DOWNY MILDEW OF ISABGUL (PLANTAGO OVATA FORSK)

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ABSTRACT

DOWNY MILDEW OF ISABGUL (*PLANTAGO OVATA* FORSK)

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Isabgul (*Plantago ovata* Forsk) is an important medicinal crop of high export potential for our country. The crop is attacked mainly by damping-off, wilt, downy mildew, leaf blight and powdery mildew. Among the diseases, downy mildew (*Peronospora plantaginis*) is a serious one causing considerable damage to the crop.

Following aspects of the disease were studied: (i) histopathology and morphology of the fungus, (ii) meteorological parameters in development of disease, (iii) phytochemical changes brought by disease and (iv) management of disease through cultural, chemical and resistance of Isabgul cultures/varieties to downy mildew fungus.

The results revealed that the fungus penetrates through stomata which grows intercellularly in host tissues.

Remification by the fungus lead to tissue necrosis and death of plant. The sporangia are borne on sterigmata of the dichotomously branched sporangiophore. The sporangia germinate by germ tube and not by zoospores. The hypha is 6.25 to 12.50 μ thick, sterigmata are 4.25 - 12.50 x 4.0 - 4.20 μ and sporangia are 32.00 - 44.00 x 17.00 - 25.00μ.

Effect of different temperatures (5 to 40°C with an increment of 5°C) studied in *vitro* revealed that 20°C is optimum for sporangial germination. Under field conditions, the temperature around 20°C and relative humidity around 89 per cent favour maximum sporangial germination between 2 a.m. and 8 a.m. and 8 p.m. and 10 p.m. These parameters during crop growth period help in development of severity of disease. Maximum disease under these conditions are observed between 75 and 95 days after sowing. The regression correlation for sporangial germination is negative with temperature and positive with relative humidity.

Biochemical studies of leaves collected from healthy and diseased plants revealed reduction in chlorophyll content by 57.71 and phenolics by 52.01 per cent due to infection by *P. plantaginis*.

Moisture and total ash contents are also reduced in leaves of diseased plants. Total nitrogen and reducing sugars showed positive correlation with disease development.

Chemical analysis of seeds collected from healthy and diseased plants indicates that the amount of nitrogen, number of black seeds and swelling factor of seed may be considered as parameters in determining the quality of seeds.

Effect of different dates of sowing coupled with various seed rates indicates that the seed rate more than 2.5 kg/ha sown in November affects the yield adversely due to severe disease development. While the crop sown with seed rate higher than 2.5 kg/ha in December increases the yield and with lower seed rate there is yield reduction due to poor plant population and growth of the crop.

The field experiments on chemical control revealed that seed treatment with Apron 35 SD 5 g/kg seeds coupled with three sprayings of Mancozeb 0.2 per cent or use of Dehusked seeds coupled with three sprayings of Metalaxyl 0.05 per cent or seed treatment with Captafol 2.5 g/kg seeds either coupled with three sprayings of Captafol 0.2 per cent or Metalaxyl 0.05 per cent could effectively control the downy mildew disease giving higher yields. All sprayings are to be applied thrice at the interval of 15 days, the first one to be applied just after the first appearance of disease.

The reaction of Isabgul varieties/cultures viz., EC 42706-1, EC 42706-2/Pl, EC 124345, Gujarat Isabgul-1, Gujarat Isabgul-2 (TS 6), Male fertile, Progeny-3 and Progeny-13 to *P. plantaginis* was positive in varied proportions. The culture EC 124345 is significantly more tolerant and significantly yields more. The culture progeny-13 is highly susceptible. The rest of the varieties/ cultures are intermediate.

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CERTIFICATE

This is to certify that the thesis entitled, "Downy mildew of Isabgul (*Plantago ovata* Forsk)" submitted by Shri Jivanbhai G. Patel in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Plant Pathology of the Gujarat Agricultural University is a record of bonafide research work carried out by him under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma or other similar title.

Anand
June 30, 1984

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C O N T E N T S

I N T R O D U C T I O N

INTRODUCTION

Amongst all the medicinal plants, Flea seed or Isabgul (*Plantago ovata* Forsk) is a crop having high export potentiality for our country. Isabgul is a plant of West Asian origin introduced in India during the Moghul period. Its name derives from two Persion words 'Isap' and 'Ghol' meaning a horse-ear, referring to its characteristic of boat-shaped seeds (Anonymous, 1976).

In the whole world, Isabgul is traditionally grown only in Mehsana and Banaskantha districts of Gujarat State as a cash crop. Now it is cultivated in Saurashtra and Kutch regions of Gujarat, southern region of Rajasthan and certain parts of Maharashtra also. In Gujarat, it was cultivated in about 25,000 hectares producing about 14,000 tonnes of seed during 1982-83.

Uses of *Plantago* species as sources of pharmaceutical drugs are reported by Shyren (1935). The husk from the seed is separated out by physical process and is used as bulk laxative. Seeds after removal of husk are by-products of Isabgul industry and are used as ice-cream stabilizer (Patel *et al.*, 1978). The seeds have also cooling and demulcent effect being used in Ayurvedic and Allopathic medicines. Seeds without husk are rich in protein and used as cattle feed. The seed and husk are used to cure inflammation of the mucous membrane of gastrointestinal and genito-urinary tracts, duodenal ulcer, gonorrhoea and piles. It can also be used as cervical dilator for termination of pregnancy (Anonymous, 1979).

About 75-85 per cent of total annual produce of North Gujarat is exported which brings crores of rupees as foreign exchange annually. India continues to hold a monopoly in its production and trade in the world (Bhagat et al., 1980). In view of its sustained demand, there is a scope to increase the production per unit area. Therefore, if India is to retain its monopoly in production of this important foreign exchange earning commodity, there is an urgent need to intensify the researches on all aspects of crop production in general and particularly on diseases and pests of this crop which have remained almost unexplored.

The crop is attacked by various fungi inciting diseases commonly known as damping-off of seedlings, wilt, downy mildew and powdery mildew. Among these, downy mildew is a dreadful disease causing enormous quantitative as well as qualitative loss and many times it becomes limiting factor in successful cultivation of this crop (Desai and Desai, 1969). Under such situations, it is very necessary to study behaviour of the pathogen and workout suitable control measures by chemicals, cultural methods and evolving resistant varieties.

As the diseases of Isabgul have not been studied at length, very little information is available about various diseases of the crop. In recent past, it has been observed that downy mildew (Peronospora plantaginis)* is attaining alarming proportion in chief Isabgul growing areas of Gujarat and causing enormous economic losses to the farmers. (Plate, 1 - A, B, C). Keeping this in view, attempts have been made to study various aspects of the disease and pathogen in detail viz. histopathology of the disease, biochemical changes in diseased plant, meteorological parameters favouring the disease, screening of various Isabgul cultures for resistance to the disease, the role of cultural practices and the use of effective chemicals in managing this serious disease.

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* The name of species used here is near identical to the species described by Underwood as per letter No. H391/81/Y12, 1983 from Francis S.M., Commonwealth Mycological Institute, Kew, Surrey, England.

P L A T E, 1

DOWNY MILDEW DISEASE IN ISABGUL FIELD:

(A) DISEASED PLANTS (PATCH) - BEFORE FLOWERING

(B) DISEASED PLANTS (PATCH) - AFTER FLOWERING

(C) HEALTHY PLANTS (PATCH)

A

B

C

REVIEW OF LITERATURE

REVIEW OF LITERATURE

2.1 Introductory

Because of less economic importance of the diseases of Isabgul much attention has not been given to study in depth. Obviously, very little literature is available. Furthermore, the crop is grown in only few places, the diseases have not been observed in an alarming proportions which otherwise would have drawn the attention of the scientists. However, an attempt has been made to review the available literature on several diseases of Isabgul in general and particularly on various aspects of downy mildew.

Most important diseases reported to affect economic cultivation of Isabgul are *Fusarium oxysporum* Schlecht emend. Synd & Hans. (Fusarium wilt); *Pythium ultimum* Trow. (Damping-off of seedlings); *Alternaria alternata*(FR.) Keissler (Alternaria leaf blight); *Peronospora plantaginis*, Underwood (Downy mildew) and *Erysiphe cichoracearum* D.C. (Powdery mildew).

Russell (1975) reported *Fusarium oxysporum* as pathogen of the mucilage plant, *Plantago ovata*. According to him, symptoms may be a pre-emergence damping-off or a rapid wilting of 120-150 days old plants, discolouration of the tap root (commencing at the tip) accompanied by the presence of large quantities of hyphae in xylem elements, vascular discolouration and cortical root rot.

Pythium ultimum . was isolated from the decayed roots of 14 days old damped-off *Plantago ovata* seedlings (Chastanger *et al*,1978). Seeds of Isabgul when sown in soil artificially inoculated with *P. ultimum* resulted in pre and post-emergence damping-off of young seedlings. Pre-treatment of seeds with CGA-48988 provided an effective measure to control this disease.

Patel *et al*.(1982) observed *Alternaria alternata* as the incitant for leaf blight disease of *Plantago ovata* caused dull green-yellowish colour at the tip of the leaves. The older and matured leaves get severly attacked and more than 50 per cent leaf area is covered by irregular necrotic zones increasing rapidly and coalescing under warm and humid climatic conditions. In advance stage, blackening of leaves followed by burning appearance and ultimately resulting into drying of leaves are important symptoms. Under severe conditions of the disease, shrivelling and blackening of seeds are also noticed. The next important disease of the crop is powdery mildew which has been found to occur occassionally (Kumarwat, 1979).

The downy mildew disease of *Plantago ovata* is incited by two different species of *Peronospora*. Desai and

Desai (1969) reported that the Peronospora plantaginis develops chlorotic areas on the upper surface of leaves and ashy-white-frost like mycelial growth on the lower surface. Later on infected leaves become chlorotic and brownish in colour followed by curling, crinkling and drying of leaves which result in poor growth of the plant and flowering.

However, Kapoor and Chowdhary (1976) observed that the Peronospora alta Fuckel is the incitant of the downy mildew disease of Isabgul. The symptoms are initiated as chlorotic streakes extending along the midribs of the leaves. Profound downy growth was observed on the lower leaf surface. Ultimately the whole leaf turns necrotic and in severe cases, the entire plant gave blighted appearance. The abundant oospore formation caused the thickening of affected leaves.

2.2 Studies on histopathology and morphology

The group of downy mildew fungi is obligate parasite, typified by invasion of host tissues intercellularly and limited intrusion into the host cells by haustoria. Fraymouth (1956) observed mycelium of Peronospora alta intercellular and haustoria hyphal infrequent. Hickman and Ho (1966) demonstrated that the stomata provide stimulus for penetration and according to them, the stomatal penetration is fairly common in the downy mildews and the greater development of sporangiophores on the lower surface of leaves may be due to large number of stomata in many host species. Francis (1981) reported conidiophores 290-450 x 8 μm; trunk 91-268 μm; branching obscurely dichotomous 4-6 times; branch ends 8-20 x 2-3 μm, slender, tapering and curved, angle usually less than right angle, conidia 26-32 x 18-24 μm, ellipsoid, pale brown -violet. Thind in 1942 measured the conidia of P. plantaginis causing disease on Plantago complexicaulis with 40-44 x 16-18 μm size.

However, literature revealed no information on histopathological aspects of Peronospora plantaginis causing disease on Plantago ovata.

2.3 Studies on meteorological parameters

The epidemiological investigations carried out by Viranyi (1975) indicated that dynamics of downy mildew infection in onion mainly depend on frequency and length of time of humid conditions in the field.

In addition, these investigations have shown that other factors like free water on the plant surface and air temperature are also responsible for the fungus development.

Palti (1975) recorded that abundant sporulation in downy mildew of cucurbits (Pseudoperonospora cubensis) on plant rich in photosynthetes, on greenish-yellow (not necrotic) lesions, in temperature range of 5-30°C (Optimum 15°C), if leaves wet for atleast 6 hours. Dispersal, mostly by wind, favoured by moderate atmospheric humidities, but sporangia rapidly lose viability at low humidity or high temperatures. Exconde (1970) concluded that rainfall and relative humidity had a significant effect on the degree of infection in downy mildew of maize.

