Dixon, 1981).

Conidia of both species are 4-5 μm x 5-7 μm in size, continuous, elliptic, hyaline and are borne in chains on short unbranched conidiophores. Conidiophores occur at right angles to the host surface. There is a stipe of one or more cells attached to the vegetative hyphae or generative cell (Walker, 1952). The conidia of Sphaerotheca fuliginea contain distinct fibrosis bodies which help to distinguish them from those of Erysiphe cichoracearum (Dixon, 1981). Chupp (1960) indicated that Sphaerotheca fuliginea produces a somewhat brownish

fruiting layer on the foliage while that of Erysiphe cichoracearum is flour white.

2.2.2. **Host range**

Spencer (1978) stated that there are about 90 genera and 750 species in the family cucurbitaceae that are susceptible to powdery mildew. Out of these only six genera and twelve species are cultivated by man and these are: watermelon (Citrullus lanatus L.), cucumber (Cucumis sativus L.), muskme on (Cucumis melo L.), gherkin (Cucumis anguria L.), dish rag gourd (Luffa Cylindrica Roem), white flowered gourd (Lageneira Siceraria), squashes and marrow (Cucurbita pepo L.), cucurbita maximia Dusch, and Cucurbita moschata poir, pumpkin (Cucurbita mixta Pang), figleaf gourd (Cucurbita ficifolia Beuche) and chayote (Sechium edule sev). He indicated that all these species are sensitive to powdery mildew. This list of cultivated cucurbits is inadequate as it excludes some important tropical cucurbits such as karela (Momordica charantia L.). Majority of cucurbits both cultivated and wild are sensitive to powdery mildew.

Other hosts of Sphaerotheca fuliginea are aubergine (Solanum melongena), bean (Phaseolus species), okra (Hibiscus esculentus), Soybean (Glycine max) and Vigna species. A large number of physiological variants have been reported (Dixon, 1981).

2.2.3. Symptoms and signs

Symptoms on cucurbits are similar to those of powdery mildews on other hosts. The first symptoms are tiny, white round superficial spots on leaves and stems which become powdery in consistency as they enlarge. These white lesions increase in number and coalesce and eventually may cover the stems and both surfaces of the leaves. Infection on young leaves may result in general chlorosis and eventually death of the leaves. Severely affected leaves become brown and shrivelled. Under ideal conditions premature defoliation may occur as the fungus covers the leaf surface (Spencer, 1978).

Fruits are usually not infected but may ripen prematurely and lack flavour. The chief effect of the pathogen is to reduce fruit quality rather than weight or fruit number (Dixon, 1981). Roots are not attacked and fungal growth on herbaceous plants usually stops above the ground line.

The sexual stage of the fungus has been found a few times. The diagnostic sign is the number of small, black, globose fruiting bodies on the plant surface. They are large enough to be seen without the aid of a hand lens (Chupp, 1960).

2.2.4. **The disease cycle**

There are several possible ways in which the two fungi can live from season to season. The sexual stage seems to be too rare to be of much value in helping the pathogen survive from season to season but it happens occasionally. The fungus survives off season on weeds or volunteer plants of the family cucurbitaceae. Conidia are blown by wind in stepwise fashion during the early part of the growing season (Roberts, 1984). If the spores of the pathogen make contact with the host under conditions of reduced light, optimum temperature and absence of moisture, germination commences within 2 hours (Spencer, 1978). A short germtube develops whose tip enlarges into a convoluted appressorium. A small penetration peg arises from the centre of the appressorium and forces it's way through the cuticle of the leaf. Once within the cell the fungus enlarges to form a balloon-like haustorium that invaginates the cytoplasm (Roberts, 1984). While the first haustorium is being established additional germ tubes are formed from other points on the same spore and hyphae are sent from the primary appressorium along the leaf surface. The mother spore remains a living part of the thallus and does not collapse after the fungus has established nutritive relations with the host. Parasitized cells do not die but they continue to nourish the fungus for several days. Although none but the epidermal cells are invaded, the fungus

adversely affects nearby mesophyll cells which turn yellow, die and turn brown. Approximately four to six days after initiation of the disease, signs of the fungus can be seen. By this time numerous penetration pegs and haustoria have developed from branches of the developing mycelium.

About four days after infection, conidiophores begin to form. The actual method of conidial abstriction is not known. Dissemination of conidia is almost exclusively by wind. Both asexual and sexual life cycles takes 5-6 days (Yarwood, 1957).

Cleistothecia if formed, occur several weeks after conidial formation. Uninucleate antheridia and ascogonia form in the centre of a colony and coil around each other. After secondary cleistothecial cell walls develop, the antheridium withers and disappears. When four or five cell layers of the wall are developed, appendages begin to arise near the base of the cleistothecium. When two layers of the outer cell wall are developed, the male and female nuclei in the ascogonium conjugate and the ascogonium divides to form the ascus. The ascus divides to form ascospores. When ascospores mature they are forcibly discharged as the cleistothecium is ruptured by the swelling asci and ascospores (Spencer, 1978).

2.2.5. Epidemiology.

Cucurbit powdery mildew is generally favoured by dry atmospheric and soil conditions, moderate temperatures, reduced light intensity, fertile soil and succulent plant growth (Yarwood, 1957).

Torelance of cucurbit powdery mildew to heat is usually lower than that of the host. Cucurbit powdery mildew is able to thrive in hot climates because vines shade the ground and mycelium develops on the underside of the leaves (Walker, 1952).

The optimum relative humidity for spore germination is near 100% but spores germinate in relative humidities as low as below 20% (Roberts, 1984; Spencer, 1978). Free water on the infection court is deleterious to spore germination. This fact implies that conidia have a high water content and extremely efficient water conservation system. Water is needed for germination of spores but it is present in the spores (Yarwood, 1957). The relative importance of cucurbit powdery mildew in different regions is correlated with rainfall in those areas. Incidence of the disease increases as rainfall decreases (Spencer, 1978).

Powdery mildew develops better in the shade than in full light. It is more severe in closely spaced plants and under

a high carbohydrate level with it's subsequent luxuriant growth. Leaves are most susceptible 2-3 weeks after unfolding. The very young folded leaves appear to be immune (Chupp, 1960).

Powdery mildew is more severe in the glasshouse than in the field because of reduced air circulation, reduced light intensities, higher temperatures and continuous cropping (Wellman, 1972).

2.2.6. Control.

Breeding of disease resistant host plants has been the most successful method of controlling any plant disease. However no variety of karela has been reported to be resistant to powdery mildew. The elimination of diseased weeds or volunteer plants of the family cucurbitaceae on which the fungus overwinters if extensively practised has slight effect in controlling the disease. Seed treatment and crop rotation have no effect in controlling the powdery mildew (Chupp, 1960).

Chemical control of powdery mildew of cucurbits has not been as thoroughly investigated as chemical control of many other plant diseases (Spencer, 1978). The fungi are vulnerable to the action of sulphur through most of their life

cycle except for cleistothecial stages (Yarwood, 1957). The difficulty with cucurbits is that many of them are sensitive to sulphur. However gourds are sulphur tolerant (Spencer, 1978). An effective sulphur application rate in the field is 4.5 kg elemental sulphur per hectare, applied when the fungus is first observed, followed by a repeat application two weeks later. In cool damp weather, 1:5:50 Bordeaux mixture may be used.

Around 1952, the first of the effective organic fungicide for control of powdery mildew of cucurbits was developed. The chemical was dinitro capryl phenyl crotonate frequently sold under the trade name karathane. The compound is also an effective miticide. Karathane acts as an eradicant fungicide, killing both mycelium and spores (Godfrey, 1952). A 1% dust at 39.1-44.7 kg/ha or a spray using a rate of 0.5 kg/ha give adequate control. It is applied when fruits are just beginning to set and at seven days interval until just before harvest.

In 1966 the first trials with benomyl (benlate) for the control of cucurbit powdery mildew were conducted by numerous investigators. Delp and Klopping (1968) first reported systemic activity with benomyl and also resistance by the pathogen when applied to the foliage. Schroeder and Provvidenti (1968) also reported these same effects when

benomyl was used a a soil drench or as seed treatment. In the seed treatment it was used as a slurry carried in a 4 % methocel solution at the rate of 2% of seed weight. One ml of this solution was used per 10g of seed. For soil drench treatment these authors used 1.5 mg per 4 inch pot of soil, initially applied to young plants in the two leaf stage and with repeated applications every other week.

Dimethirimol a pyrimidine derivative (used commercially as milcurb) is a systemic fungicide which is highly active against certain powdery mildews. It was shown by Elias et al (1968) to be effective and it gives long lasting protection when applied to the soil. Bent (1970) found dimethirimol to be toxic to spores of S. fuliginea in vitro. Since 1969 the chemical has been widely used in Britain and Holland but in both these countries emergence of resistant strains was reported. As a consequence it's use had been abandoned (Marsh, 1977).

Trifoline (CELA W 524) was reported by Fuchs et al (1971) to be systemically active against cucurbit powdery mildew when applied as pre or post infectional treatment. When used as a spray at 200 ppm it gives good control.

The systemic fungicide pyrazophos was found by de' Waard (1974) to have a greater specific protectant and curative

action than any other organophosphorus compound he tested against cucumber powdery mildew. This compound is used as a spray at 90-300 ppm depending on the level of infection pressure. There is a waiting period of 3 days between last application and harvest.

Triadimefon (bayleton) is a systemic fungicide which has been recommended for use against powdery mildew of vegetables. It is recommended to be used at 0.25-0.5 kg/ha. Results obtained from experiments in Kenya suggested that bayleton may be used to control the powdery mildew of karela. However it was observed that plants sprayed with bayleton developed very tiny necrotic spots on leaves which were signs of phytotoxicity (Chweya and Buruchara, 1989). Triadimenol (bayfidan) is recommended by the manufacturers for control of cucurbit powdery mildew. If the causal fungus is E. cichoracearum it is used at 0.0025% or 15-30g/ha while for S. fuliginea it is used at 0.01-0.0125% or 100-125 g/ha.

Other systemic fungicides tested effectively are ethirimol, triarimol applied at 10 ppm on a 14 day schedule, barbiturate secobarbital, dodemorph, carbendazim used as a spray at 250 ppm (0.5 kg/1000 l) applied at monthly intervals, thiophanate methyl used at 500 ppm (1kg/1000 l), quinomethionate and buprimate (Spencer, 1978).

A novel control procedure for the small scale commercial grower with abundant water supply has been suggested by Yarwood (1939) who found that rainfall through mechanical action decreases the disease incidence. Small scale growers can control powdery mildew by simply spraying the leaves with an ordinary garden hose in the afternoon (Spencer, 1978).

Spencer (1977) proposed standard methods for evaluation of fungicides for the control of cucurbits powdery mildew. The procedures adopted are effective in determining efficacy of the fungicide, systemic activity and suitability in terms of phytotoxicity and yield enhancement.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Propagation of the pathogen.

Karela seeds were sown, two seeds per plastic pot size (super -5) filled with moist sterilised mixed soil composed of soil, manure and sand in a ratio of 3:2:1 respectively. The pots were kept in a glasshouse at Kabete Field Station and they were watered twice a week.

Karela leaves infected with powdery mildew were collected from farmer's fields around Kibwezi, Makindu and Kiboko in Makueni district. The leaves were packed in transparent polythene bags and transported to the laboratory where they were stored in a refrigerator at about 5°C.

The plants in the glasshouse were inoculated with powdery mildew spores by shaking the infected leaves over them at the first two true leaves stage. The plants were used to multiply the pathogen and this was enhanced by lightly rubbing the infected leaves on uninfected leaves.

3.2 Identification and morphology of the causal organism of karela powdery mildew.

Direct observations of the fungus were made. Powdery mildew colonies on karela leaves were scraped to separate the fungus from the host using a dissecting needle, mounted in a drop of cotton blue in lactophenol on a slide, covered with a coverslip and examined under a light microscope. Microscopic examinations of the fungus mycelia, conidiophores and conidia were made at magnifications of 6.3 x 16, 6.3 x 40 and 6.3 x 100.

Dry infected karela leaves were collected from the plants in the glasshouse. They were lightly rubbed on to a glass slide using a soft brush to remove the fungal structures, mounted in cotton blue in lactophenol and examined under a light microscope for the presence of cleistothecia. The above procedure was repeated using dry pumpkin leaves infected with powdery mildew.