Waite (1971) observed that 11.7 per cent of the conidia of P. trifoliorum germinated at 5°C, decreasing to 0 per cent at 30°C, while light did not decrease germination but inhibited germ tube growth.

Dalmacio and Raymundo (1972) indicated that Sclerospora philippinensis does not sporulate every night. The presence of thin film of moisture over the surfaces of infected leaves was determining factor in spore production but sporulation was always observed when RH was 90 per cent or over.

Bashi and Aylor (1983) concluded that temperature, RH and sunlight were the most important factors in determining sporangial survivability of P. destructor and P. tabacina.

Palti and Rotem (1973) suggested that classical forecasting techniques in temperate zones are based on the determination of environmental factors that promote disease development and on the quantitative expression of these factors. They have also summarized that agricultural practices such as irrigation and year round cultivation of crops render semi-arid areas more suitable for development of downy mildews and late blight than might be supposed by weather criteria alone. Further, the complex interrelations to be attributed to factor affecting disease development make their regional forecasting impracticable. This is due to the absence of clearcut "Zerotime" for the beginning of the disease development, the limited significance of temperature, the unpredictable occurrence of hot and dry spells, intense sub-tropical rainfall and the dominance microclimatic factors. On the otherhand, timing of control operations can be aided by regional determination of disease free periods in crops sown in different seasons and by prediction of the length of such periods in individual fields according to their age and density, irrigation regime and proximity to sources of inoculum. It is very well recognized in plant pathology that the use of chemotherapy, either alone or in combination with other control methods in absence of the information on disease epidemiology, is not going to help the farmers especially to get successful control of the disease.

2.4 Studies on phytochemical changes

The rate of respiration in downy mildew infected leaves of bajra increased by 149.5 per cent than that in the healthy ones (Mandahar and Grag, 1974).

According to Jain and Arya (1978), healthy leaves of bajra contained almost double quantity of ascorbic acid than the infected ones with downy mildew.

The chemical analysis of diseased and healthy leaves of Isabgul on 70th and 90th days from sowing evinced more quantity of phenolics in healthy leaves than diseased one; (Shah *et al*., 1981). It was around 4 and more than 3 per cent on 70th and 90th day respectively in healthy leaves as compared to around 3 and less than 2 per cent on 70th and 90th day respectively in diseased leaves.

Thanki and Talati (1983) concluded that the husk obtained from black seeds of Isabgul is of poor quality as compared to healthy one, eventhough black seeds revealed higher swelling factor. However, they have not given any reason for blackening of seeds.

2.5 Studies on disease management

Chemical

It is universally acknowledged that after breeding for disease resistance, chemical control of plant disease is the best and most satisfactory method of plant disease management (Anonymous, 1983). Palti and Cohen (1980) indicated that success of control by protectant chemical depends largely on proper timing of applications, moreover, rate of leaf formation and favourable factors determine the frequency of applications. According to Desai and Desai (1969), application of aureofungin can control downy mildew disease of Isabgul.

With the recent development of systemic fungicides, the use of these compounds applied as seed, soil or foliar treatments may be the plausible means of combating downy mildew disease. The development of systemic fungicides is an important mile-stone in this field particularly for a developing country like India where farmers cannot afford frequent applications of conventional fungicides. A systemic fungicide has the advantage that it is able to enter the plant system and is retained by the host for a longer period and remains unaffected by the external adverse weather conditions especially the rains. Therefore, such groups of chemical compounds made its appearance on the scene (Anonymous, 1983).

The compound currently known as Metalaxyl was known in past as CGA-48988, Apron 35 SD or Ridomil has been found effective against downy mildews of tobacco, onion, spinach, rose, cucurbits, brassica seedlings and lettuce crops

(Johnson *et al*, 1979; Teviotdale *et al*; 1980; Jones and Dainello, 1983; Folk, 1983; Palti and Cohen, 1980 and Hartill, 1982).

Cultural

Dense seed-bed stands of onion, tobacco or tomato are more likely to be attacked by downy mildew fungi than that in spaced out seed beds. This is more so when an old crop is in the vicinity. Plants spaced out in the field can be predicted to remain free of disease at least as long as dense stands in the same neighbourhood are not attacked (Palti and Rotem, 1973). The research work carried out under AICRP (M&AP) suggested that the seed rate of Isabgul should not be more than 4 kg/ha as the seed rate higher than that tends to the disease incidence (Anonymous, 1980).

Resistance

Spurling (1973) stated that in Malawi, local cultivars of pumpkin and cucumber are rarely affected by *P. cubensis* but introduced cultivars are sometimes destroyed completely. Simanca *et al*.(1977) tested 360 cultivars and lines of cucumbers in Cuba under fungicidal protection, natural infection and artificial inoculation. Only five of them exhibited high resistance to *P. cubensis*. Thirteen other cultivars possessed moderate resistance.

Nagarajan *et al*.(1970) tested 3961 entries of sorghum against downy mildew. Out of these 48.2 per cent were found resistant, 17.7 per cent intermediate and 34.1 per cent susceptible.

Only recently, the work on reaction of different varieties of Isabgul to downy mildew has been undertaken. Kapoor and Chowdhary (1976) have reported briefly the reaction of varieties and crosses of Isabgul to downy mildew and have observed severity of disease almost on all varieties and crosses of this crop.

MATERIALS AND METHODS

MATERIALS AND METHODS

3.1 Introduction

The studies on various aspects of downy mildew disease of *Plantago ovata* were undertaken.

(i) Histopathology and morphology of *Peronospora plantaginis*

The studies on mode of penetration, infection and relation with host cells; measurements of hyphae, sporangiophores, sporangia and sterigmata were undertaken.

(ii) The effects of temperature and humidity on sporangial germination and their overall impact on disease incidence were studied in laboratory and field.

(iii) Variation in moisture, ash, nitrogen, chlorophylls and phenolics percentages in leaves and seeds; moisture, nitrogen, swelling factor and percentage of black seeds from healthy and diseased plants were studied.

(iv) The efforts were also made to manage the disease by (a) use of fungicides, (b) cultural practices and (c) search of resistant germplasm.

3.2 Histopathology

3.2.1 Host parasite relationship

To carry out the histopathological studies diseased leaves of the variety 'Gujarat Isabgul-1' were collected from the field. Leaves were then cut into small pieces after collection and were subjected to process for microtome sections as described by Sass (1964). The microtome sections in ribbons were examined.

3.2.2 Morphology

With the help of camera lucida the pictures of sporangiophore, sterigmata, sporangia and germinating sporangia were depicted. After staining hypha, sterigmata and sporangia were measured with the help of stage micrometer. Such one hundred measurements for each were taken.

3.3 Meteorological parameters

3.3.1 The influence of temperature on sporangial germination

Fresh sporangia were collected from the diseased leaves in the early morning to study the effect of temperature on sporangial germination. The suspension was prepared in tap water and kept at 0°C, 5°C, 10°C, 15°C, 20°C, 25°C, 30°C, 35°C and 40°C and the percentage germination was worked out by observing a set of six slides prepared from each temperature, replicated twelve times.

3.3.2 Role of temperature and humidity on germination of sporangia under field conditions

During the peak period of disease, 80 days after sowing, at every 2 hrs. of interval the disease samples were collected from the field from 6.0 a.m. onward on three consequ tive days and the presence of germinating sporangia on Isabgul leaf tissues were checked under microscope. Field temperature and Relative humidity were recorded at the time of collection of samples.

3.3.3 Role of temperature and humidity on disease initiation and development of the disease

In maize brown stripe downy mildew, once the primary infection becomes established, then its spread is possible through sporangia, although, their viability is of short duration (Singh, 1969). Keeping this in view, one hundred plants were tagged randomly in the field of Gujarat Isabgul-1 to determine the role of initial inoculum on diseased plants and subsequently on further development and severity of the disease. The plants so tagged were graded for disease index 0-4 regularly at 15 days interval, beginning from the first appearance of disease till the time of harvest. Furthermore, the observations regarding temperature and humidity were also recorded in term of meteorological weeks. The yield data were also recorded individually for all the hundred tagged plants. The disease severity was graded by using the following scale (Plate 2A, B).

Disease ratings	Percentage of necrotic leaf area of a plant
0	Completely healthy (No infection)
1	Trace to 25
2	26 to 50
3	51 to 75
4	More than 75

Disease index was worked out according to the following formula.

$$\text{Disease index} = \frac{\sum (\text{Class value x Frequency})}{\text{Total no. of plants observed}}$$

3.4 Phytochemical changes

Gujarat Isabgul-1 variety was grown on college farm during the year 1982-83 as a general crop. Few days before harvest several healthy and diseased plants were marked for the study. Leaves and seeds from five healthy and five diseased plants were cleaned and collected separately in polythene bags and weighed to determine the fresh weight (Plate 3A, B). Care was taken to avoid loss of moisture till the time of determination of weights. The leaves and seeds so weighed were then transferred to an oven and dried at 65°C till constant weight of each was obtained.

PLATE, 2

(A) INTENSITY OF DOWNY MILDEW DISEASE

0 - COMPLETELY HEALTHY (NO INFECTION)

1 - TRACE TO 25 PER CENT NECROTIC LEAF AREAS/PLANT

2 - 26 TO 50 PER CENT NECROTIC LEAF AREAS/PLANT

3 - 51 TO 75 PER CENT NECROTIC LEAF AREAS/PLANT

4 - MORE THAN 75 PER CENT NECROTIC LEAF AREAS/PLANT

(B) INTENSITY OF DOWNY MILDEW DISEASE ON ISABGUL LEAVES.

A

B

PLATE, 3

(A) LEFT - DISEASED PLANTS

RIGHT - HEALTHY PLANTS

(B) LEFT - SEEDS FROM DISEASED PLANTS

RIGHT - SEEDS FROM HEALTHY PLANTS

A

B

The differences between fresh and dry weights were taken as the moisture content. The moisture content thus obtained has been expressed as per cent moisture on fresh weight basis. Further, per cent content of ash, nitrogen, chlorophylls, phenolics and reducing sugars of leaf; per cent nitrogen content of seed, swelling factors of seed and per cent of black seed were determined. The method followed for each component is briefly given below.

3.4.1 Ash

The oven dried 1 g. samples of leaves collected from healthy and diseased plants in previously weighed silica basin were digested separately in a muffle furnace at about 500-550°C for about an hour. It was then cooled in a desiccator and weighed until weighed constant.

3.4.2 Nitrogen

Nitrogen was estimated in leaf and seed samples collected from healthy and diseased plants by using standard Kjeldahl method. The procedure is given below.

Procedure: 200 mg of sample was digested with 5 ml of concentrated sulphuric acid in presence of catalyst(mixture of potassium sulphate and copper sulphate). The solution obtained was transferred quantitatively in distillating assembly and 20-30 ml 40 per cent sodium hydroxide solution was added. The liberated ammonia was collected in saturated 20-25 ml of 2 per cent boric acid solution and titrated with 0.02 N standard NaOH solution using bromocresol green and methyl red indicator (mix indicator). The end point was blue to colourless and the burette reading was recorded. The percentages of nitrogen were calculated by using the following formula.

$$\text{Per cent Nitrogen} = \frac{X \times N \times 0.014 \times 100}{W}$$

X = Burette reading

N = Normality of NaOH

W = Wt. of sample.

3.4.3 Total chlorophylls

Chlorophylls were extracted and estimated from 1 g dry powder of healthy and diseased leaves in acetone using 'Omega' homogenizer as per the procedure of AOAC (1980). After filteration, filterate was made to 100 ml with acetone and 10 ml of filterate was extracted with ether thrice (15 ml each time) using separating funnel. Ether extract was further washed with distilled water and the volume was made to 50 ml with solvent ether. The optical density of the solution was read at 640 nm and 660 nm and total chlorophylls (mg/l) were determined using the following formula. The values were converted into per cent chlorophylls.