3.3 Symptoms and signs of powdery mildew of karela.

Eight karela plants were grown in plastic pots and kept in a greenhouse. After one month three plants were inoculated with powdery mildew spores from infected karela

leaves. Three plants were inoculated with spores from pumpkin leaves infected with powdery mildew. Three plants were not inoculated and were maintained as controls. The control plants were kept in a separate glasshouse. Daily observations of symptoms and signs were made.

3.4 **Histopathology.**

3.4.1 **Prepenetration stages of the pathogen.**

Detached karela leaves were wiped with dry cotton wool. They were dusted with powdery mildew spores from infected leaves and put in a moist chamber. The chambers were kept on a laboratory bench in the light at room temperature of 24°c average.

After 10, 24, 30, 48, 54, 72, 78 and 96 hours of inoculation five 1 cm long leaf tip pieces were cut from each incubated leaf. The pieces were cleared and stained using Bruzzese and Hasan clearing and staining technique (Burchill, 1981). They were immersed in clearing aniline blue stain in stoppered universal bottles. The composition of clearing aniline blue stain is given below.

Composition of aniline blue stain for Bruzzese and Hasan technique.

Chemical	Amount
Ethanol 95%	300.00 ml
Chloroform	150.00 ml
Lactic acid 90%	125.00 ml
Phenol	150.00 g
Chloral hydrate	450.00 g
Aniline blue (powder)	0.60 g

After 48 hours the leaf pieces were removed and put in chloral hydrate clearing solution (2.5g /c.c) for 12 hours. They were rapidly immersed in distilled water and mounted in clear lactophenol. The cleared leaf pieces were observed under a light microscope at a magnification of 6.3 x 40.

3.4.2 **Tissue colonization.**

Thin transverse and horizontal sections of infected leaves were cut using sharp razor blades. The sections were cleared in 0.1 m sodium hydroxide for 3-5 minutes and stained in cotton blue in lactophenol. The sections were transferred into clear lactophenol to remove excess stain. They were mounted in clear lactophenol and observed under a light microscope.

3.5 Factors influencing spore germination.

3.5.1 Relative humidity.

To establish the relationship between relative humidity and spore germination, the method described by Clayton (1942) was used. Saturated solutions of $cacl_2$, $cacl_2 . 6H_2O$, $Ca(No_3)_2 . 4H_2O$, $(NH_4)_2SO_4$ and $Na_2HPO_4 . 12H_2O$ were prepared. The theoretical relative humidities obtained with the saturated salt solutions are 0%, 32.0% 55%, 81% and 95% respectively. Distilled water was used to provide 100% relative humidity. Twenty ml of each solution was poured into a petri dish in replicates of three and undissolved crystals were maintained. A watch glass was inserted in each petri-dish and a glass slide placed on it.

Uninfected karela leaves were wiped with dry cotton wool and cut into pieces of approximately 1.5 x 0.5 cm avoiding the major veins. Conidia of the mildew fungus were brushed onto the leaf pieces. Three pieces were placed on a glass slide and put in each petri-dish. The petri-dishes were sealed with a tape and placed at random in an incubator at 28°C.

After 24 hours, the leaf pieces were removed and cleared separately using the Bruzzese and Hasan technique. The pieces were mounted in clear lactophenol. Three microscopic fields were taken at random for each leaf piece and percentage spore

germination was determined.

3.5.2 Temperature.

Uninfected karela leaves were wiped with dry cotton wool and cut into pieces of approximately 1.5 x 0.5 cm. Conidia of the mildew fungus were brushed onto the leaf pieces. Five pieces were placed on a glass slide which was put in a petri-dish fitted with a moist filter paper.

Two such dishes were kept in an incubator at 5±2°C. Twenty four hours later the dishes were withdrawn from the incubator. The leaf pieces were stained and cleared using Bruzzese and Hasan technique. For every leaf piece, three microscopic fields were taken at random and examined for spore germination. Percentage spore germination was taken for each microscopic field.

The above procedure was repeated at 10°C, 15°C, 20°C, 25°C, 30°C, 35°C and 40°C.

3.6 Chemical control of powdery mildew of karela

The standard methods for evaluation of fungicides for control of cucurbits powdery mildew proposed by Spencer (1977) were used. The following six fungicides were evaluated in the laboratory, in a glasshouse and in the field for their effectiveness in controlling the powdery mildew.

Trade Name	Common Name	Chemical Name
Bayleton	Triadimefon (BAY MEB 6447)	1-(4-Chlorophenoxy)-3,3-di-methyl-1-(1H -1,2,4-triazole-1-yl)-2-butanone
Bayfidan	Triadimenol (BAY KWG 0519)	ß-(4-chlorophenoxy)-α-(1,1-dimethylethyl) -1H-1,2,4-triazole-1-ethanol
Baycor	Bitertanol	ß-{ [1,1-biphenyl]-4-yloxy} -α-(1,1-dimethylethyl)-1 H-1,2,4 - triazole -1-ethanol
Cercobin	Thiophanate methyl	dimethyl (4,4-0-phenylene-bis (3-thioallophanate)
Daconil	Chlorothalonil	Tetrachloroisophthalonitrile
Folicur	Tebuconazole (BAY HWG 1608)	α-tert-butyl-α-(para-chlorophenethyl)-1 H-1 2,4-triazole-1-ethanol

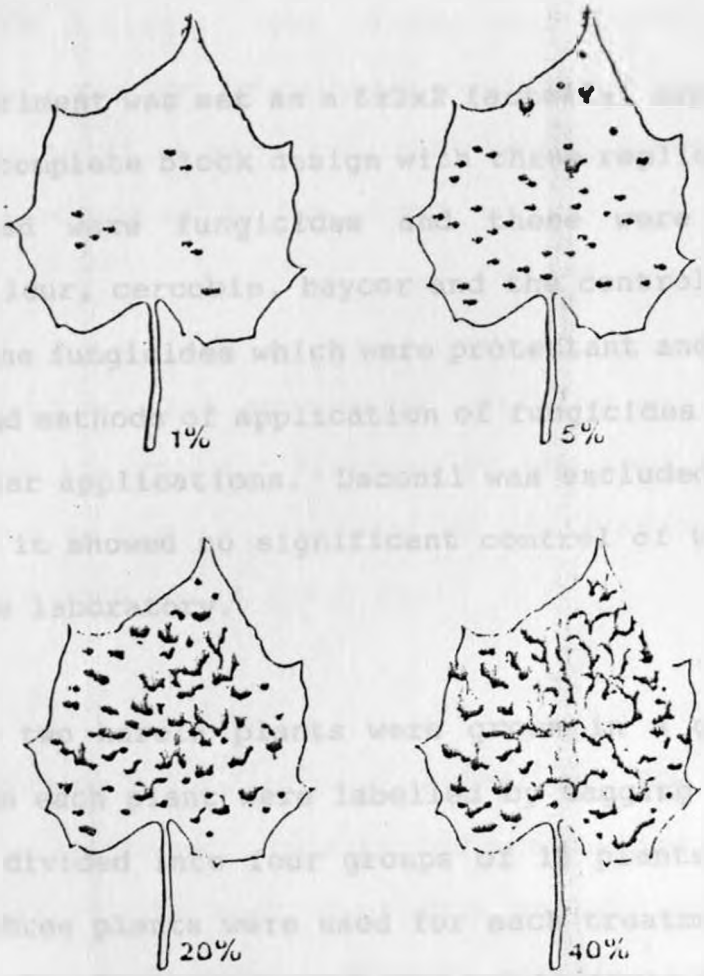
3.6.1 Laboratory evaluation of fungicides.

The leaf disc method was used. Heavily infected karela leaves were shaken over detached healthy leaves. Triangular leaf tips of approximately 1.5 cm long were cut from the inoculated leaves. Three pieces were floated upper surface up on 10 ml solution of each of the six fungicides in a petri-dish. Each fungicide was tested at concentrations of 0, 50, 125, 200, 275, 350, 425 and 500 ppm. The amounts which were measured for each fungicide concentration are shown below.

	Fungicide					
	Bayleton	Bayfidan	Folicur	Daconil	Cercobin	Baycor
	Triadimefon 25% w.p	Triadimenol 250 E.C.	Tebuconazole 250 E.C	Chloro-thalonil 75% w.p	Thiophanate -methyl 50% w/v	Bitertanol 300 E.C
Concentration (ppm)	(g)	(ml)	(ml)	(g)	(ml)	(ml)
0	0	0	0	0	0	0
50	0.002	0.002	0.002	0.00067	0.0010	0.00167
125	0.005	0.005	0.005	0.00167	0.0025	0.00417
200	0.008	0.008	0.008	0.00267	0.0040	0.00667
275	0.011	0.011	0.011	0.00367	0.0055	0.00917
350	0.014	0.014	0.014	0.00467	0.0070	0.01167
425	0.017	0.017	0.017	0.00567	0.0085	0.01467
500	0.020	0.020	0.020	0.00667	0.0100	0.01667

The petri-dishes were covered and kept at random in good light on a laboratory bench for 7 days. The 6x8 factorial experiment was set in a randomized complete block design with

four replicates. The mildew was assessed on a disease severity scale shown in Figure 1, after 7 days.



Powdery mildew infection assessment grades.

Grade	0	1	2	3	4	5
Percentage disease	0	0-1	2-5	6-20	21-40	>40

Figure 1.

Standard diagrams to show four levels of karela Powd
mildew infection.

3.6.2. Test for fungicides efficacy, their effective method of application and mode of activity.

The experiment was set as a 6x2x2 factorial experiment in a randomized complete block design with three replicates. The factors tested were fungicides and these were bayleton, bayfidan, folicur, cercobin, baycor and the control, modes of activity of the fungicides which were protectant and eradicant activities and methods of application of fungicides which were root and foliar applications. Daconil was excluded from this test because it showed no significant control of the powdery mildew in the laboratory.

Seventy two karela plants were grown in a glasshouse. Ten leaves on each plant were labelled by tagging them. The plants were divided into four groups of 18 plants each. In each group three plants were used for each treatment as well as control. The fungicides were applied at their recommended rates. The four groups of plants were treated as follows.

Group 1:

It was used to test protectant activity of the fungicides when applied on foliage. Fifty ml were sprayed for each treatment. After 24 hours the plants were inoculated with powdery mildew spores. Inoculation was repeated after one week. Disease severity was assessed after two weeks on the labelled leaves using the scale of 0-5 shown in figure 1.

Group 2:

It was used to test eradicant activity of the fungicides when applied on foliage. The plants were inoculated with powdery mildew spores and inoculation repeated after one week. After two weeks 50 ml of each fungicide was sprayed. Disease severity was assessed after two weeks.

Group 3:

It was used to test protectant activity of fungicides when applied at the roots. Fifty ml of the test fungicides was poured into each pot. Inoculation and disease severity assessment were done as in group one.

Group 4:

It was used to test eradicant activity of the fungicides when applied at the roots. Inoculation was done as with group two. Fifty ml of the test fungicide was poured into the rooting medium of each plant. Disease severity was assessed after two weeks.

3.6.3. Primary evaluation of fungicides.

Five fungicides, bayleton, bayfidan, folicur, baycor and cercobin were evaluated. They were selected based on their efficacy in the preceeding experiments. Each fungicide was used at the recommended concentration and 100 ppm above and below the recommended rate as shown below.

Fungicide	Recommended rate	-100 pm	+ 100 ppm
Bayleton 25% w.p	5g per 5 l 250 ppm	3g per 5 l 150 ppm	7g per 5l 350 ppm
Bayfidan 250 E.C	5ml per 5l 250 ppm	3ml per 5l 150 ppm	7ml per 5l 350 ppm
Folicur 250 E.C	2.5ml per 5l 125 ppm	0.5ml per 5l 25 ppm	4.5ml per 5l 225 ppm
Baycor 300 E.C	5ml per 5l 300 ppm	3.3ml per 5l 200 ppm	6.7ml per 5l 400 ppm
Cercobin 50% w/v	20ml per 5l 2000 ppm	19ml per 5l 1900 ppm	21ml per 5l 2100 ppm

Karela seedlings were grown in propagation polythene bags size No.2, one seedling per bag and they were kept in a sheltered nursery. Four replicate seedlings were used for each treatment and control.

The seedlings were sprayed at the age of five weeks with the fungicides using a knapsack sprayer. Fifty ml of the test fungicide solution was poured into the rooting medium at the same time. After 24 hours the plants were inoculated with the powdery mildew spores by shaking infected leaves over them.

The disease incidence and severity were assessed after one week. Disease incidence was determined as the percentage

of infected leaves per plant. The second, fourth, sixth, eighth and tenth node leaves were assessed for disease severity using the scale of 0-5 grades (Figure 1).

3.6.4 Field evaluation of fungicides

The experiment was conducted at Kibwezi dryland irrigation Project site. The site is in Makueni District, in the Eastern Province of Kenya. It is located about 7 Km south of Kasayani market on Kibwezi-Kitui road. It lies at an average altitude of about 800 a.s.l. Latitude 2° 17'00"s and longitude 38° 01' 36" intersect in the farm. It has deep chromic luvisols with good drainage and sandy to clay texture (Ekirapa and Muya, 1991). The Kibwezi area has an average annual rainfall of between 600 - 800 mm distributed bi-modally. The lowest mean monthly temperature is 12°c and highest mean monthly temperature is 30°c (Shirim et al, 1982). The average temperature estimated for the area is about 24°c and falls in agro-climate Zone V, described as semi-arid (Sombroek et al 1982).

The experimental plot lay on a relatively flat area and measured 21 x 35 metres. The experiment was done in four replicate plots per treatment using randomized complete block design. The fungicides tested were selected based on their efficacy in the preceeding experiments and were bayleton, bayfidan, folicur and cercobin. The blocks measured 8 x 9 metres and each had ten rows with five karela plants per row. Five rows per block were used as plots for each fungicide and control while the rest of the skipped rows were used as spreader rows.

Karela seeds were directly sown in holes in which furadan, a nematicide had been applied. A spacing of 1 x 2 metres was used. Drip irrigation pipes were laid out. The crop was thinned to leave one plant per hole and five plants per row. The crop was trained using wires fastened on poles of 2.5 metres and stretched along the rows (Plate 1).

The spreader rows were inoculated six weeks after sowing by shaking heavily infected karela leaves over them. The fungicides were sprayed two weeks later at their recommended rates. The treatments were randomly allocated to the plots in each block.

Disease incidence was assessed as the percentage number of infected leaves per plant and disease severity was assessed using the scale shown in figure 1. The first disease assessment was done one week after treatments application and within the same week the second treatments application was done. Third treatments application was done two weeks later. The second disease assessment was done two weeks after the third treatment.

Harvesting was started about two months after planting and was done after every two weeks until the plants could no longer produce marketable fruits or had died. Five harvests were done and the yield was taken in grams per plant.

CHAPTER FOUR

4. RESULTS

4.1. Morphology and identification of the causal fungus of powdery mildew of karela.

The fungus grew on the plant surface and it only colonised the epidermal cells of the plant. The mycelium grew closely appressed to the plant surface without following any specific course on the leaf surface (Plate 2). The mycelium was hyaline and septate with a simple central pore. Hyphal cells were thin walled and longer in length than width (Plate 3).

Conidiophores were erect bearing chains of immature conidia. They were macronematous that is distinct from the vegetative hyphae. They were produced approximately at right angles to and at the middle of the hyphal cells (Plate 4). Conidiogenous cells were basal and incorporated in the main axis of the conidiophore. They were broader and thicker walled than hyphal cells (Plate 5). They produced conidia initials which enlarged into conidia as more initials were produced below thus producing a conidiophore which broadens towards the tip (Plate 6.).

Plate 1: An established field of karela plants

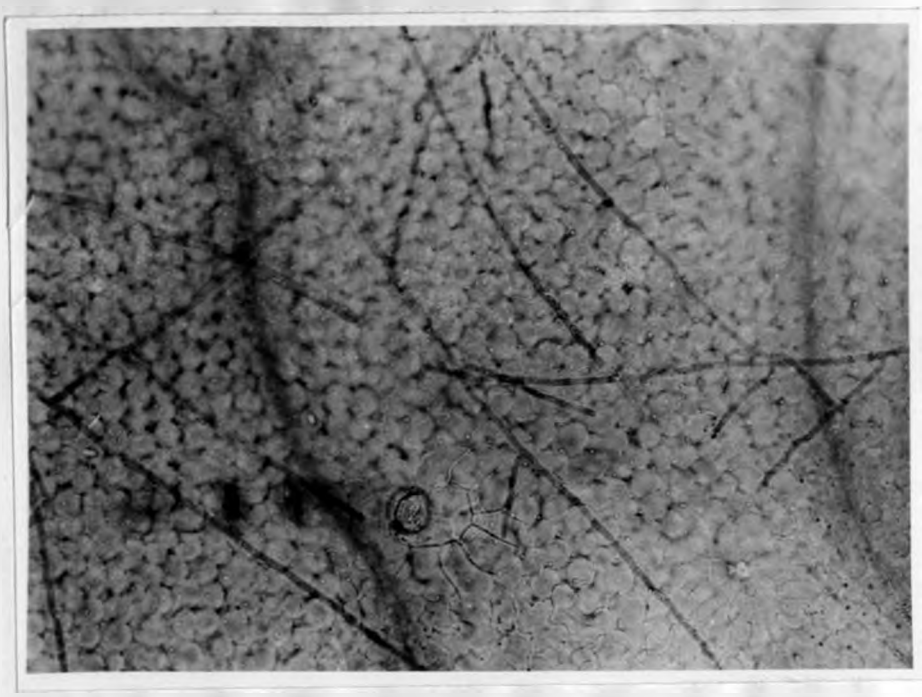


Plate 2: Mycelium of the causal fungus of powdery mildew of karela growing on a leaf surface. Magnification 6.3 x 16.

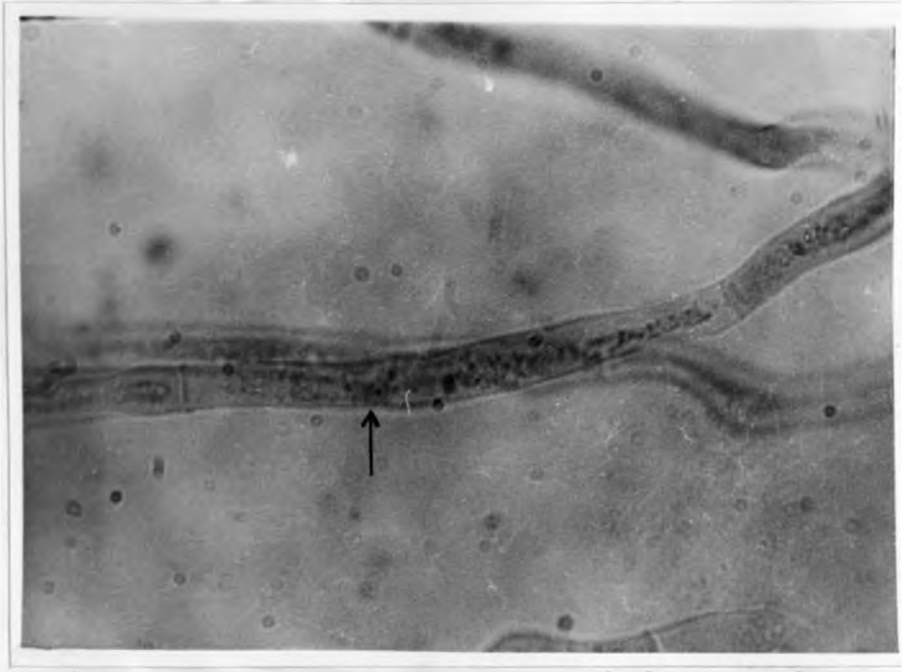


Plate 3: Hyphal cell (arrow) of the causal fungus of powdery mildew of karela. Magnification 6.3 x 100.

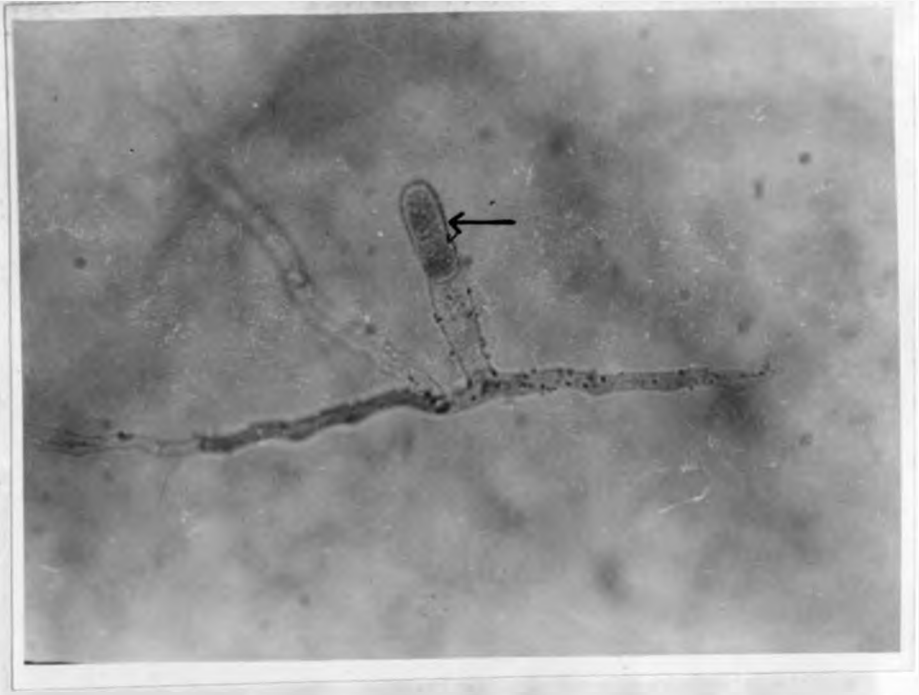


Plate 4: A conidiophore (arrow) produced approximately at a right angle and at the middle of a hyphal cell. Magnification 6.3 x 40.

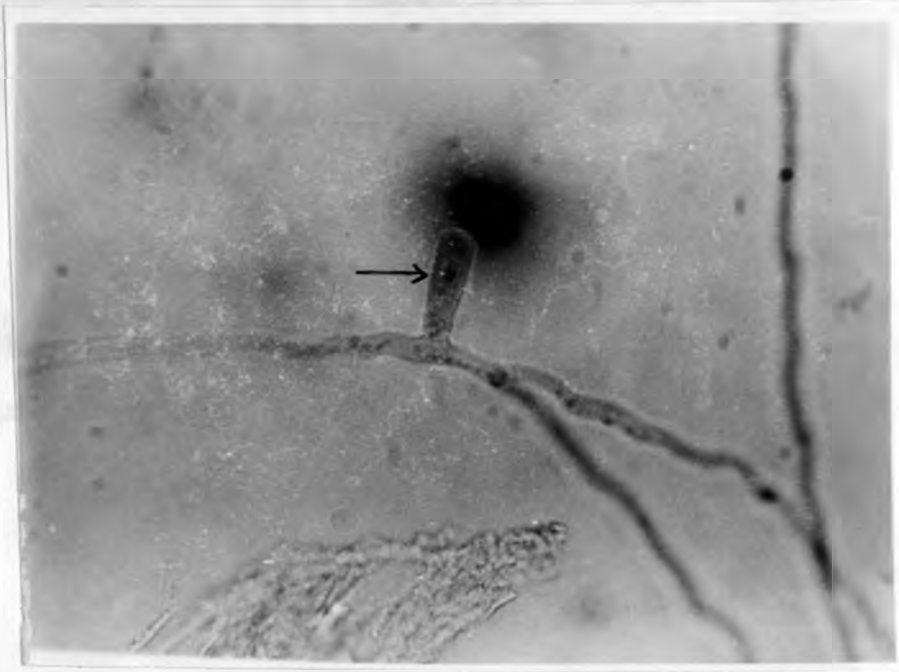


Plate 5: A conidiogenous cell (arrow). Magnification 6.3 x 40.

Plate 6: The shape of conidiophore. Larger, older conidia are found towards the tip. Magnification 6.3 x 16.