Total chlorophylls = (7.2 + OD at 660 nm) + (16.8 + OD at 640 nm)

3.4.4 Total phenolics

Total phenolic compounds from healthy and diseased leaves of *P. ovata* were extracted from 100 mg dry powder of each with 20 ml 80 per cent V/V chilled ethanol using 'Omega' homogenizer. The extract was centrifuged at 5000 RPM for 15 minutes and process was repeated twiceusing 10 ml 80 per cent V/V ethanol each time. Successive extracts were combined and volume was made to 50 ml with 80 per cent (V/V) ethanol. Phenolics were estimated by Folin method of Swain and Hillis (1959). A suitable aliquot (1 ml) of the ethanolic extract was diluted with distilled water to 8 ml; then 0.5 ml of Folin phenol reagent was added and the contents were well mixed. After 3 minutes, 1 ml saturated sodium bicarbonate solution was added and the final volume was made upto 10 ml with distilled water. The tube was thoroughly shaken and the colour development was measured at 700 nm after standing for 60 minutes. A standard curve was prepared using chlorogenic acid and all the concentrations were expressed in terms of percentage of this compound.

3.4.5 Determination of reducing sugar using Somogyi's copper reagent method

Exactly 0.5 gm sample of powdered dry leaves(collected from diseased and healthy plants) was weighed in 100 ml conical flask. After adding 50 ml distilled water and 0.5 gr. calcium carbonate, the mixture was boiled for 15 minutes with a funnel in the neck of the flask. Then it was cooled at room temperature and 8 ml of neutral saturated lead acetate solution was added. After 15 minutes, sufficient quantity of anhydrous disodium phosphate(powder) was added to precipitate excess lead. The solution was mixed thoroughly, transferred quantitatively in 100 ml volumetric flask and diluted exactly to 100 ml with distilled water.

After allowing the precipitates to settle down the supernatant solution was filtered through What'man No. 42 filter paper. Avoiding first few drops, the filterate was tested for the absence of lead with disodium phosphate.

Twenty five ml of filterate was placed in 100 ml conical flask and 10 ml of copper iodometric reagent 50 was added to it. The solution was boiled for exactly 15 minutes. Then it was cooled (36°C) for 5 minutes under running water. Two ml of 2.5 per cent potassium iodide and potassium oxlate solution and 10 ml of 1 N H_2SO_4 were added to it. After waiting for 10 minutes, the solution was shaken to dissolve cuprous oxide. The solution was then titrated with 0.01 N Sodium thiosulphate solution adding 1 ml starch solution as an indicator near the end point. Blank was also run in a similar way. Percentage sugar was calculated using standard curve prepared from standard glucose solution.

3.4.6 Swelling factor

Under severe condition of the disease shrivelling and blackening of seeds are commonly noticed. The seed coat (husk) of Isabgul seed is mucilagenous and is a bulk laxative. The mucilagenous part which is seed coat (husk) of Isabgul seeds swells on absorption of water. Indian Pharmacopoeia (Anonymous 1966) has prescribed swelling factor to determine quality of Isabgul seeds. It is a measurement of volume of 1 g of Isabgul seeds swelling with water after 24 hrs. The mucilage constitutes over a 30 per cent of the whole seed. This showed that mucilage of the husk has very definite advantage. Thanki and Takti (1983) have claimed that higher the husk percentage, lower nitrogen percentage and less number of black seeds in a unit of sample are desirable for getting good quality seed for the industry. Keeping this in view, swelling factor for the seeds collected from healthy as well as diseased plants were determined by method recommended in Indian pharmacopoeia (Annonymous 1966) as given below.

One gram seed was placed in 25 ml graduated stoppered cylinder and distilled water was added upto 20 ml. Agitated immediately for thorough wetting and allowed to stand for about 20 minutes. It was again agitated for uniform distribution of swollen seeds. Volume of the swollen seeds was recorded after allowing to stand for 24 hrs.

3.4.7 Black seeds

At the time of harvest the seeds were collected from each of the five healthy and diseased plants. The number of black seeds was counted from the seeds collected from each plant and per cent of black seeds was accessed.

3.5 Disease management

3.5.1 Disease management by chemicals

Experiments to control the disease were carried out at two locations (1) on Agronomy farm of B.A. College of Agriculture, Gujarat Agricultural University, Anand Campus Anand for four years (1979-80 to 1982-83) and (2) in the Isabgul area in North Gujarat for two years (1980-81 to 1981-82) at Gujarat Agricultural University Research Station, Vijapur.

Seed treatment with Apron-35-SD, Captafol and Carbendazim as well as Dehusk seeds were used as main treatments. Whereas, Metalaxyl, Chlorothalonil, Captafol and Mancozeb were used for spraying as sub-treatments in field trial.

Experimental details

1. Experimental design - Split plot
2. Replications - Three
3. Plot size: Gross - 2 m x 1.2 m
   Net - 1 m x 1 m
4. Seed rate - 10 kg/ha
5. Variety - Gujarat Isabgul - 1 (a susceptible variety)
6. Date of sowing - December 1st week

The variety Gujarat Isabgul-1 was selected to carry out field experiments as it is susceptible.

7. The experiment was laid out in an area where the downy mildew disease generally appears every year in severe proportions.
8. Treatments:

Main treatments

No.	Fungicide	Dose gm/kg seed	Active Ingredient
1	Apron-35 SD	5.0	(Methyl D, L-N.C.2,6-dimethyl-Phenyl) -N-(2' methoxyacetyl) - alaninate.
2	Carbendazim 50 % WP	2.0	2-(Methoxy-Carbomoyl) benzimidazole
3	Captafol 80% W	2.5	N(1, 1,2,2, - tetrachloroethyl) thiocis 4 cyclohexene - 1,2 dicarboximide
4	Dehusked seeds	-	-
5	Control	-	-

<u>Sub-treatments</u> (Fungicidal sprayings)

No.	Fungicide	Concent-ration %	Active Ingredient
1	Metalaxyl-25 WP	0.05	(Methyl D, L-NC$_2$, 6-dimethyl-phenyl) -N-(2'methoxyacetyl) alaninate.
2	Captafol-80 W	0.20	N(1,1,2,2 - tetra chloroethyl) thiocis - 4 cyclohexene - 1, 2 dicarboximide.
3	Chlorothalonil -75 W	0.10	Tetra chloroisophthalonitrite
4	Mancozeb	0.20	Manganese ethylene bis dithio carbamate
5	Control	-	-

All other operations were kept common for all the treatments. First spraying was carried out every year at both the locations during first week of February, sixty days after sowing and subsequently at the interval of fifteen days. Downy mildew disease was rated on 35 plants randomly selected from each plot during the third week of March every year at both the locations. The disease severity was determined by using the grade 0-4 as described earlier. Every year during the experimentations at both the locations, 10 plants were tagged randomly in each plot and data on number of tillers, total spikes, effective spikes, non effective spikes and mean length of spike were recorded at the time of harvest. Crop in the experimental plots was harvested separately during first week of April. Spikes were threshed and grains were takenout individually from each plot at both locations. The data were subjected to statistical analysis.

3.5.2 Disease management by varying seed rates and dates of sowing.

The effect of seed rate and date of sowing was studied in field experiments on the farm of Medicinal and Aromatic Project, GAU, Anand Campus, Anand for two years (1979-80 to 1980-81).

Experimental details

1. Experimental design	:	Split plot
2. Replications	:	Four
3. Plot size: Gross	:	8 m x 3 m
Net	:	6 m x 2 m
4. Variety	:	Gujarat Isabgul-1 (a susceptible variety)

5. The experiment was laid out in an area where the downy mildew disease generally appears every year in severe proportion.

6. Treatments

(i) Main treatments (Date of sowing)

1. 1st November
2. 16th November
3. 1st December
4. 16th December
5. 31st December

(ii) Sub treatments (seed rate)

1. 1 kg/ha
2. 2.5 kg/ha
3. 4.0 kg/ha
4. 5.5 kg/ha
5. 7.0 kg/ha

Ninety days after sowing in each of sowing date, (Main treatments) the downy mildew incidence was rated on 40 plants selected randomly from each of the sub plots. The crop under each sowing dates from each plot was harvested separately after 120 days of sowing. The yield data from each plot were recorded. The data were analysed statistically for both the years.

3.5.3 Reaction of Isabgul varieties/cultures to downy mildew.

The best way to control the disease is to have a variety resistant or tolerant to the disease. Hence a search was made to locate resistant germplasm. The germplasm collection at the medicinal plant scheme was critically examined for resistant to downy mildew disease. Seven such cultivars located showed resistance in the field. These seven strains along with susceptible one were further tested for three years (1979-80 to 1981-82) in an endemic area. The details of the trial are given below -

Experimental details

1. Experimental design : R.B.D.
2. Replications : Four
3. Plot size: Gross : 1.5 m x 1.5 m
   Net : 1 m x 1 m
4. Seed rate : 10 kg/ha
5. Date of sowing : December 1st week
6. Varieties/cultures : Eight
   i) Male fertile
   ii) E.C. 42706-2/Pl
   iii) Progeny-3
   iv) Progeny-13
   v) E.C. 42706-1
   vi) E.C. 124345
   vii) Gujarat Isabgul-2(TS-6)
   viii) Gujarat Isabgul-1 (a susceptible variety)

The crop of most susceptible culture (Progeny-3) to downy mildew was raised all around the experimental block to a width of one meter with 5 kg seed rate/ha. Moreover, the inoculum (sporangial suspension) was sprayed twice between 50 to 70 days after sowing to ensure disease development. The spraying was carried out during late evening. The sporangial suspension for inoculation was prepared from diseased leaves collected in late afternoon and placed in moist chamber for about 12 to 15 hrs. When the crop was at the flowering stage (i.e. after 90 days of sowing) thirty five plants were randomly selected from each plot and were graded for disease intensity as per scale referred earlier.

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

The results of research work undertaken on downy mildew of Isabgul incited by *Peronospora plantaginis* with respect to the (i) histopathology (a) host-parasite relationship and (b) morphological attributes, (ii) effect of meteorological parameters in relation to sporangial germination and disease development (iii) bio-chemical changes brought out due to disease and (iv) management of disease through (a) cultural practices (b) chemicals and (c) germplasm screening are presented.

4.1 Histopathology

4.1.1 Host parasite relationship

The histopathological studies conducted with leaves infected by *P. plantaginis* revealed that the sporangia on host leaf surface upon germination, produced germ tubes and penetrated through stomata (Plate, 4 A). After penetration, fungal haustorium (Plate, 4 B) became hyphoid and then grew, normally adjacent to the host wall giving rise to hypha (Plate, 4 C). Thenafter intercellular mycelium formed (Plate, 4 D) haustoria invading adjacent cells. Haustoria are generally cylindrical, long tenuous and curling and vary in size. They were observed often in clusters in mesophyll and lower epidermal walls and resulted in severe pathogenesis which progressively led to tissue necrosis and death of plants. Sporangia and sporangiphores emerged