Conidia were detached from the conidiophore as they matured and they aggregated into clumps on the leaf surface. They got detached either singly or the conidiophore broke to release them in a group (Plate 7). They were liberated either by separation of the septum between two conidia or by fracture across the wall of a sterile, thin walled intermediate cell (Plate 8 and 9).

Conidia were elliptical in shape and measured 24-42 μm x 13-20 μm in size. They had the basal end narrower than the other. This is the side which got detached from the conidiophore (Plate 10).

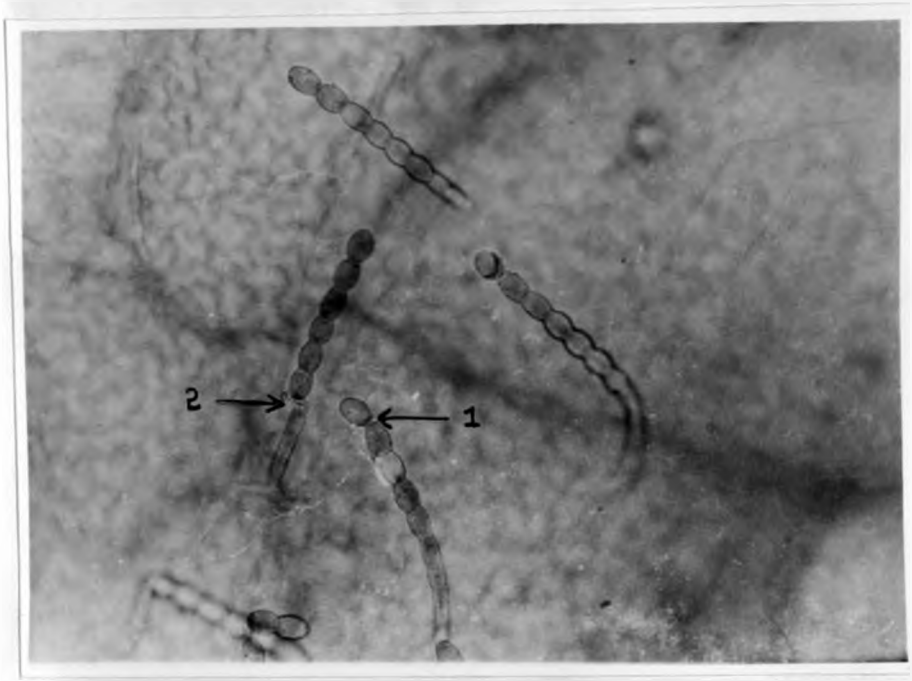


Plate 7: Conidiophores liberating conidia.

1. Conidium being released by detachment of the septum.
2. Conidiophore breaking to release a group of conidia.

Magnification 6.3 x 16.

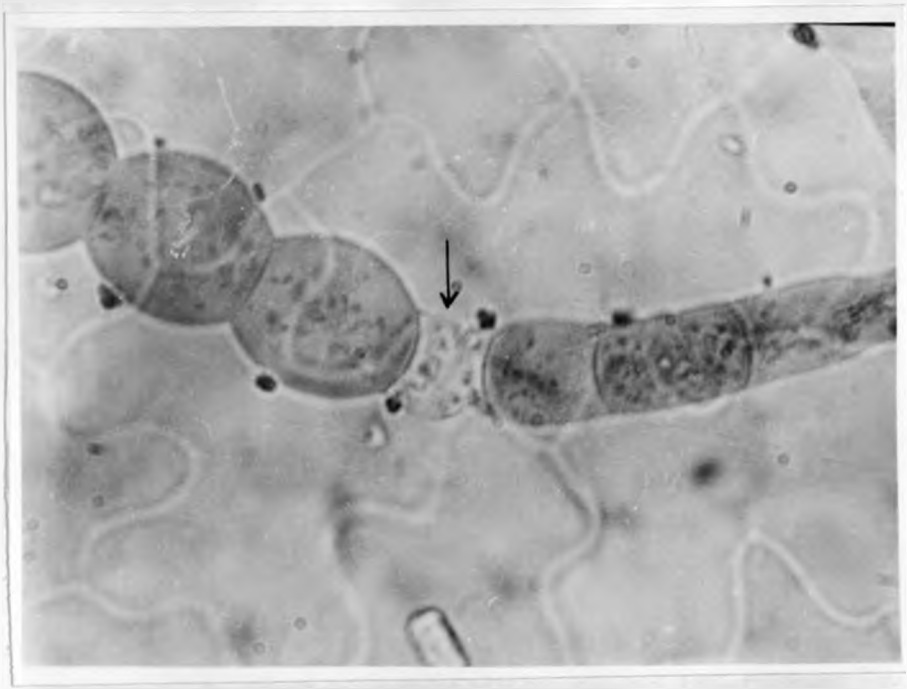


Plate 8: Sterile intermediate cell (arrow) produced between a conidiogenous cell and conidium on a conidiophore.
Magnification 6.3 x 100.

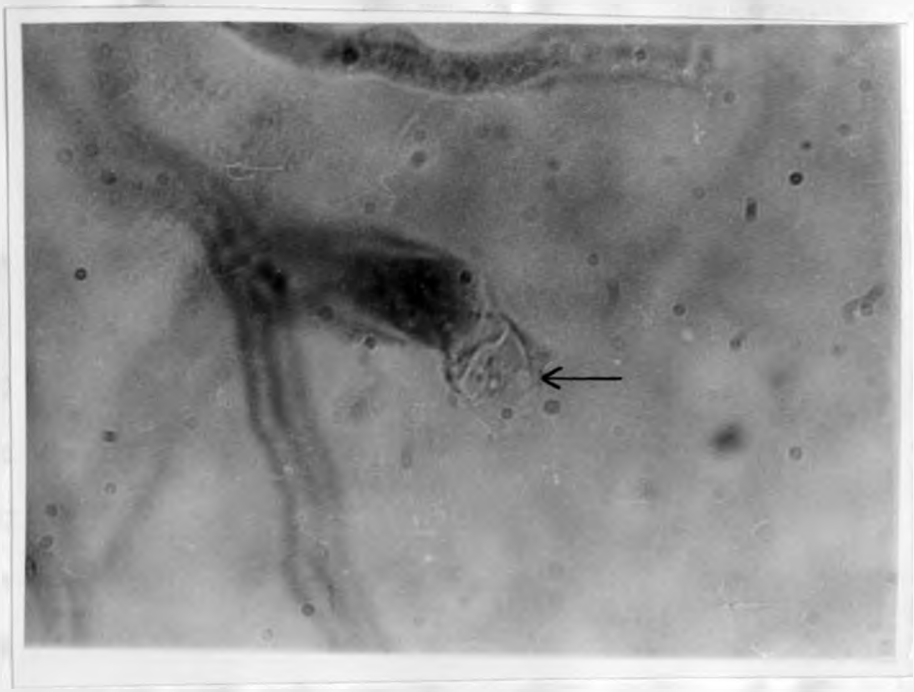


Plate 9: Conidiophore cell bearing the remnants of an intermediate cell (arrow).
Magnification 6.3 x 100.

Plate 10: Conidia on a leaf surface.
Magnification 6.3 x 40.

Cleistothecia were globose in shape and measured 68-100 μm in diameter. They were thick walled with three layers (Plate 11). Each cleistothecium contained a single ascus which bore eight ascopores (Plate 12). They did not have conspicous appendages. Cleistothecia extracted from pumpkin leaves infected with powdery mildew had conspicous appendages (Plate 13) and contained two asci.

Based on the above characteristics of cleistothecia the fungus infecting karela was identified as belonging to genus Sphaerotheca and not genus Erysiphe. Cleistothecium of the former genus contain one ascus while that of the latter contain two asci.

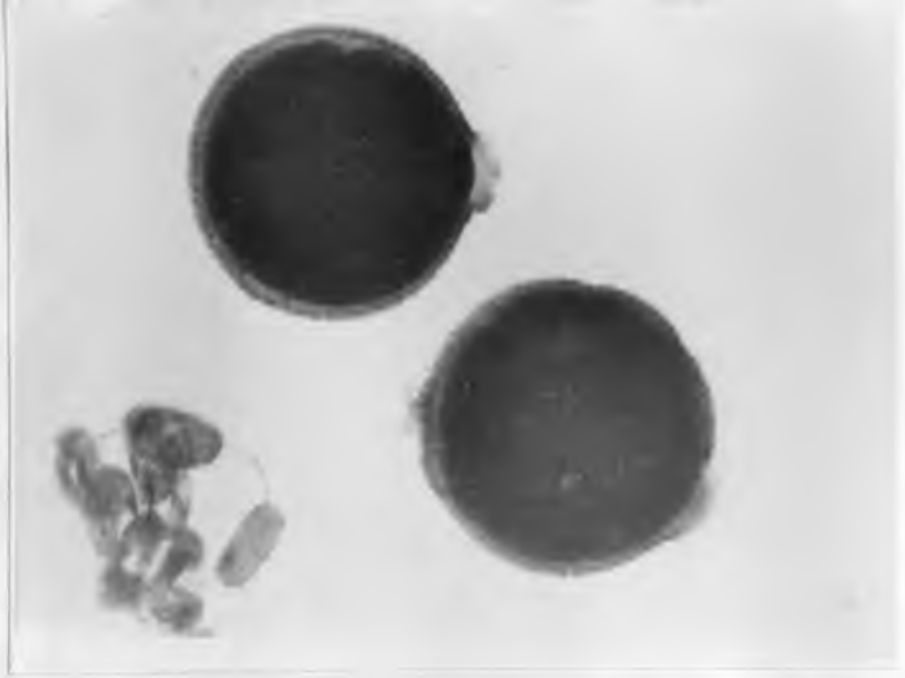


Plate 11: Cleistothecia of the causal fungus of powdery mildew of karela.
Magnification 6.3 x 40.

Plate 12: Cleitothecium bearing one ascus
Magnification 6.3 x 40.

Plate 13: Cleistothecium extracted from pumpkin leaves infected with powdery mildew.
Magnification 6.3 x 16.

4.2 Symptomatology.

The first symptoms of powdery mildew infection appeared on the leaves five to seven days after inoculation as tiny, round white lesions. The initial lesions were flavy and not powdery. The lesions increased in size and number with time. After 10-12 days the old lesions started to appear powdery. This was due to a mass of aggregated white conidia produced. The leaves remained green. However some lesions developed a chlorotic background.

The lesions merged producing a scatter of white powdery colouring on the leaves (Plate 14). The young tender leaves were not infected. However they were infected as they matured. Infection was severe on sheltered leaves especially those near the base of the plants. Both surfaces of the leaves were infected. The lesions on the lower surface were more powdery in consistency.

Severely infected leaves turned chlorotic (Plate 15). They later developed necrotic irregular patches starting along the margins. After 3 weeks the infected leaves became completely necrotic except the young tender leaves. The dead dry leaves remained attached to the plant in a crumbled state such that the plants appeared defoliated (Plate 16).

Stems and fruits were also infected. They developed the white lesions which merged to cover the whole surface but they appeared more flavy than powdery. Seedlings inoculated at 3-6 true leaves stages developed the symptoms, collapsed and died within two weeks. Plants inoculated with conidia from dry leaves did not develop the symptoms. Only plants inoculated with conidia from fresh infected leaves developed the disease symptoms.

The plants which were inoculated with conidia from pumpkin leaves infected with powdery mildew did not develop the disease symptoms.

Plate 14:

Infected karela plants showing the white powdery colouring on Leaves.

Plate 15:

Severely infected chlorotic karela leaves.

Plate 16:

Severely infected karela plant.

4.3 Histopathology.

Conidia germinated after 24 hours. Each conidium produced one or two germtubes (Plate 17 and 18). Germination was polar. After 30 hours some germtubes had terminated forming globose appressoria (Plate 19). However, other germtubes grew, branched and developed into mycelium with no appressoria observed. The germinated conidia did not collapse but remained intact. The mycelium established itself on the leaf surface growing in all directions.

After 72 hours, small conidiogenous cell initials had developed relatively at the middle of hyphal cells. The conidiogenous cell first appeared as a bulge on a hyphal cell, grew in size and a septum developed separating it from the mother hyphal cell (Plate 20). After 96 hours, young conidiophores bearing conidia had developed (Plate 21).

The fungus colonised the epidermal cells producing globose haustoria in the cells (Plate 22).