PLATE. 4

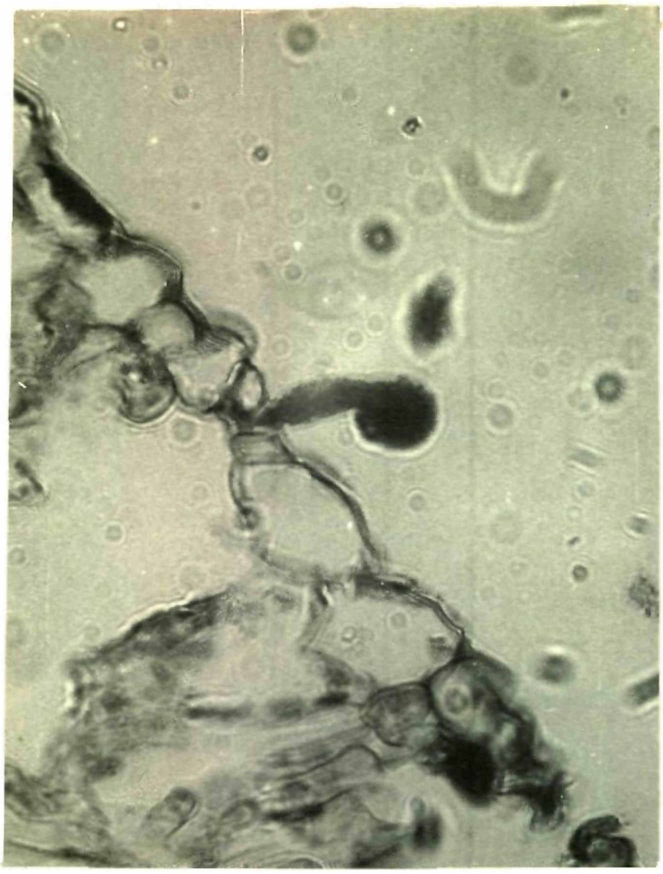
LEAF INFECTION

(A) GERMINATING SPORANGIUM AND PENETRATION THROUGH STOMATA

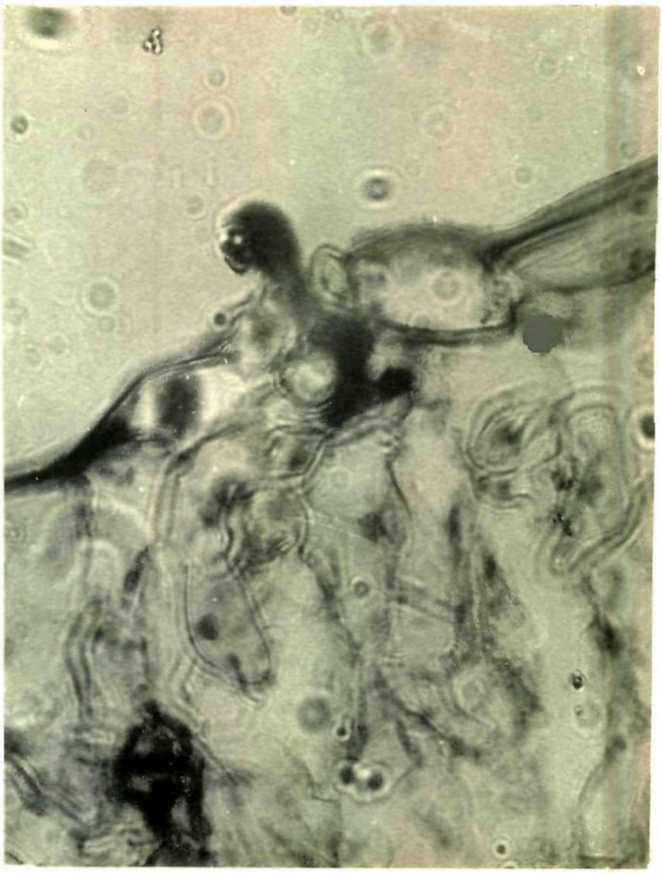
(B) DEVELOPMENT OF HAUSTORIUM

(C) DEVELOPING HYPHAE

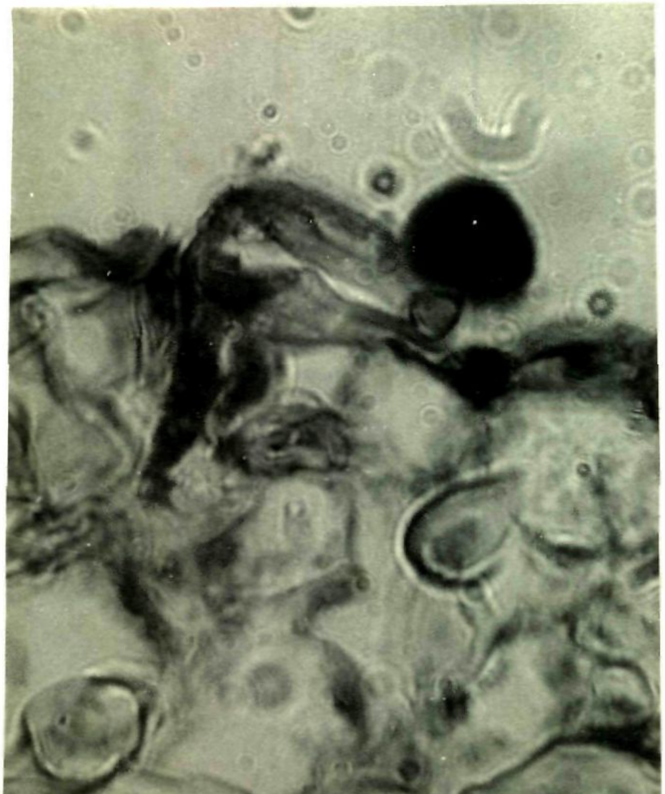
(D) DEVELOPMENT OF MYCELIUM



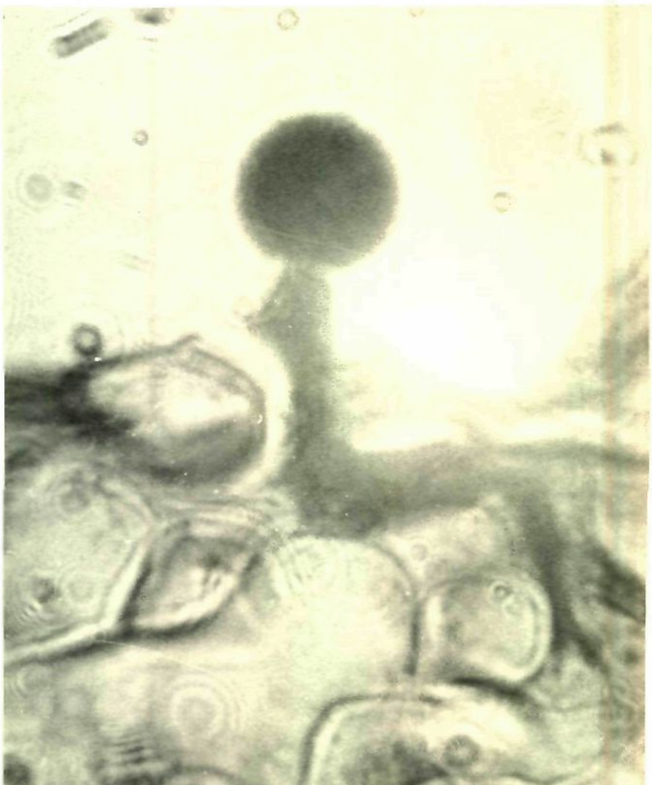
A



B



C



D

through stomatal opening before death of the tissues. Zoospore formation was never observed. The studies also revealed that the fungus can penetrate either directly or through stomata. Although, it has been reported that the fungus penetrates directly (Iwata, 1943 and Preece et al. 1967), it has been observed here that the fungus penetrates only through stomata under the studies as has been reported by Gregory (1912); Campbell (1935); Suchults(1937); Wolf (1947) and Waite and Cannon (1974).

4.1.2 Morphological characters

The coenocytic fungal hypha was intercellular in host tissues with breadth ranging from 6.25 to 12.50 μ (average 8.5 μ) (Table 1). Sporangiophores appeared slender and "tree like" with an erect 'trunk', gray to pale violet in colour emerging singly or in clusters from stomata on the lower surface and were dichotomously branched (Plate 5 and Fig. 1 a). The terminal branches or sterigmata were pointed more or less straight, and measured 4.25-12.30 μ x 4.00-4.20 μ (average 6.81 x 4.17 μ). Sporangia were sub-hyaline, broadly elliptical to sub-globose and usually measured 32-44 x 17-25 μ (average 37 x 21 μ) (Fig. 1 b). Sporangium upon germination produced germtube (Fig.1 c). The measurements reported here for branch ends (Sterigmata) and sporangia are very much similar to those reported for P. plantaginis by Eliasson (1915) as referred by Francis

P L A T E, 5

SPORANGIOPHORE WITH SPORANGIUM

FIG. 1

FUNGAL MORPHOLOGY

a. BRANCHED SPORANGIOPHORE

b. SPORANGIA

c. GERMINATING SPORANGIA

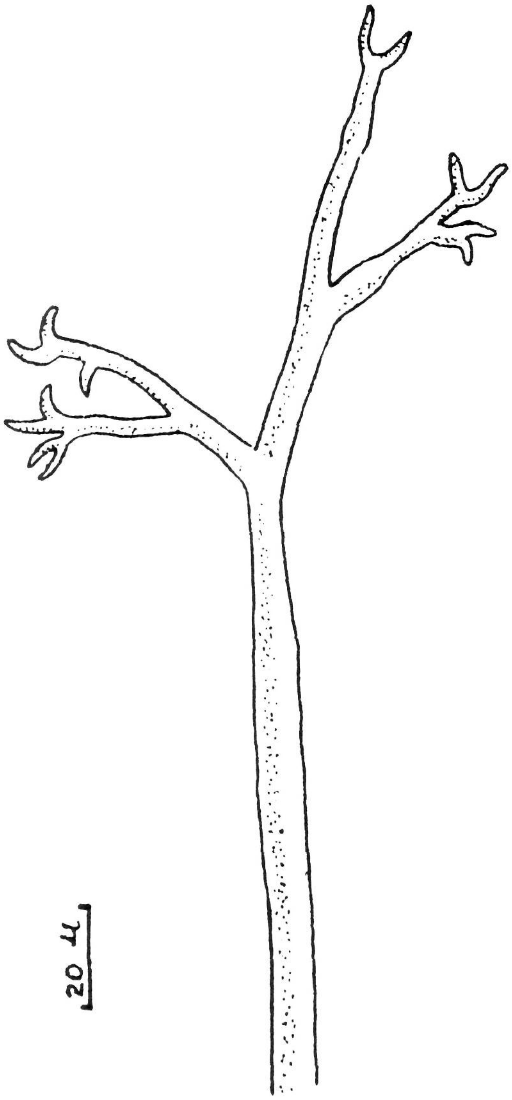


Fig. a

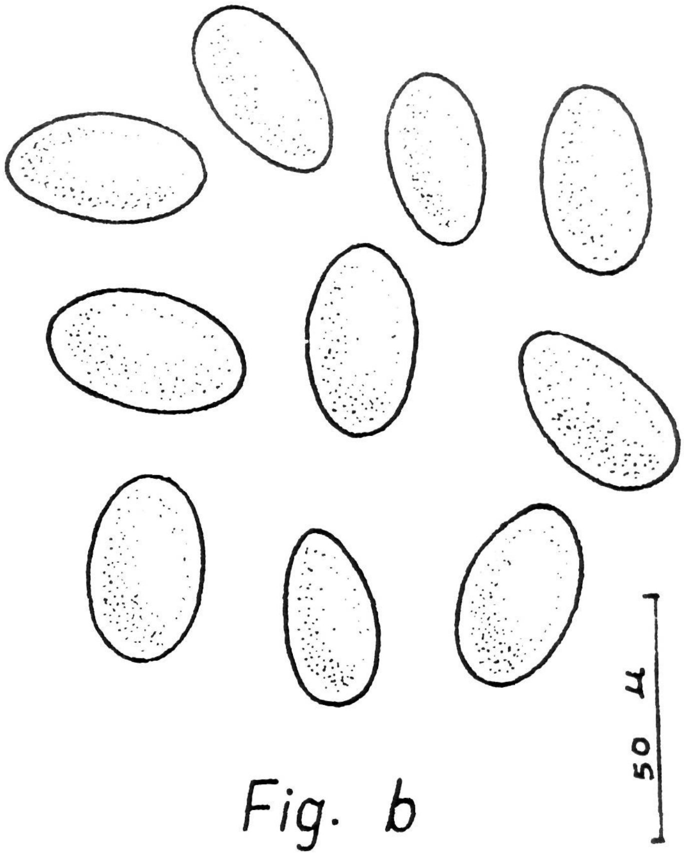


Fig. b

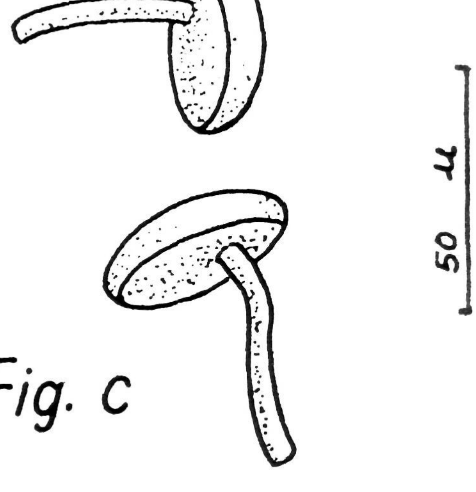


Fig. c

(1981). The conidial width was 16-18 μ according to Francis (1981), while it was 17.00-25.00 μ in the present study.

Table 1. Dimensions of different structures of P. plantaginis on Gujarat Isabgul-1.

Structure	Number of observation	Length (μ)	Width (μ)
Hyphal	100	-	6.25 - 12.50 (8.50)
Sterigmata	100	4.25 - 12.50 (6.81)	4.00 - 4.10 (4.17)
Sporangia	100	32.00 - 44.00 (37.00)	17.00 - 25.00 (21.00)

NB: Figures in parentheses are mean values.

4.2 Meteorological parameters

4.2.1 Influence of temperature on sporangial germination *in vitro*.

The study on effect of temperature on sporangial germination revealed significant differences among sporangial germination at various temperatures. The sporangial germination was significantly more at 20°C than those at other temperatures tried (Table, 2 and Fig. 2). Thus optimum temperature for sporangial germination is 20°C. Thereafter it decreased either with increase or decrease in temperature

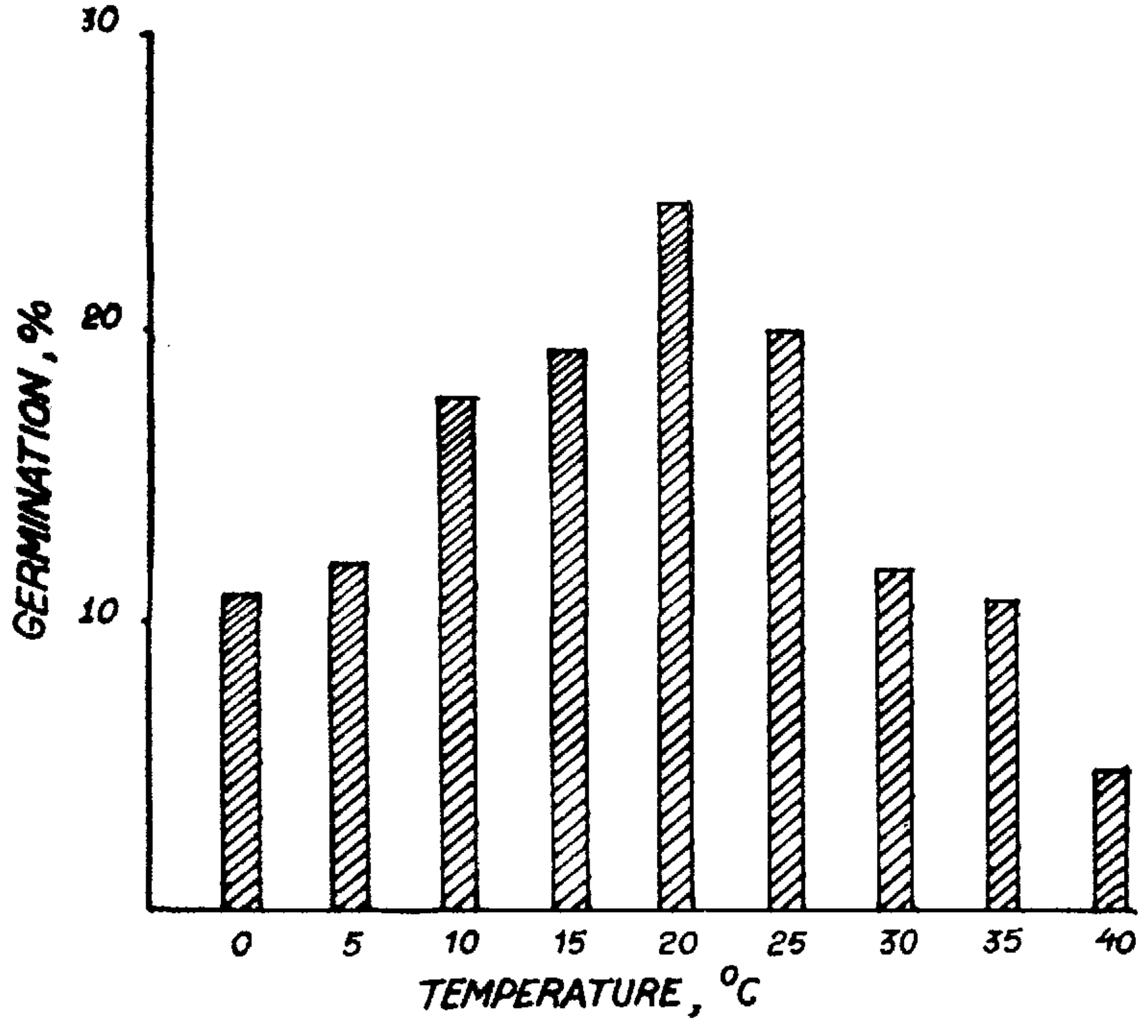
Table 2. Influence of temperature on sporangial germination of *P. plantaginis* in *vitro*.

(average of 12 replications)

Temperature, °C	Mean sporangial germination, %
0	11.52
5	12.08
10	18.44
15	19.51
20	24.68
25	20.44
30	12.88
35	11.91
40	5.20
S.Em.	1.23
C.D. 0.05	3.47
C.V. %	28.1

levels (Fig. 2). Singh *et al.*(1970) have also observed that the optimum temperature for sporangial germination to be 20-22°C and decreased with increase or decrease in temperature in case of *Sclerophthora rayssiae*.

FIG. 2: EFFECT OF TEMPERATURE ON SPORANGIAL GERMINATION IN VITRO


30
20
10
GERMINATION, %
0
5
10
15
20
25
30
35
40
TEMPERATURE, °C

4.2.2 Influence of temperature and relative humidity on sporangial germination in field

The results presented in Table, 3 and Fig. 3 indicated that the maximum sporangial germination (41.57%) was observed at 17.5°C temperature and 88.50 per cent relative humidity at 06.00 a.m.; while it was minimum (17.27%) at 42.5°C temperature and 32.5 per cent relative humidity at 2.00 p.m. In general, temperature around 20°C and relative humidity around 89 per cent favoured sporangial germination between 02.00 and 08.00 a.m. and at 10.00 p.m. On the other hand the sporangial germination (40.11 %), second highest, was observed at temperature 22°C and relative humidity 66.50 % at 08.00 p.m. which seems to be exception. The probable reason could be either the prevalence of cloudy atmosphere particularly during that period and emmision of long wave radiation from the cloud or some other abiotic factors might have played vital role in giving higher percentage of sporangial germination. While prevalence of low relative humidity during that period was caused by advection of vapour to other places as a result of high wind velocity. The regression correlation for sporangial germination was negative (-0.89) with temperature and positive with relative humidity (+0.82).

Table 3. Influence of temperature and relative humidity (RH) on sporangial germination of P. plantaginis in field.

Time of sporangial collection hrs.	Temperature, °C *	RH % *	Sporangial germination, %**
06.00 a.m.	17.5	88.50	41.57
08.00 a.m.	20.0	83.52	37.96
10.00 a.m.	27.5	55.00	28.36
12.00 noon	36.0	42.50	21.02
02.00 p.m.	42.5	32.50	17.27
04.00 p.m.	43.0	46.04	22.30
06.00 p.m.	30.5	45.02	27.62
08.00 p.m.	22.0	66.50	40.11
10.00 p.m.	20.5	79.00	36.63
12.00 midnight	23.0	81.50	28.95
02.00 a.m.	21.5	89.00	32.15
04.00 a.m.	20.0	89.50	35.67

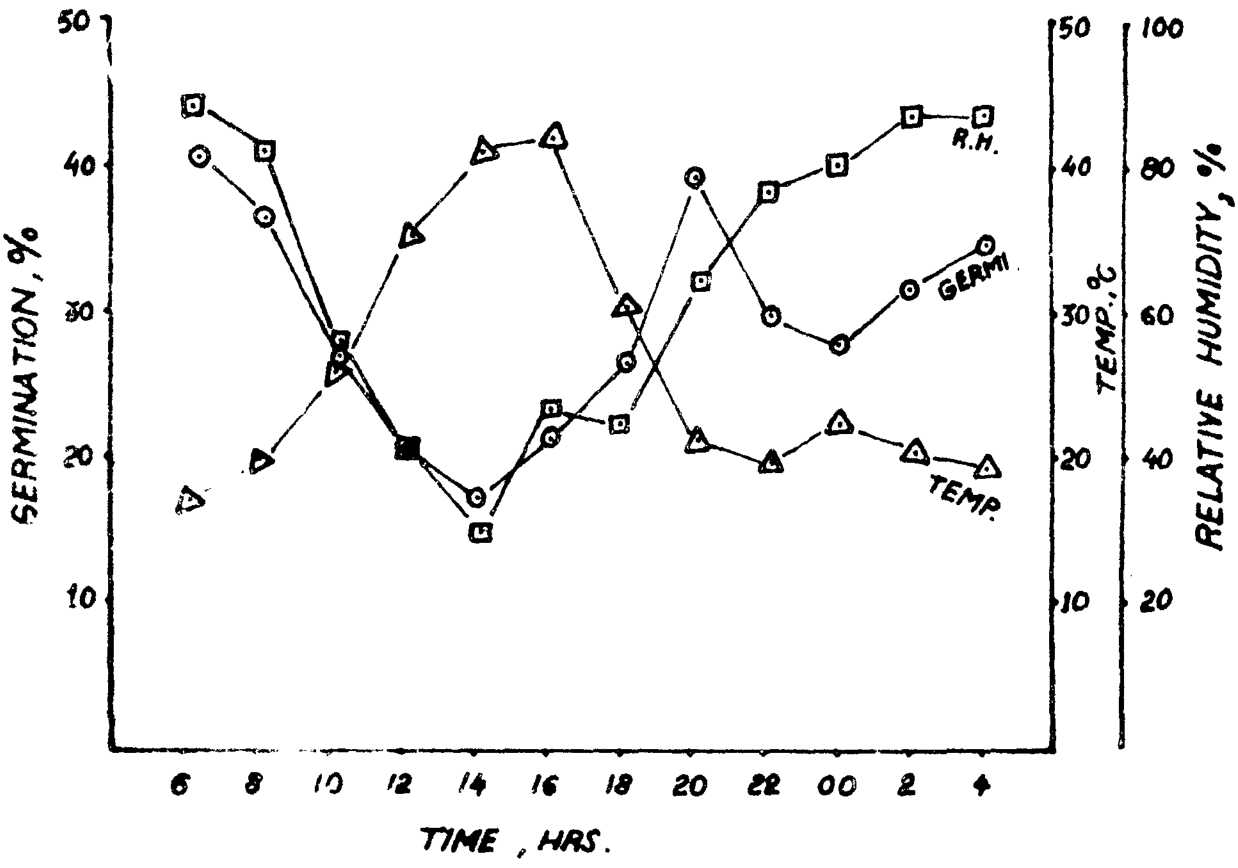
* Average of three consecutive days

** Average of 18 slides; 25-40 sporangia examined in each slide: (a set of 6 slides each day for three consecutive days).

Co-efficient of correlation with temp: - 0.89

Co-efficient of correlation with RH : + 0.82

FIG. 3: EFFECT OF TEMPERATURE AND RELATIVE HUMIDITY (RH) ON SPORANGIAL GERMINATION IN FIELD



50
40
30
20
10
GERMINATION, %
50
40
30
20
10
TEMP. °C
100
80
60
40
20
RELATIVE HUMIDITY, %
R.H.
GERMI
TEMP.
6
8
10
12
14
16
18
20
22
00
2
4
TIME , HRS.

4.2.3 Effect of temperature and relative humidity on disease development in field

The results obtained under the study indicated that meteorological parameters had great influence on disease development. The disease was first observed on 50th day after seeding. However, the massive development of downy mildew appeared only after 75 days which was correlated with the progressive rise in temperature (minimum and maximum) and relative humidity. Disease intensity also increased with the decrease in the range of temperature (minimum and maximum) along with increase in relative humidity (Table 4, Fig. 4).