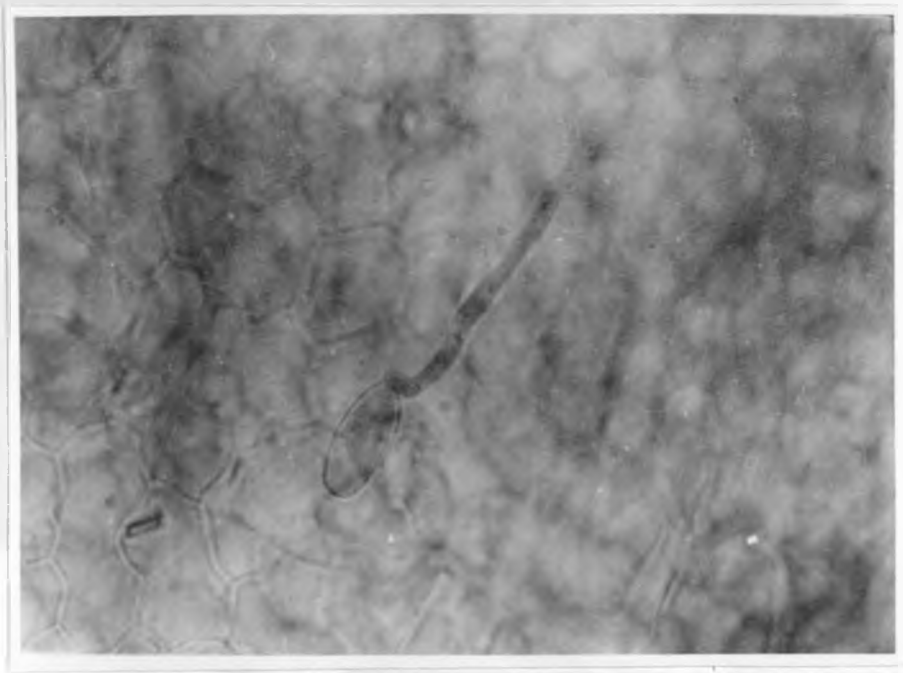


Plate 17: A germinating conidium with one germtube.
Magnification 6.3 x 40.

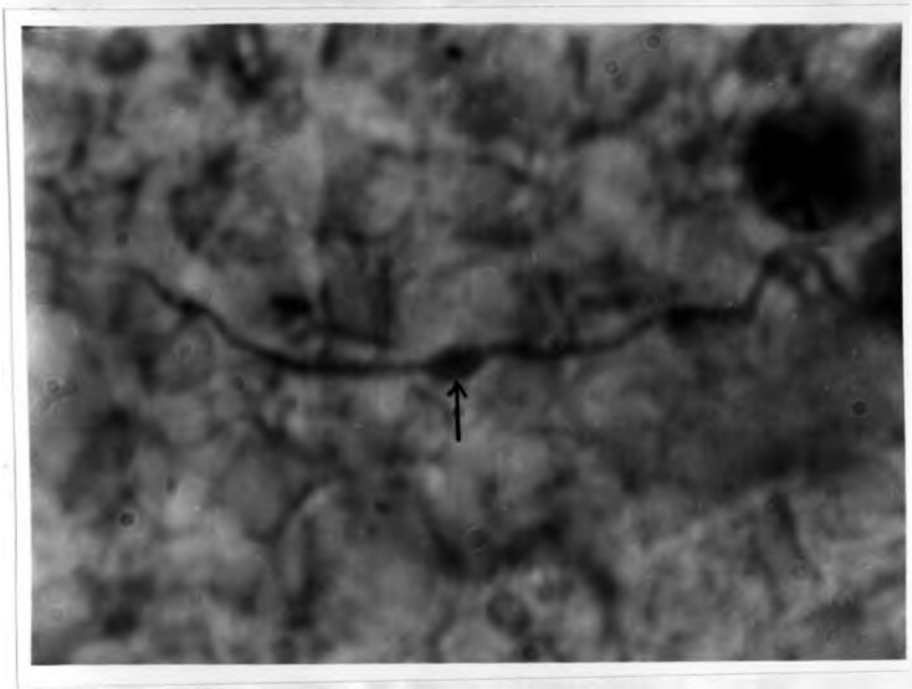


Plate 18: A germinating conidium (arrow) with two germtubes.
Magnification 6.3 x 40.

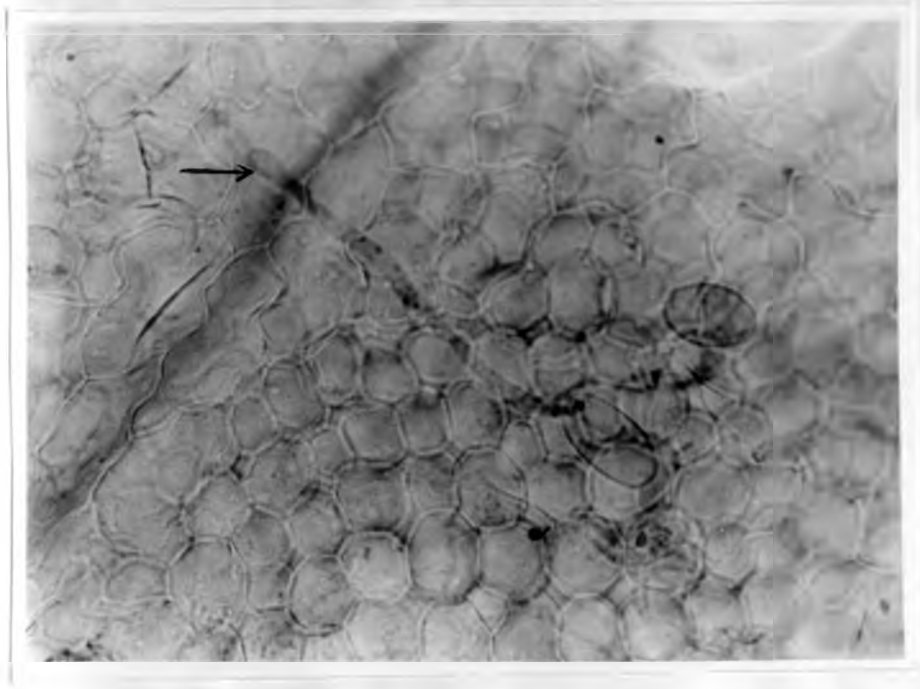


Plate 19: A germtube (arrow) terminating in an appressorium.
Magnification 6.3 x 40.

Plate 20: Developing conidiophores after 72 hours of inoculation.

1. A bulge on a hyphal cell.
2. A conidiogenous cell.

Magnification 6.3 x 40.

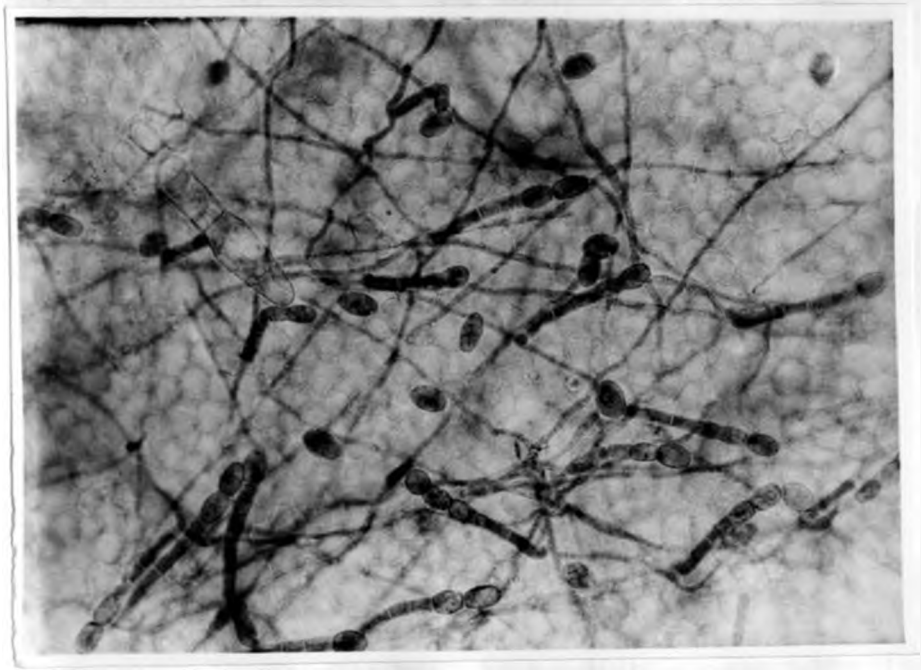


Plate 21: Conidiophores on a leaf surface after
96 hours of inoculation.
Magnification 6.3 x 16.

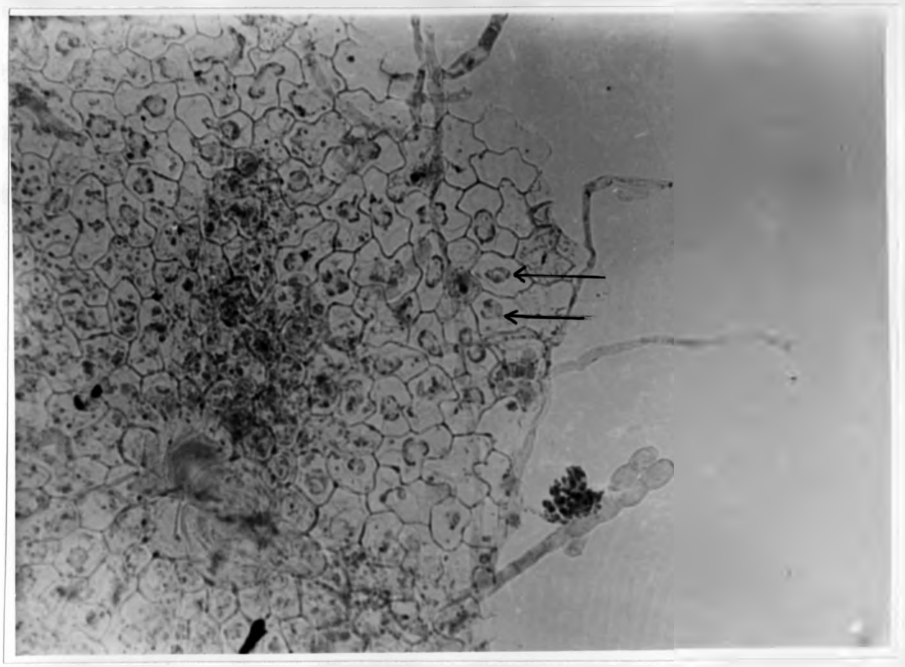


Plate 22: Leaf horizontal section showing haustoria (arrows) in the epidermal cells. Magnification 6.3 x 16.

4.4. Factors influencing spore germination.

4.4.1. Relative humidity.

Percentage spore germination increased with increase in relative humidity. Germination started at 55% relative humidity and increased at higher relative humidities of 81%, 95% and 100%. No spore germination was observed at 0% and 32% relative humidities. The mean spore germination percentages for the relative humidity levels are shown in Table 1. There were significant differences between mean spore germination percentages at 0.1% level of significance (Table 1).

Table 1.

Percentage spore germination of the causal fungus of powdery mildew of karela at different relative humidities after 24 hours.

Percentage relative humidity	Percentage spore germination *
0	-
32	-
55	4.05[a]
81	15.43[b]
95	30.14[c]
100	50.40[d]

* Average of nine replications.
C.V = 26.33%.
LSD at 5 per cent = 4.16.
LSD at 1 per cent = 5.56.
LSD at 0.1 per cent = 7.27.
Figures followed by different letters are significantly different at 0.1% level of significance.

4.4.2. Temperature

No spore germination observed at 5°C, 10°C and 15°C. Germination started at 20°C and increased as temperature was raised to 25°C and 30°C. No spore germination was observed at 35°C and 40°C. The mean spore germination percentages at the different temperatures are shown in table 2. Mean percentage germination at 20°C was significantly different from those at 25°C and 30°C at 0.1% level of significance. However there was no significant difference between mean spore germination percentages at 25°C and 30°C (Table 2).

Table 2.

Percentage spore germination of the casual fungus of powdery mildew of karela at different temperatures after 24 hours.