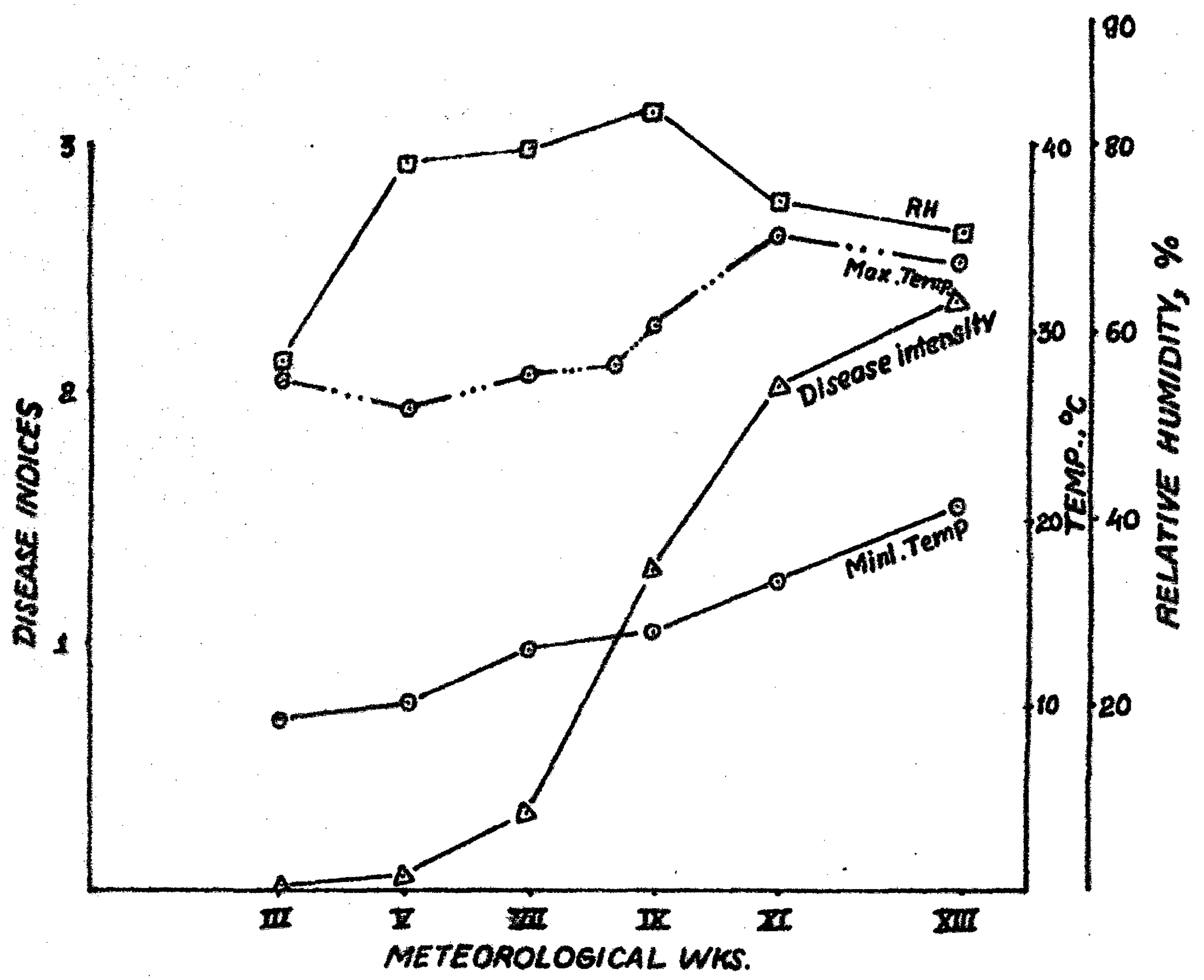
Table 4. Effect of temperature and relative humidity on disease development in field.

Date of observation	Temperature, °C Minimum	Temperature, °C Maximum	Relative humidity,%	Disease index *(0-4)*
15.1.83	9.3	27.4	57	0.00
30.1.83	10.0	25.7	79	0.05
15.2.83	13.3	28.8	80	0.30
3.3.83	13.5	30.7	34	1.40
18.3.83	16.6	35.2	74	2.07
2.4.83	20.1	34.3	71	2.37

* 0 - Free; 4 - Maximum disease intensity

** Observations based on 100 plants.

FIG. 4: EFFECT OF TEMPERATURE AND RELATIVE HUMIDITY (RH) ON DISEASE DEVELOPMENT IN FIELD



DISEASE INDICES
3
2
1
RH
Max.Temp.
Disease intensity
Mini.Temp
40
30
20
10
TEMP., °C
90
80
60
40
20
RELATIVE HUMIDITY, %
III
V
VII
IX
XI
XIII
METEOROLOGICAL WKS.

This trend continued till the crop matured. The steady increase in both temperature (minimum temperature 9.3 to 20.1 °C and maximum temperature 27.4 to 34.3 °C) and relative humidity (57 to 84 per cent) during the crop period favoured the formation of sporangia and their germination. This resulted in severe infection. Consequently the disease intensity also increased. Thereafter, eventhough there is a reduction in relative humidity at 15 days before the crop harvest, it did not retard the disease incidence as the external meteorological factors had very little effect on the pathogen which has already established in the host. Hence the maximum intensity of the disease appeared in the crop of 80-85 days. Thus, it is evident that the effect of temperature on sporangial germination in laboratory and in field is more or less identical.

Bashi and Aylor (1983) attribute light as a factor responsible for lower germination of sporangia but as has been observed here the increase in temperature and decrease in relative humidity as the day rises is more responsible for lower germination than light.

The crop yield decreased with the increase in disease severity (Table 5 and Fig. 54). The minimum yield (1.10 g/plant) was obtained from diseased plants graded 4 while maximum yield (7.3 g/plant) recorded from healthy plants. Heavily infected leaf will have lower

Table 5. Effect of disease intensity on yield

Disease intensity	Average yield, g/plant
0	7.30 (1)
1	3.87 (17)
2	1.64 (35)
3	1.03 (38)
4	1.10 (9)

NB: Figures in parentheses indicate number of plants.

rate of photosynthesis than that of healthy leaf. This is an universally accepted fact that the diseased plant will yield poor.

4.3 Biochemical changes

The results reported in Table 6,revealed that the moisture content, total chlorophylls (a + b), total phenolics and ash content decreased significantly in diseased leaves. The increase of reducing sugar in diseased leaves was not significant. Increase of nitrogen in diseased leaves was significant. The maximum reduction was recorded in total chlorophylls content (57.76 per cent) followed by total phenolics (52.01 per cent) while reduction in moisture and ash contents was to the tune of 19.02 and 12.42 per cent respectively.

FIG. 5: EFFECT OF DISEASE INTENSITY ON YIELD

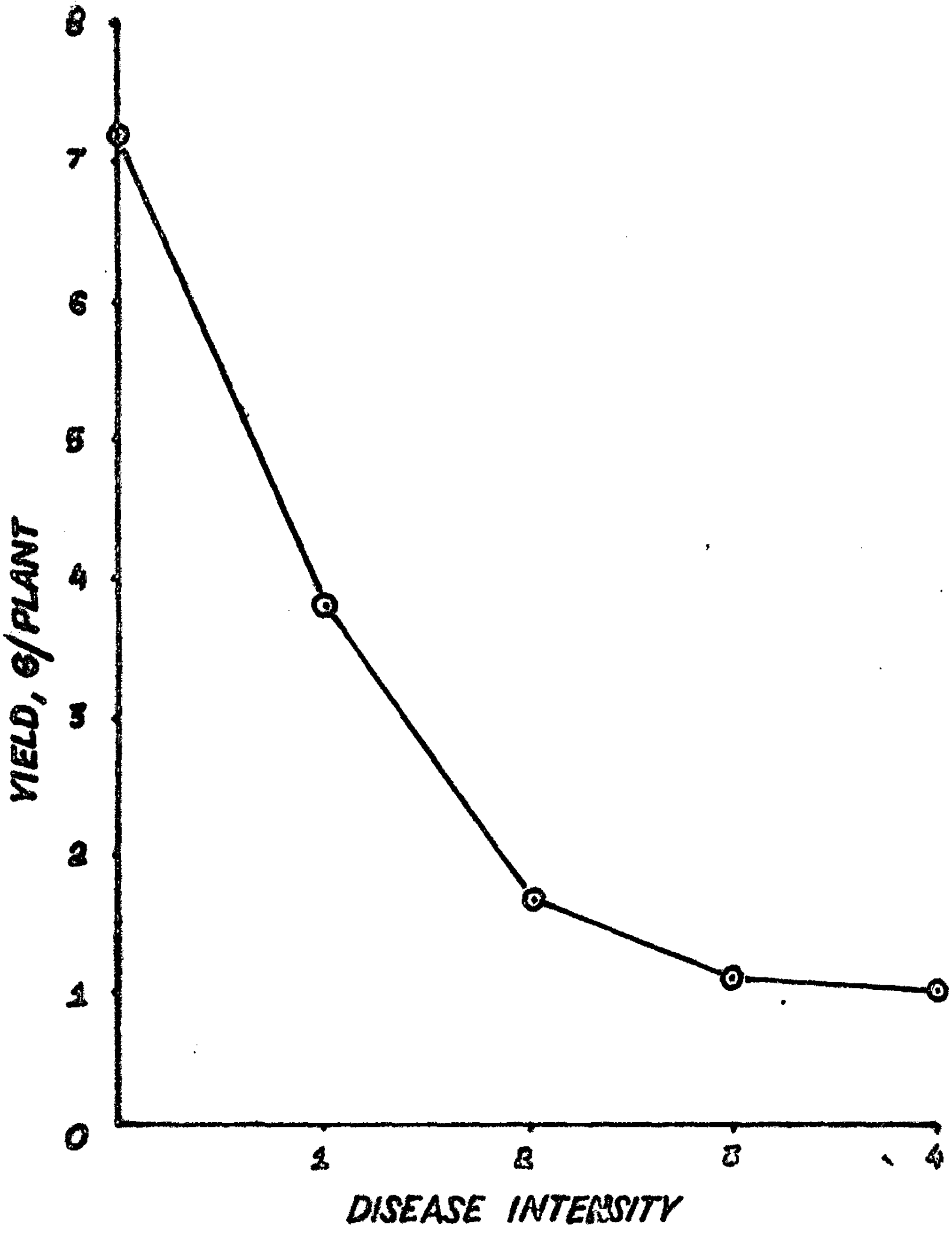

YIELD, G/PLANT
DISEASE INTENSITY

Table 6. Biochemical changes in diseased and healthy leaves of Isabgul plants

Constituent / Type of leaves	Moisture, %	Ash, %	Nitrogen, %	Reducing sugars, %	Total chlorophylls, mg/1	Total phenolics, %
Healthy	82.11	18.44	1.41	0.66	2.32	2.98
Diseased	66.49	16.15	2.41	0.72	0.98	1.43
Percent increase (+) or decrease (-)over healthy	-19.02	-12.42	+70.92	+9.09	-57.76	-52.01
't' value	7.77*	2.65*	6.18*	2.22	9.14*	8.61*

* Significant at 5% level

Reduction in chlorophylls due to the infection of P. plantaginis may be compared with the results Shukanau et al. (1978) who also observed reduction in amount of chlorophylls (a, b, A+B), carotenoids and photo-chemical activity of chloroplasts in peduncles and leaves of onion plants infected by Peronospora schleidenii. The decrease was related to degree of infection and age of onion plant. Similarly Talieva (1980), working with Allium sp. infected by P. destructor reported progressive tissue necrosis ultimately resulting into death of onion plants by sporulation of P. destructor. In contrast to this, Thornton and Cooke (1974) did not get such effects with cabbage cotyledons infected by P. parasitica. The loss of chlorophylls reported in the present investigation in diseased leaves may probably be due to interference of pathogen in the normal chlorophyll synthesis by limiting physical/chemical factors necessary for bio-synthesis of chlorophylls and disturbing the metabolic events of healthy leaves. Balasubramanian (1981) has demonstrated reduction in iron and manganese and increase in chloride, zinc, copper and phosphorus in downy mildew infected sorghum leaves. He has associated these changes in nutrients with reduction in chlorophyll a and b in infected leaves. It is widely recognized that nutrient uptake by plant root is regulated by the activity of photosynthetic parts of plants

(Hatrick and Bowling, 1973). Increase in total nitrogen and reducing sugars in infected leaves may be visualized in light of reduction of chlorophylls by P. plantaginis in Isabgul leaves whereby affecting the plant growth and nutrient uptake. Such increased rate of protein and total nitrogen in case of onion plants infected by P. destructor was also recorded by Talieva (1980). On the other hand, Krik and Koshevskii (1977) observed drastic reduction in sugar content of diseased pods of pea (P. pisi) against increase in reducing sugar by P. plantaginis (Table,6). However, this is possible because this observation was with leaves being the source of assimilation as compared to the pods which are the bowls of these assimilates.