Temperature (°C)	Percentage spore Germination *
5	-
10	-
15	-
20	15.70[a]
25	28.70[b]
30	31.06[b]
35	-
40	-

* Average of 10 replications.
C.V = 40.24%.
LSD at 5 per cent = 6.79.
LSD at 1 per cent = 9.02.
LSD at 0.1 per cent = 11.71.
Figures followed by different letters are significantly different at 0.1% level of significance.

4.5 Chemical control of powdery mildew of karela.

4.5.1 Laboratory evaluation of fungicides.

All the fungicides gave some control of the powdery mildew. Bayleton was the most effective with the lowest mean disease severity score of 0.48 followed by folicur (0.56), bayfidan (0.72), cercobin (1.10), baycor (1.30) and daconil (1.76) respectively. There were no significant differences between bayleton and folicur, between folicur and bayfidan and between cercobin and baycor at 5% level of significance. At 1% level there was no significant differences between bayleton, folicur and bayfidan (Table 3).

There was a general decline in disease severity with all the fungicides as their concentrations were raised from 0 ppm, 50 ppm, 125 ppm, 200 ppm, 275 ppm, 350 ppm, 425 ppm to 500 ppm. (Table 3 and Figure 2).

Table 3:

Effect of different fungicides at different concentrations on control of powdery mildew of karela in the laboratory: Disease severity score*.

Concentration (ppm)	Fungicide					
	Daconil	Bayleton	Bayfidan	Baycor	Folicur	Cercobin
0	3.08^{a}	2.67^{cd}	2.17^{fg}	2.75^{bc}	2.33^{ef}	2.50^{de}
50	2.17^{fg}	0.50^{qrs}	1.25^{lm}	2.00^{gh}	0.67^{pq}	1.67^{ij}
125	1.83^{hi}	0.33^{stu}	0.83^{op}	1.50^{jk}	0.58^{qr}	1.33^{kl}
200	1.50^{jk}	0.25^{tuv}	0.67^{pq}	1.33^{kl}	0.33^{stu}	1.25^{lm}
275	2.17^{fg}	0.08^{v}	0.50^{qrs}	1.08^{mn}	0.33^{stu}	0.92^{no}
350	1.42^{kl}	0	0.33^{stu}	0.83^{op}	0.25^{tuv}	0.58^{qr}
425	0.83^{op}	0	0	0.58^{qr}	0	0.41^{rst}
500	1.08^{mn}	0	0	0.33^{stu}	0	0.17^{uv}

* Average of four replications.

LSD at 5 per cent = 0.21.

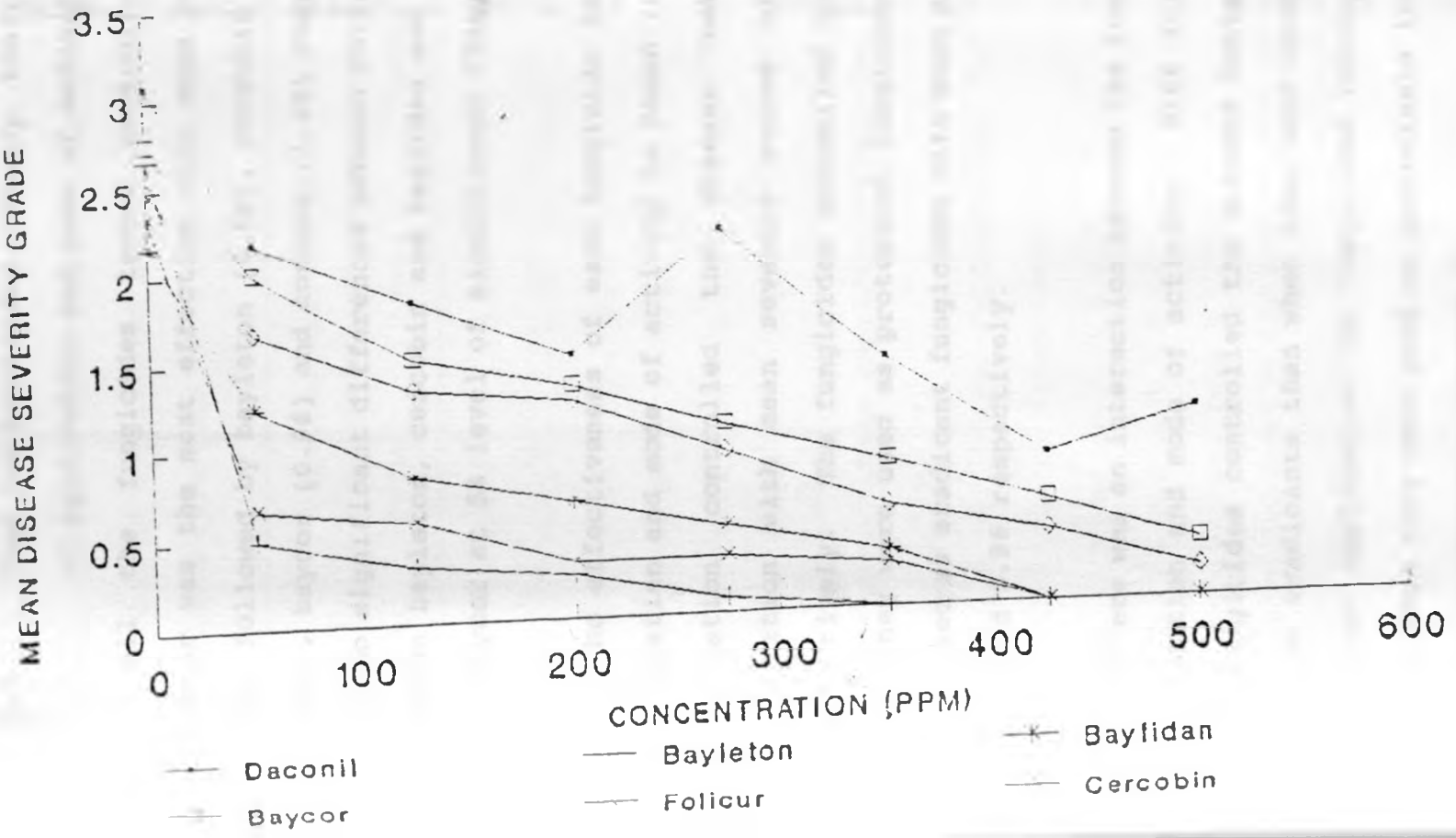
LSD at 1 per cent = 0.27.

LSD at 0.1 per cent = 0.35.

Figures followed by the same letter are not significantly different at 5% level of significance.

FIGURE: 2

EFFECT OF FUNGICIDES AT DIFFERENT CONCENTRATIONS ON CONTROL OF POWDERY MILDEW OF KARELA IN THE LABORATORY.



4.5.2 Test for fungicides efficacy, their effective method of application and mode of activity.

All the fungicides tested controlled the disease. Folicur was the most effective with mean severity score of 0.49, followed by bayleton (0.58), cercobin (0.83), bayfidan (0.84), baycor (0.96) and control (1.28) respectively. There were no significant differences between folicur and bayleton, between bayleton, cercobin and bayfidan and between bayfidan and baycor at 5% level of significance (Table 4).

The effectiveness of each fungicide for each method of application and mode of activity is shown in table 4. Foliar application controlled the disease better than root application with mean severity scores of 0.51 and 1.14 respectively. The fungicides controlled the disease better when they were used as protectant fungicides than when they were used as eradicant fungicides with mean severity scores of 0.70 and 0.96 respectively.

There was an interaction between the fungicides method of application and mode of activity. With foliar application, the fungicides controlled the disease better when they were used as eradicants than when they were used as protectants. With root application the fungicides controlled the disease better when they were used as protectants than when they were used as eradicants (Figure 3).

Table 4:

Effectiveness of different fungicides for different methods of application and modes of activity on control of powdery mildew of karela: Disease severity score.

Fungicide	Method of application*		Mode of activity**	
	Foliar	Root	Protectant	eradicant
Bayleton	0.27^{gh}	0.88^{cd}	0.42^{fgh}	0.73^{cde}
Bayfidan	0.45^{fgh}	1.23^{ab}	0.63^{def}	1.05^{bc}
Folicur	0.22^{h}	0.77^{cde}	0.30^{gh}	0.68^{def}
Cercobin	0.52^{efg}	1.15^{b}	0.72^{cde}	0.95^{c}
Baycor	0.52^{efg}	1.40^{ab}	0.77^{cde}	1.15^{b}
Control	1.12^{b}	1.43^{a}	1.35^{ab}	1.20^{ab}

* Average of three replications and two modes of activity of fungicides.

** Average of three replications and two methods of application of fungicides.

LSD at 5 per cent = 0.27

LSD at 1 per cent = 0.36

LSD at 0.1 per cent = 0.47

Figures followed by the same letter are not significantly different at 5% level of significance.

Table 4:

Effectiveness of different fungicides for different methods of application and modes of activity on control of powdery mildew of karela: Disease severity score.

	Method of application*		Mode of activity**	
Fungicide	Foliar	Root	Protectant	eradicant
Bayleton	0.27^{gh}	0.88^{cd}	0.42^{fgh}	0.73^{cde}
Bayfidan	0.45^{fgh}	1.23^{ab}	0.63^{def}	1.05^{bc}
Folicur	0.22^{h}	0.77^{cde}	0.30^{gh}	0.68^{def}
Cercobin	0.52^{efg}	1.15^{b}	0.72^{cde}	0.95^{c}
Baycor	0.52^{efg}	1.40^{ab}	0.77^{cde}	1.15^{b}
Control	1.12^{b}	1.43^{a}	1.35^{ab}	1.20^{ab}

* Average of three replications and two modes of activity of fungicides.

** Average of three replications and two methods of application of fungicides.

LSD at 5 per cent = 0.27

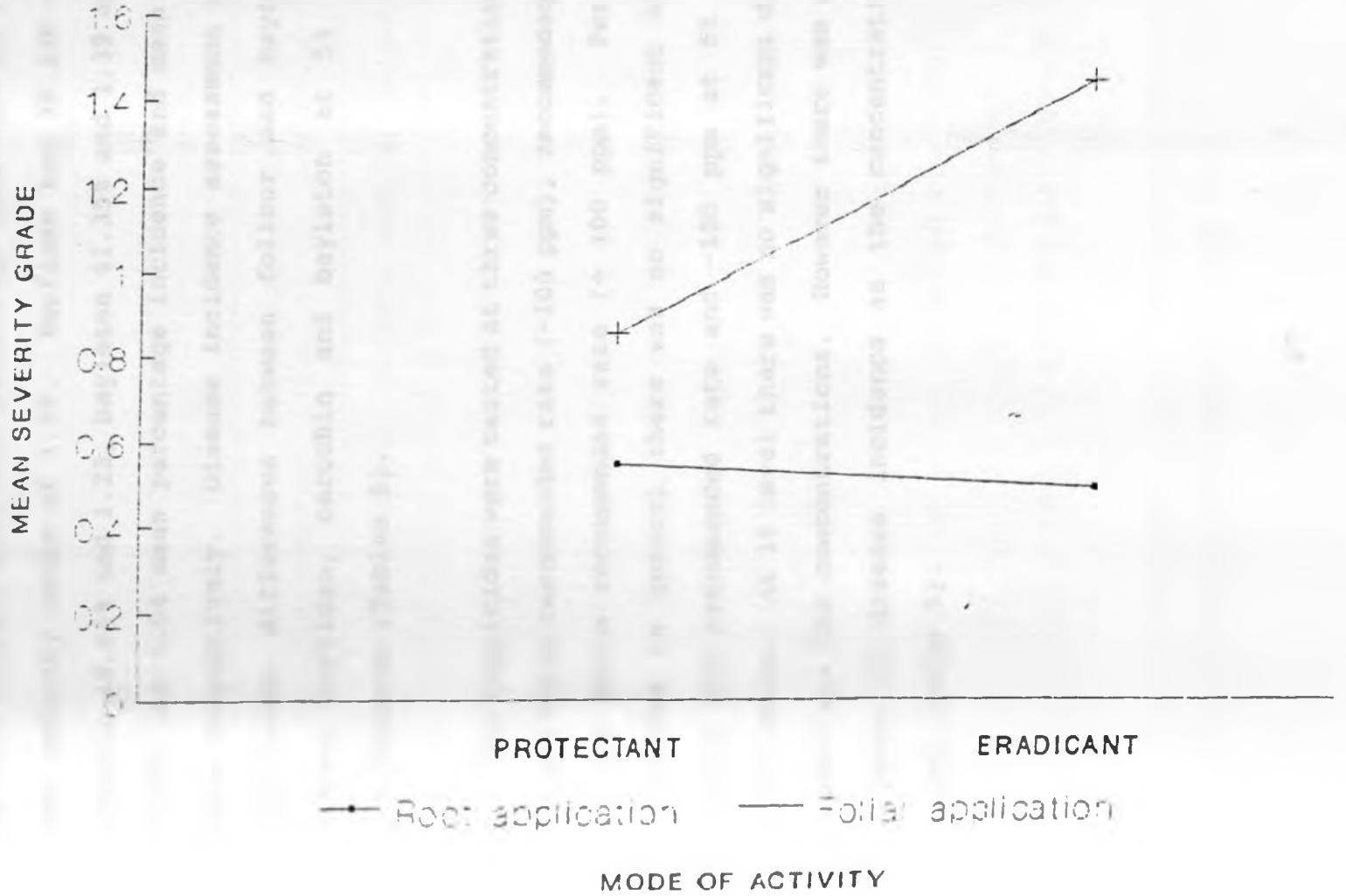
LSD at 1 per cent = 0.36

LSD at 0.1 per cent = 0.47

Figures followed by the same letter are not significantly different at 5% level of significance.