Phenolic compounds are reported to be important for imparting resistance to disease (Gaumann, 1950; Cruick Shank and Swain, 1956; Biehnet et al, 1968). Shah and Dalal (1980) observed that the total phenolics fall rapidly during the development of the plant to relatively low concentrations at the time when P. plantaginis attacks the leaves. It was also suggested that the cultures which maintained higher levels of phenolics at the time of infection are more tolerant to downy mildew disease. Shetty and Ahmed (1980) also correlated high amount of phenolics with exhibition of natural resistance to downy mildew of certain sorghum cultures. Similarly in *Allium* spp. Talieva (1980) demonstrated correlation of phenolic substances to peroxidase activity and observed higher rate in cultivars resistant to *P. destructor*. In contrast to this Talieva *et al.* (1973) reported higher chlorogenic acid content in infected plants than in healthy plants. However, the results obtained in the present investigation clearly pointed out the correlationship between total phenolics and downy mildew disease giving higher per cent phenolics in healthy leaves (2.98 per cent) than that in diseased (1.43 per cent) leaves. Similar observations are also reported by Shah and Dalal (1980) and Talieva (1980).

Seeds collected from diseased plants showed significant increase (34.55 per cent) in total nitrogen. Yet the increase (88.78 per cent) was nonsignificant in per cent black seeds (Table,7). The swelling factor recorded for seeds collected from diseased plants was also lower than the seeds collected from healthy plants. Similar results were obtained by Thanki and Talati (1983) who demonstrated that with increase in nitrogen per cent in the seeds and number of black seeds in the sample, there was decrease in swelling factor. They have concluded that higher husk percentage, lower nitrogen and less number of black seeds in a gram of sample are desirable parameters for determining good quality seeds. In the present investigation, the seeds

Table 7. Comparison of seeds from healthy and diseased Isabgul plants.

Characters / Type of seed	Nitrogen %	Black seeds, %	Swelling factor ml/g
Healthy	2.46	4.19	10.80
Diseased	3.31	7.91	10.50
Percent increase (+) or decrease (-) over healthy	+34.55	+88.78	-2.78
't' value	4.44*	1.76	1.18

* Significant at 5% level

collected from healthy plants showed significantly less nitrogen, no significant difference in number of black seeds and slightly higher swelling factor. Thus it is evident that seeds collected from healthy plants have all the desirable parameters for good quality seeds.

If we could understand the mechanisms of resistance and susceptibility, it would be possible to utilize these in the control of plant disease. Preliminary work reported here on the undergoing biochemical changes in the host due to infection of P. plantaginis indicated that they have played important role in the physiology of disease development in Isabgul. For example, it is evident that increased amount of chlorophylls and total phenolics have negative effect in contrast to positive effect of nitrogen and reducing sugars on the development of downy mildew disease in Isabgul. Further work on the nitrogen content of seeds collected from healthy and diseased plants suggested that the amount of nitrogen in the seeds may be considered as one of the parameters for good quality seeds in addition to the number of black seeds in the seed sample.

4.4 Management of disease

4.4.1 Disease management by chemicals

With respect to various seed treatment the differences were nonsignificant for control of downy mildew disease at Anand and Vijapur locations (Table 8.1). However, on an average, the seed treatments either with Apron 35 SD @ 5.0 g/kg seeds or Captafol @ 2.5 g/kg seeds were promising for getting high yield and less disease intensity.

Significant differences were observed amongst various fungicidal spray applications for disease intensity at both the locations and for yield only at Anand. Metalaxyl (Ridomil 25 WP) spray at 0.05 per cent concentration had significantly less disease indices of 0.71 and 0.64 at Anand and Vijapur respectively than those observed with Captafol, Chlorothalonil and Mancozeb. Thus resulted in significantly more yield (1519 kg/ha) at Anand than those rent seed treatments in combination with fungicidal sprays on disease intensity and yield of Isabgul.

	Disease index (0-4)*		Number of tillers		Number of spikes		Number of effective spikes		Length of spikes, cm		Yield, kg/ha	
	Anand @	Vijapur +	Anand @	Vijapur +	Anand @	Vijapur +	Anand @	Vijapur +	Anand @	Vijapur +	Anand @	Vijapur +
	1.44	1.37	3.6	3.2	22.4	19.1	18.9	16.8	2.93	3.09	1350	927
	1.27	1.30	3.7	3.2	22.9	18.3	19.4	16.8	3.04	3.01	1358	893
	1.36	1.26	3.7	3.5	22.4	21.9	18.4	20.1	2.89	3.04	1315	925
	1.38	1.21	3.8	3.2	23.5	17.9	19.5	16.3	3.05	3.01	1354	936
	1.42	1.26	3.6	3.5	22.4	21.7	18.7	18.7	2.86	3.24	1320	875
	0.04	0.07	0.1	0.2	0.8	1.4	0.8	1.2	0.04	0.10	38.00	43.80
	ns	ns	ns	ns	ns	ns	ns	ns	0.12	ns	ns	ns
	21.7	29.1	26.8	27.7	28.2	38.6	33.6	37.4	10.8	17.9	21.9	26.4
	0.71	0.64	3.8	3.5	23.0	20.4	19.5	18.6	3.04	3.13	1519	973
	1.48	1.31	3.7	3.2	22.8	19.6	19.3	17.6	2.92	3.07	1290	945
	1.50	1.43	3.7	3.3	21.9	17.0	17.8	15.2	2.90	3.06	1272	910
	1.25	1.23	3.9	3.3	24.0	20.6	20.0	18.2	2.97	3.09	1365	893
)	1.93	1.79	3.6	3.4	22.0	21.4	18.3	19.2	2.94	3.03	1251	835
	0.03	0.06	0.1	0.1	1.1	1.3	1.0	1.2	0.05	0.07	34.0	36.50
	0.09	0.17	ns	ns	ns	ns	ns	ns	ns	ns	93.00	ns
	19.1	25.3	24.4	16.8	37.2	35.1	39.8	36.0	12.1	11.7	19.5	21.9
MxS)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	Sign.
M)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Sign.	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

e intensity; @ Pooled data of four years i.e.1979-80 to 1982-83; + Pooled data of two years i.e. 1980-81 to 1981-82 obtained as a result of spraying with other fungicides. The yield differences at Vijapur were nonsignificant. However, spraying with metalaxyl 0.05 % yielded maximum. The disease indicies in untreated plots were significantly more and yields were least. The seed treatment and fungicidal spray had no significant effect on total number of tillers, total number of spikes and total number of effective spikes at both the locations, except that the mean length of spikes was significantly more at Anand than at Vijapur. The interaction (year x seed treatment) effect was nonsignificant at both the locations indicating that the conditions prevailing during the year had no variable effects on seed treatments. However, interaction (year x fungicidal spraying) effect for disease index was significant only at Anand. Looking to the overall average disease indices, the year 1980-81 had least (0.89) disease index (Table 8.2) while it remained almost consistent for the years 1979-80, 1981-82 and 1982-83. This clearly indicates that the environmental parameters prevailing during 1980-81 may be unfavourable for the disease development. Whereas fungicidal effects on control of downy mildew disease during 1979-80, 1981-82 and 1982-83 was more or less similar. With regards to yield, the interaction (seed treatment x fungicidal spraying) effect was significant at Vijapur only (Table 8.3). The seed treatment with Apron-35 SD @ 5 g/kg seed coupled

Table 8.2. Significant interaction of year x spraying for disease index at Anand.

Year / Spraying	Disease index (0-4)*			
	1979-80	1980-81	1981-82	1982-83
Metalaxyl	0.93	0.33	0.62	0.96
Captafol	1.80	1.02	1.46	1.63
Chlorothalonil	1.82	0.87	1.56	1.76
Mancozeb	1.61	0.83	1.32	1.24
No fungicidal spray(Control)	1.91	1.42	2.20	2.18
S.Em.	0.05	0.06	0.08	0.08
C.D. 0.05	0.14	0.17	0.24	0.22
C.V. %	11.5	24.5	22.9	18.9

* 0 - Free ; 4 - maximum disease intensity.

with mancozeb 0.2 % spray, dehusked seed with metalaxyl (Ridomil 25 WP) 0.05% spray, Captafol 2.5 g/kg seeds with captafol 0.2% spray and Captafol 2.5 g/kg seeds with Metalaxyl 0.05 % spray gave maximum yield than that obtained with rest of treatment combinations. The interaction between year, seed treatment and fungicidal spray was nonsignificant. This indicates that the seasons during different years had no effect either on seed treatment or fungicidal spraying and their combinations. Thus it is

Table 8.3. Significant interaction of seed treatment x spraying for yield kg/ha at Vijapur.

Spraying / Seed treatment	Metalaxyl	Captafol	Chloro-thalo-nil	Manco-zeb	No fungicidal spray
Dehusk seeds	1083	893	862	987	810
Apron-35 SD	913	925	852	1088	688
Carbendazim	880	937	985	822	1003
Captafol	1060	1080	898	870	770
No seed dressor (Control)	927	892	955	700	903
S.Em.	82				
C.D. 0.05	230				
C.V. %	21.9				

obvious from the data presented that seed treatment with Apron 35 SD @ 5 g/kg seeds coupled with three spraying (15 days interval) of Mancozeb 0.2% or use of dehusked seed with three spraying (15 days interval) of Metalaxyl 0.05% or seed treatment (2.5 g/kg seed) as well as three spraying (15 days interval) of Captafol 0.2% or seed treatment with Captafol 2.5 g/kg seeds with three spraying (15 days interval) of metalaxyl 0.05% can effectively control downy mildew disease giving higher yields. For want of cost of Apron 35 SD and metalaxyl, it was not possible to work out the cost benefit ratios. It has also been reported by several workers that downy mildew caused by Peronospora sp. can be effectively controlled through chemicals. Metalaxyl was reported to be effective against Peronospora destructor on onion (Wilson, 1980); Peronospora on rose (Folk, 1983); Peronospora tabacina on tobacco (Fosllnum Johnson et al, 1978); Peronospora parasitica on brassica (Hartill, 1982); Peronospora sorghi on sorghum (Anahosur and Patel, 1983); Mancozeb (Dithane M-45) was also reported effective against Peronosclerospora sorghi on sorghum (Balasubramanian, 1976). While both Mancozeb and Captafol (Difolatan) were also reported effective against Peronospora destructor on onion (Mirakur et al, 1977).

4.4.2 Effect of dates of sowing and seed rates on disease intensity and yield

The differences amongst the yields obtained from plots sown on different dates were significant (Table 9.1). Crop seeded on 16th December suffered significantly less from downy mildew disease as compared to the plots sown on other dates while disease indices were significantly more in the plots seeded on 1st November. The disease intensity was moderate in crop sown on other dates.

Crop yield was significantly less in the plot seeded on 31st December, than the rests barring that from crop

Table 9.1. Effect of sowing dates and seed rates on downy mildew disease and yield of Isabgul.

(Pooled for two years)

Sowing date (D)	Disease index (0-4)*	Yield, kg/ha
1st November	2.25	818
16th November	1.95	910
1st December	1.98	841
16th December	1.57	898
31st December	1.94	743
S.Em.	0.06	34
C.D. 0.05	0.19	100
C.V. %	21.5	25.8
Seed rate kg/ha (R)		
1.0	1.29	848
2.5	1.85	879
4.0	2.02	854
5.5	2.23	842
7.0	2.29	788
S.Em.	0.4	22
C.D. 0.05	0.11	ns
Interaction	Ns	Sign.
C.V. %	13.0	16.2
Year effect	Sign.	Sign.
y x t effect	Sign.	ns

* 0 = Free; 4 = Maximum disease intensity.

sown on 1st November and 1st December. While maximum yield was recorded from the plot seeded on 16th November. This was at par with yields obtained from plots sown on 16th December, 1st December and 1st November.