FIGURE: 3

RESPONSE CURVES FOR SIGNIFICANT INTERRACTION BETWEEN METHODS OF APPLICATION AND MODES OF ACTIVITY OF FUNGICIDES.



4.5.3 Primary evaluation of fungicides.

All the fungicides controlled the disease. Folicur had the best control with mean percentage incidence of 34.57% and mean severity score of 1.09. Bayfidan had 38.33% and 1.45, Cercobin 40.84% and 1.23, bayleton 41.35% and 1.39 and baycor 49.53% and 1.94 mean percentage incidence and mean severity score respectively. Disease incidence assessment showed no significant differences between folicur and bayfidan and between bayfidan, cercobin and bayleton at 5% level of significance (Tables 5).

The fungicides were tested at three concentration levels, 100 ppm below recommended rate (-100 ppm), recommeded rate and 100 ppm above recommended rate (+ 100 ppm). For all the fungicides in general there was no significant difference between the recommended rate and -100 ppm at 5% level of significance. At 1% level there was no significant difference between all the concentrations. However there was a general decrease of disease incidence as the concentrations were raised (Table 5).

Table 5:

Effect of different fungicides at three concentrations on control of powdery mildew of karela: Percentage disease incidence and severity score*.

	Concentration					
	100 ppm below recommended rate		Recommended rate		100 ppm above recommended rate	
Fungicide	Incidence	Severity	Incidence	severity	incidence	severity
Bayleton	38.61^{cde}	0.90^{defgh}	34.60^{ef}	1.15^{bcdef}	36.08^{def}	1.00^{cdefg}
Bayfidan	40.86^{cd}	1.35^{bcd}	30.62^{fg}	1.20^{bcde}	25.73^{ge}	0.75^{efgh}
Folicur	33.42^{ef}	0.60^{gh}	25.08^{e}	0.40^{h}	23.66^{e}	0.85^{defgh}
Baycor	48.79^{ab}	1.50^{bc}	50.97^{a}	1.65^{ab}	42.24^{c}	2.10^{d}
Cercobin	43.85^{bc}	1.25^{bcde}	40.54^{cd}	0.65^{fgh}	22.87^{e}	0.50^{gh}

* Average of four replications.

Disease incidence.

LSD at 5 per cent = 5.69

LSD at 1 per cent = 5.57

LSD at 0.1 per cent = 9.86

Disease severity.

LSD at 5 per cent = 0.55

LSD at 1 per cent = 0.73

LSD at 0.1 per cent =0.95

For each parameter, figures followed by the same letter are not significantly different at 5% level of significance.

4.5.4. **Field evaluation of fungicides.**

Field results for the disease assessment and yield are given in table 6. All the fungicidal treatments gave some control of powdery mildew of karela.Folicur was more superior to bayleton, bayfidan and cercobin. Disease incidence assessment showed no significant difference between bayfidan and cercobin. Disease severity assessment showed no significant difference between bayleton, bayfidan and cercobin both at 5% level of significance (Table 6).

Plants sprayed with folicur showed signs of phytotoxicity. The fungicide caused leaf curling and puckering (Plate 23). However the plants tended to recover from this effect even after subsequent sprays and this effect did not significantly affect the yield. The other fungicides showed no signs of phytotoxicity on the plants. Plants sprayed with fungicides had more luxuriant foliar growth than unsprayed plants (Plates 24, 25, 26).

There were no significant differences between total plot yield sprayed with the four fungicides, bayleton, bayfidan, folicur and cercorbin and between plants sprayed with bayleton, bayfidan and unsprayed control plants. However total yield of plants sprayed with folicur and cercobin were significantly different from that of unsprayed control plots at 5% level of significance (Table 6).

There was no direct relationship between plot yield and the disease infection. However there was a general decrease of yield as disease severity and incidence increased (Figures 4 and 5).

Table 6.

Effect of fungicidal sprays on control of powdery mildew of karela and yield during the period May-August 1992 at Kibwezi.

Fungicide	Incidence as Percentage of leaves infected per plant *1	Severity as disease infection score *2	Yield (tons/ha)	Percentage Yield increase over control
Bayleton	27.22[b]	1.55[b]	1.99[ab]	48.51
Bayfidan	28.83[bc]	1.55[b]	2.19[ab]	63.43
Folicur	21.79[a]	0.70[a]	2.62[a]	95.52
Cercobin	30.96[c]	1.47[b]	2.77[a]	106.72
Control	58.14[d]	2.88[c]	1.34[b]	-

* Plot average for two assessments and six replications.

1. LSD at 5 per cent = 3.65
   LSD at 1 per cent = 4.98
   LSD at 0.1 per cent = 6.74
2. LSD at 5 per cent = 0.40
   LSD at 1 per cent = 0.54
   LSD at 0.1 per cent = 0.74

For each parameter, figures followed by the same letter are not significantly different at 5% level of significance.

Plate 23. Phytotoxicity effect of folicur on karela plants.

Plate 24: Karela plant in the field sprayed with folicur.

Plate 25: Karela plant in the field sprayed with bayleton.

Plate 26: Unsprayed control karela plant in the field infected with powdery mildew.

FIGURE: 4

REGRESSION OF YIELD OF KARELA AGAINST MEAN POWDERY MILDEW SEVERITY

(y=3.20 - 0.62X)

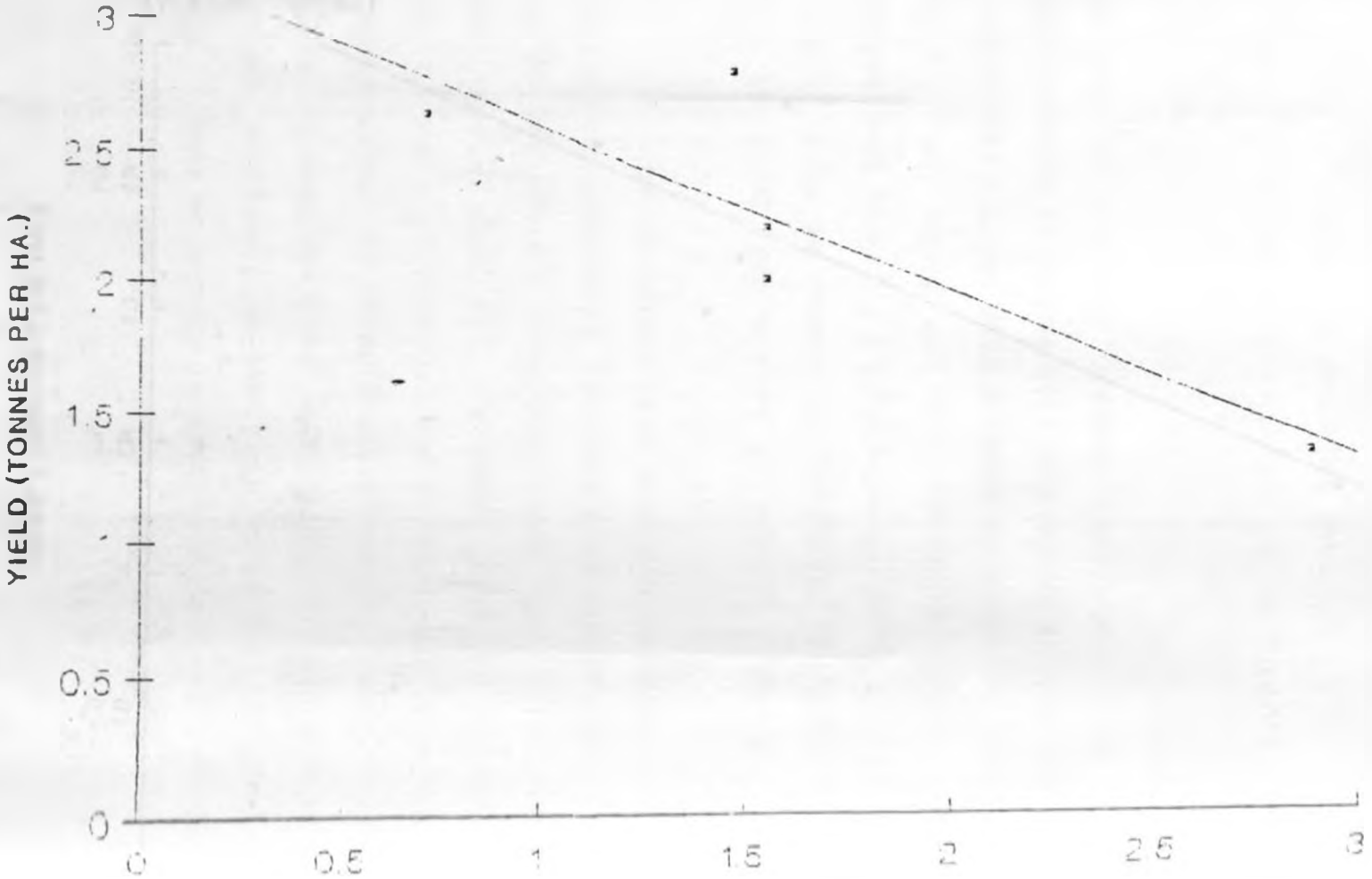
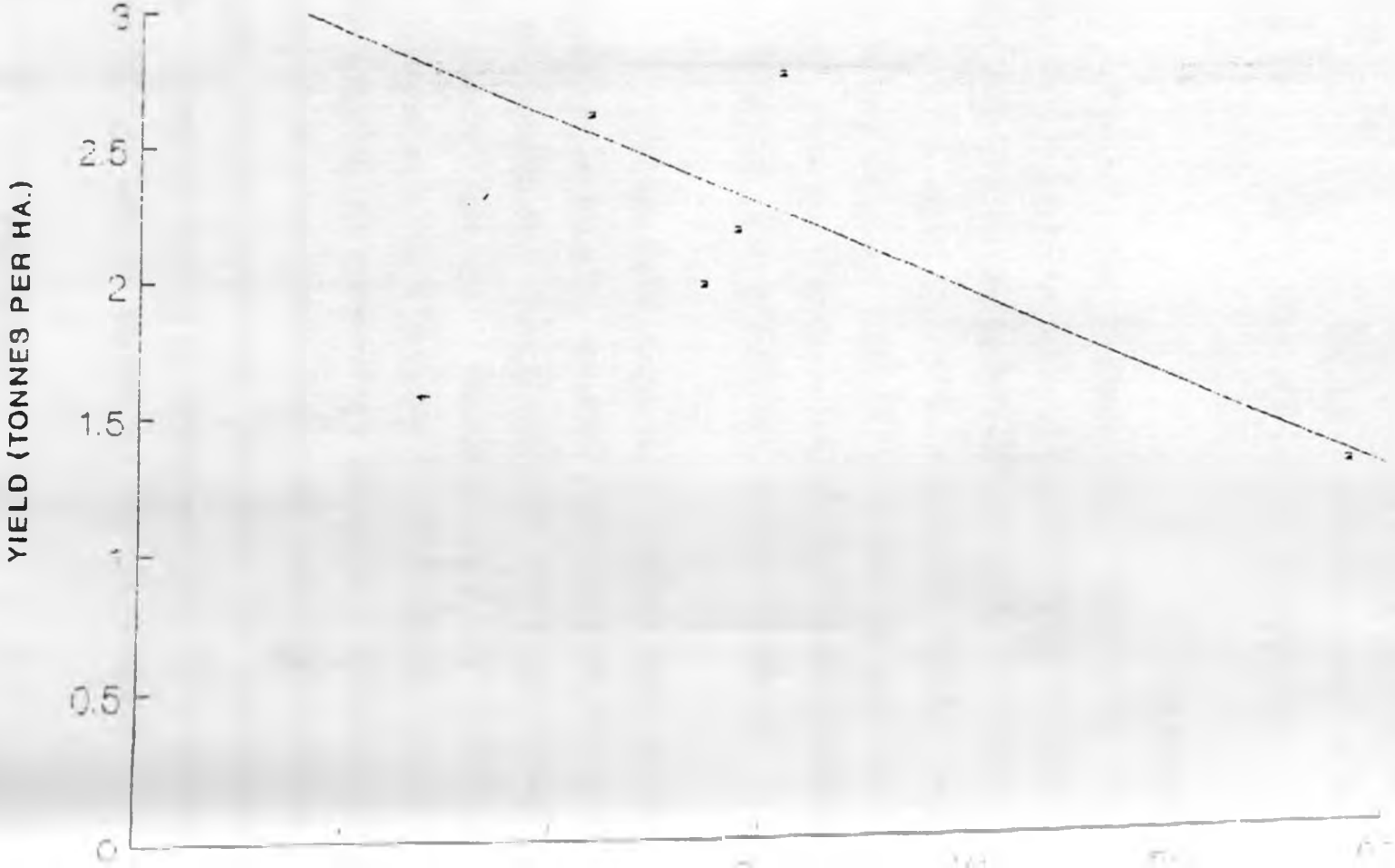


FIGURE: 5

REGRESSION OF YIELD OF KARELA AGAINST MEAN POWDERY MILDEW INCIDENCE

(y=3.26 - 0.03x)



5 DISCUSSION

Powdery mildew of karela is one of the major limiting factors in the production of karela in Kenya. Epidemics are prevalent in warm and semi-arid regions particularly in Eastern and coast provinces where the crop is cultivated under irrigation. Although the disease is economically important, little information is available on it's nature, etiology and control in this country. In this study, the pathogen identification, symptomatology, histopathology and chemical control aspects were studied. Environmental factors favouring spore germination were also investigated.

Powdery mildew of karela was found to be caused by Sphaerotheca fuliginea (schlecht ex Fr.) Poll. This was confirmed by two observations. Firstly, the cleistothecium of the fungus contained only one ascus and therefore it was placed in the genus Sphaerotheca and not Erysiphe. Secondly, karela plants inoculated with spores of Erysiphe cichoracearum Dc. ex Mecat. from pumpkin did not develop the disease symptoms.

Conidia of the fungus were larger in size measuring 24-42 µm x 13-20 µm than reported by Walker (1952), and Spencer (1978). They reported the size to be 4-5 x 5-7 µm.

However the size corresponded with that reported by Kranz, Schmitterer and Koch (1977) and Dixon (1981). They reported the size of conidia of powdery mildew of cucurbits fungi to be 25-37 x 14-25 μm. Cleistothecia of Sphaerothecia fuliginea were found to be common in the infected plants observed. They were found on dry infected karela leaves collected from the field and from the glasshouse.

Symptoms and signs of powdery mildew of karela were as reported by other researchers but working on different host plants (Spencer, 1978, Walker, 1952; Dixon, 1981). The symptoms appeared after one week as tiny, white, round, flavy, superficial spots on leaves, stems and fruits which became powdery in consistency as they enlarged. The lesions increased in number and coalesced to cover the plant surfaces. The young tender leaves were not infected. This indicated a kind of resistance by these leaves. Chupp (1960), reported that these young leaves are almost entirely immune to powdery mildew infection.

Conidia germinated after 24 hours producing one or two germtubes. Appressoria were formed after 30 hours. After germination the mother spore remained a living part of the thallus and did not collapse after the fungus had established nutritive relations with the host. The fungus colonised the epidermal cells through haustoria one in each cell. Branching

of hyphae was found to be acute and regular. Conidiophores formed on the superficial mycelium after four days as was also reported by Spencer (1978). Conidia were produced by a conidiogenous cell that elongated and divided into two cells where the proximal cell developed into a barrel shaped conidium. Conidia ripened from the apex of the conidiophore downwards. They were released either singly or in a group.

Spore germination was influenced by relative humidity and temperature. The spores germinated within 55-100% relative humidity range. At 55% relative humidity, percentage spore germination was 4.05% which rose to 50.4% at 100%. Chupp (1960), reported the minimum relative humidity for spore germination to be 46%. Spencer (1978), reported 55% while Dixon (1981), reported the minimum relative humidity to be as low as 20%.

The spores germinated within a temperature range of 20-30°C. At 20°C, percentage spore germination was 18.99% and at 30°C it was 31.06%. These results are similar to what has been reported by other workers. Dixon (1981), found the optimum temperature to be 28°C with a range of 22-31°C. Roberts (1984), reported the optimum temperature for spore germination to be 25-30°C. It was evident that even at the optimum temperature and humidity the fungus produced a large proportion of non-viable spores because a leaf thoroughly

dusted with spores developed isolated colonies. These were probably the immature spores and over-mature spores which had lost viability.

All the fungicides tested with the exception of daconil controlled powdery mildew of karela. In the preliminary tests bayleton and folicur were found to have better control of the disease than the other fungicides. However, bayleton was not significantly different from cercobin. Baycor was inferior to the other fungicides. In the field folicur was superior to the other fungicides. Bayleton, bayfidan and cercobin showed no significant differences between them. Folicur showed signs of phytotoxicity on plants by causing leaf curling and puckering after the first spray but the plants recovered the effect even after subsequent sprays. Bayleton did not show any signs of phytotoxicity as reported by Chweya and Buruchara (1989).

The intensity of powdery mildew on karela plants had no significant influence on yield. Plots sprayed with folicur, bayleton, bayfidan and cercobin had no significant differences in their yield despite significant differences in disease incidence and severity between these plots. Furthermore plots sprayed with bayleton, bayfidan and unsprayed control plots had no significant differences in their yield. However, unsprayed control plots had significantly lower yield than the

plots sprayed with folicur and cercobin. The influence of the disease on yield is probably due to poor foliar growth caused by premature drying of infected leaves other than direct effect of the disease on fruit production. Dixon (1981), also reported that the main effect of the powdery mildew is to reduce fruit quality rather than weight or number of fruits.

6 CONCLUSIONS.

Powdery mildew of karela (Momordica charantia L.) is caused by Sphaerotheca fuliginea (Schlecht ex Fr.) Poll. The fungus grows superficially on plant surfaces and colonises only the epidermal cells. The spores germinate by producing one or two germtubes and it occurs within a narrow range of temperature and relative humidity. The optimum temperature for spore germination is 25-30°C and relative humidity of 100%. It is therefore favoured by warm and humid weather. The spore germtubes grow and terminate into globose appressoria from which a penetration peg pierces the plant's waxy layer and cuticle and enters into the epidermal cells. Haustoria are formed one in each cell and through them the fungus derives nutrients from the host plant.

Asexual spores are conidia. Conidiophores are formed on the superficial mycelium, 4 days after inoculation. Conidia are released by abstriction or by rupture of an intermediate sterile cell. They are barrel shaped and measure 24-42μm x 13-20μm in size. The fungus produces the spores abundantly such that a mildewed plant at a glance appears white and powdery. Sexual reproduction also occurs. The fungus produces spherical cleistothecia from which ascospores are released.

The disease has an incubation period of 5-7 days provided environmental conditions are favourable for spore germination and disease development. Initial symptoms are round, white colonies which enlarge and merge to cover the plant surfaces. Severely infected leaves become chlorotic and premature drying and defoliation occurs. The old leaves are more susceptible than the young leaves.

Powdery mildew of karela can be controlled effectively by application of tebuconazole (BAY HWG 1608) which is sold under the trade name folicur. Although plants sprayed with this fungicide show signs of phytotoxicity, they tend to recover from this effect and the crop yield is not significantly reduced. Triadimefon (bayleton), triadimenol (bayfidan) and thiophanate methyl (Cercobin) are also effective against the powdery mildew. They are not phytotoxic to karela plants but not as effective as folicur. Chlorothalonil (daconil) and bitertanol (baycor) do not effectively control the disease.

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APPENDICES

Appendix 1.

Percentage spore germination at different relative humidities after 24 hours. Analysis of Variance.

Source	df	SS	MSS	F
Treatments	5	18320.36	3664.07	190.14***
Error	48	925.05	19.27	
Total	53.	19245.41		

Appendix 2.

Percentage spore germination at different temperatures after 24 hours.

Analysis of variance.

Source	df	SS	MSS	F
Treatments	7	13235.19	1890.74	131.21***
Error	72	1037.76	14.41	
Total	79	14272.95		

Appendix 3.

Effect of different fungicides at different concentrations on control of powdery mildew of karela in the laboratory: Disease Severity. Analysis of variance.

Source	df	SS	MSS	F
Blocks	3	0.60	0.2	1.11
Treatments	47	140.05	-	-
F	5	39.08	7.82	36.66***
C	7	93.51	13.36	74.09***
FC	35	7.46	0.21	1.18
Error	141	25.43	0.18	
Total	191	166.08		

F = Fungicides C= Concentrations

Appendix 4.

Effect of different fungicides, their method of application and mode of activity on control of powdery mildew of karela : Disease severity.

Analysis of variance.

Source	df	SS	MSS	F
Treatments	23	16.52	-	-
F	5	4.73	0.95	8.71***
M	1	7.16	7.16	65.90***
A	1	1.25	1.25	11.54**
FM	5	0.58	0.12	1.06
FA	5	0.68	0.14	1.25
MA	1	1.90	1.90	17.51***
FMA	5	0.22	0.04	0.40
Error	48	5.21	0.11	
Total	71	21.73		

F= Fungicides

M = Method of application of fungicides

A = Mode of activity of fungicides

Appendix 5.1

Effect of fungicides at three concentrations on control of powdery mildew of karela: Percentage disease incidence.

Analysis of variance.

Source	df	SS	MSS	F
Blocks	3	141.46	47.15	0.73
Treatments	19	10649.14	-	-
F	4	1941.68	485.42	7.53***
C	3	7360.88	2453.63	38.07***
FC	12	1346.58	112.22	1.74
Error	57	3673.89	64.45	
Total	79	14464.48	-	-

F = Fungicides

C = Concentrations

Appendix 5.2

Effect of fungicides at three concentrations on control of powdery mildew of karela : Disease severity.

Analysis of variance.

Source	df	SS	MSS	F
Blocks	3	0.38	0.13	0.84
Treatments	19	43.53	-	-
F	4	6.69	1.67	11.10***
C	3	31.38	10.46	69.40***
FC	12	5.46	0.45	3.02***
Error	57	8.59	0.15	-
Total	79	52.50		

F = Fungicides

C = Concentrations

Appendix 6.1

Effect of fungicides on control of powdery mildew of karela in the field: Percentage disease incidence.

Analysis of variance.

Source	df	SS	MSS	F
Blocks	5	353.25	70.65	7.68
Treatments	4	4872.73	1218.18	132.35***
Error	20	184.09	9.20	-
Total	29	5410.06	-	-

Appendix 6.2

Effect of fungicides on control of powdery mildew of karela in the field: Disease severity.

Analysis of variance.

Source	df	SS	MSS	F
Blocks	5	0.27	0.05	0.49
Treatments	4	14.79	3.70	33.73***
Error	20	2.19	0.11	_
Total	29	17.24	-	-

Appendix 6.3

Yield (g) of karela plots sprayed with different fungicides. Analysis of variance.

Source	df	SS	MSS	F
Blocks	5	4795574.20	959114.84	1.54
Treatments	4	7227170.03	1806792.21	2.90*
Error	20	12450029.97	622501.50	-
Total	29	24472774.20	-	-