With regards to seed rates, significant differences were obtained amongst various seed rates for disease indices. The disease index increased significantly with increase in seed rates. Though the disease intensity was maximum in the plots receiving seed rate of 7.0 kg/ha, it was at par with 5.5 kg seed rate. As expected plots seeded with 1.0 kg/ha had least disease intensity.

Though the yield differences were nonsignificant among various seed rates, maximum yield was obtained from plots receiving 2.5 kg/ha seeds. Thereafter with increase in seed rates, the yields decreased, lowest being recorded in plots seeded with 7.0 kg/ha seeds. It also decreased when seed rate was reduced from 2.5 kg/ha to 1.0 kg/ha.

The interaction effect between sowing dates and seed rates was nonsignificant for disease index, while it was significant for yield (Table 9.2). The plots seeded with 1.0 kg/ha seeds on 16th November recorded significantly more yield than the rest of the combinations barring 5.5 kg/ha seed sown on 16th December and 2.5 kg/ha seed sown on 16th November.

Table 9.2. Significant interaction of sowing date x seed rate for yield.

Seed rate, kg/ha / Sowing dates	1.0	2.5	4.0	5.5	7.0	Mean
1st November	829	925	856	728	756	818
16th November	1088	979	909	782	790	910
1st December	869	891	829	821	795	841
16th December	836	842	868	1066	879	898
31st December	617	758	809	814	718	743
Mean	848	879	854	842	788	

S.Em.	C.D. 0.05	C.V. %
48	135	16.2

Further, it is interesting to note that the yields increased with increase in seed rate upto 5.5 kg/ha in plots sown on 16th and 31st December. The overall increase in yield was recorded in plots sown on 16th and 31st December. These late sowings are not congenial for growth and development of the crop as well as for disease development as compared to the crop sown in November. This might have resulted in reduced yields. However, increase in total yield/unit area was perhaps because of increase in plant population (due to high seed rate) and /or less disease development because of unfavourable meteorological parameters.

While significantly less yields were obtained in the plots receiving 1.0 kg/ha seed sown on 31st December than the rest barring 7.0 kg/ha seeds sown on 31st December and 5.5 kg/ha seeds sown on 1st November. Thus it is evident from the results that the crop seeded around 16th December gives more yield with less disease intensity. However, crop seeded around 16th November suffered little more disease but there was no adverse effect on yield. On the contrary, yield was maximum due to luxurient growth of the crop.

For seed rates, the use of 2.5 kg/ha gave optimum yield of 879 kg/ha with disease index of 1.85 while increase in the seed rates decreased yield with corresponding increase in disease intensity. Seed rate less than

2.5 kg/ha when used, showed minimum disease index (1.29) with lower yield as compared to seed rate of 2.5 kg/ha. This may be due to less plant population.

Further, it is evident that the crop sown in November is more prone to downy mildew as compared to the crop sown in December. It has been also reported that seed rate should not be more than 4 kg/ha as higher seed rate increased the disease incidence with reduced yield (Anonymous, 1980).

4.4.3 Reaction of different isabgul varieties/cultures to downy mildew

Data on disease indices on various varieties tried during 1979-80 to 1981-82 (Table 10.1) revealed that the reaction of the pathogen in different varieties/cultures varied significantly. However, none of the variety/culture was found completely free from the disease. The disease intensity ranged from 0.77 to 2.10. The culture E.C.124345 gave significantly less disease index (0.77) than the rests. The disease indices of other cultures were at par.

The culture E.C. 124345 having significantly less disease index, yielded significantly more than the rest of the cultures. The next in order was E.C. 42706/Pl, being at par with progeny 3, progeny 13, male fertile and

Table 10.1. Evaluation of different varieties/cultures for yield and disease intensity.

Varieties/cultures	Yield, kg/ha	Disease index (0-4)**
E.C. 42706-1	846	2.07
E.C. 42706-2/P1	1059	1.89
E.C. 124345	1288	0.77
Gujarat Isabgul-2(T.S.6)	952	1.81
Male fertile	945	2.08
Progeny-3	934	2.05
Progeny-13	938	2.10
Gujarat Isabgul-1	807	2.04
S.Em.	65.80	0.11
C.D. 0.05	185.50	0.32
Year effect	*	*
Year x (treatment culture/ variety effect)	ns	*
C.V. %	22.5	11.0

*Significant at 5% level

** 0-Free; 4-Maximum disease intensity

Gujarat Isabgul-2 (T.S.6). This is possible because different genotypes have variable potentiality for yield. The Gujarat Isabgul-1 yielded minimum (807 kg/ha) as has susceptible reaction to disease. The year effect was significant for both yield and disease index while, year X varieties/cultures effect was significant for disease index and nonsignificant for yield.

The disease indices for different varieties/cultures under screening trial during different years also varied (Table 10.2). Moreover, overall disease intensity during 1981-82 was higher whereas it was comparatively less during 1979-80 and 1980-81. The culture E.C. 124345 had consistently least disease intensity (less than one) while culture progeny-13 had consistently more disease intensity (more than two). This may be due to the variations in prevalence of temperature (minimum and maximum) and relative humidity at the peak period of disease development during crop phase (Table 10.3). Shah and Dalal (1980) while screening various Isabgul cultures observed lowest downy mildew disease index in relation to phenolics in the leaves in culture E.C. 124345. They suggested maintenance of higher amount of phenolic compounds at the time of disease initiation was probably one of the reasons for low disease intensity in culture E.C. 124345. Importance of phenolic compounds in disease resistance of the crop

Table 10.2. Significant interaction of year x cultures/ varieties for disease intensity.

Year / Cultures/ varieties	Disease index (0-4)* 1979-80	1980-81	1981-82
EC-42706-1	2.12	1.77	2.31
EC-42706-2-P1	1.73	1.56	2.38
EC-124345	0.88	0.44	0.98
GI-1	1.96	1.72	2.44
TS.6(G.I.2)	1.26	1.73	2.44
Male fertile	2.02	1.80	2.42
Progeny-3	1.84	1.85	2.46
Progeny-13	2.00	1.86	2.43
S.Em.	0.11	0.11	0.08
C.D.0.05	0.30	0.32	0.23
C.V. %	13.0	13.8	7.1

* 0-Free, 4-Maximum disease intensity.

Table 10.3. Mean meteorological parameters prevailing during the peak period of disease development (65 to 95 days of the crop period).

Meteorological parameters	Year 1979-80	1980-81	1981-82
Minimum temperature, °C	12.54	12.92	13.76
Maximum temperature, °C	31.90	31.44	29.04
RH, %	59.20	68.40	78.60

has also been discussed earlier in details. However, it is quite premature to out line any definite reason for resistance of E.C. 124345 culture to downy mildew over other cultures because of multiplicity of factors governing the development of disease.

If such a resistant/tolerant variety is sown then it is easy to manage crop health by less number of sprays than those required for a susceptible variety. Thus not only the cost of production can be reduced, but can reduce the spread of the inoculum/disease also.

SUMMARY AND CONCLUSION

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Isabgul (*Plantago ovata* Forsk) is an important medicinal crop of high export potentiality for our country. The crop is attacked by mainly damping-off (*Pythium ultimum*, Trow), wilt (*Fusarium oxysporum*, Schlecht, emend synd & Hans); downy mildew (*Peronospora plantaginis*, Underwood); leaf blight (*Alternaria alternata* (FR.) Keissler) and powdery mildew (*Erysiphe cichoracearum* D.C.). Among these diseases downy mildew is a dreadful disease which causes considerable damage to the crop quantitatively as well as qualitatively.

Investigations into various aspects of downy mildew of isabgul caused by *P. plantaginis* were carried out.

1. The host-parasite relationship studies carried out revealed that the sporangia upon germination produce hyphoid germ tubes which penetrate in host through stomata and then grow normally nearby the host cells giving rise to hyphae. The coenocytic hyphae grows intercellularly in host tissues. Sporangiphore appears as slender and tree like emerging singly or in clusters from stomata. Zoospores were never observed. Ultimately, the host tissue becomes necrotic and in severe cases the plant dies. The hypha is 6.25 to 12.50 μ thick, sterigmata are 4.25 - 12.50 μ x 4.00 - 4.20 μ ; sporangia are 32-44 x 17-25 μ in size.

2. The sporangial suspension prepared in tap water was incubated at 0°C, 5°C, 10°C, 15°C, 20°C, 25°C, 30°C, 35°C and 40°C. It revealed that the sporangia germinate by germ tube significantly more at 20°C and least at 40°C. Thus the temperature 20°C is optimum for sporangial germination.

Further the study of effects of temperature and relative humidity on sporangial germination under field conditions revealed that the temperature around 20°C and relative humidity around 89 % favour sporangial germination between 2 a.m. and 8a.m. and at 10 p.m. The regression correlation for sporangial germination is negative (-0.89) with temperature and positive (+0.82) with relative humidity. The downy mildew disease increased 75 days after sowing which is correlated with progressive rise in temperature and relative humidity. The prevalence of temperature around 20°C and relative humidity about 90 % during and prior to the peak period of disease development help in severe infection, which ultimately results in severe disease conditions. Maximum disease develops during the 75 to 95 days age of the crop.

3. Investigations on biochemical changes of leaves collected from healthy and diseased plants revealed that these changes have considerable impact on the physiology of downy mildew disease development in Isabgul.

(i) The chlorophyll content reduces by 57.71 per cent and phenolics by 52.01 per cent due to infection by *P. plantaginis*.

(ii) Moisture and total ash contents also reduce in the leaves of diseased plants.

(iii) Total nitrogen and reducing sugars show positive correlation with disease development.

(iv) Chemical analysis of seeds collected from healthy and diseased plants indicated that the changes have considerable impact on seed quality. The nitrogen contents and per cent black seeds increased in the seeds collected from diseased plants. While swelling factor was slight to lower in case of seeds collected from diseased plants as compared to those from healthy ones.

4. The seed rate higher than 2.5 kg/ha increases the disease severity in November sowing which ultimately reduces the yield significantly. With lower seed rate the plant population decreases and the disease severity consequently reduces. As a result the yield is maintained. However, December sown crop generally escapes from disease due to low temperature and low RH. Therefore, the yield is reduces in case of low seed rate and increases with higher seed rate in December sowing.

5. The various combinations of four seed treatments (Dehusked seeds, Apron 35 SD, Captafol and Carbendazim) and spraying with four fungicides (Metalaxyl, Mancozeb, Captafol and Chlorothalonil) in a field trial during the years 1979-80 to 1982-83 at Anand and 1980-81 to 1981-82 at Vijapur revealed that the seed treatment with Apron 35 SD 5 g/kg seeds coupled with three sprayings of Mancozeb 0.2% or use of dehusked seeds with three spraying of Metalaxyl 0.05% or seed treatment with Captafol 2.5 g/kg seeds coupled with the three spraying of Captafol 0.2% or Metalaxyl 0.05% reduce the downy mildew disease and yields higher than other combinations.

The first spray treatment is given just after the appearance of disease and in all three spraying are applied at an interval of 15 days.

6. The Gujarat Isabgul-1, Gujarat Isabgul-2 (T.S.6) Male fertile, Progeny-3, Progeny-13, E.C. 124345, E.C. 42706-1 and E.C. 42706-2/P1 were inoculated artificially with the sporangial suspension of P. plantaginis twice prior to 75 days of crop growth revealed that the culture E.C. 124345 is having significantly less disease index and significantly yields more than any other culture. The culture progeny-13 is highly susceptible to downy mildew disease. The varieties/cultures Gujarat Isabgul-2 (TS.6) E.C. 42706-2/P1, Gujarat Isabgul-1, Progeny-3, E.C.42706-1 and Male fertile are intermediate in reactions.

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