Journal of Research and Development
Peer Reviewed International, Open Access Journal.
ISSN : 2230-9578 | Website: https://jrdrvb.org Volume-17, Issue-9| September 2025

Original Article

Bio mechanisms changes of Downy mildew fungi *Pseudoperonospora cubensis* on Cucurbits

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Manuscript ID:
JRD -2025-170904
ISSN: 2230-9578
Volume 17
Issue 9|
Pp. 22-25
September 2025
Submitted:15 Aug. 2025
Revised: 25 Aug . 2025
Accepted: 10 Sept. 2025
Published: 30 Sept. 2025

Abstract

Impact of downy mildew on chlorophyll, nitrogen, protein and reducing sugar content of cucurbits was studied. Chlorophyll a, chlorophyll b pigment of the infected leaf was found to be decreased due to downy mildew. Loss in nitrogen, protein and reducing sugar contain due to infection of downy mildew were also observed. Downy mildew caused by Pseudoperonospora cubensis significantly affects the physiological and biochemical composition of cucurbits. The present study evaluated its impact on chlorophyll, nitrogen, protein, reducing sugars, phenols, and starch in infected leaves. Results revealed a marked reduction in chlorophyll a and b, nitrogen, protein, and reducing sugar contents across most cucurbit species, with the greatest protein loss recorded in Cucumis melon. Conversely, phenolic content increased consistently in infected plants, indicating a possible defense response. Interestingly, starch levels remained largely unaffected or showed a slight increase under infection. These findings highlight the biochemical alterations induced by P. cubensis, providing insight into host–pathogen interactions and contributing to strategies for disease management in cucurbits.

Keywords- *Downy mildew fungi, biochemical, common name, crop leaves Pseudoperonospora cubensis, cucurbits, biochemical changes, chlorophyll, phenols.*

Introduction

Downy mildew of cucurbits is caused by *Pseudoperonospora cubensis* is obligate bio trophic member of Oomycete Babadoost (2016). This disease occurrence in wild and cultivated plants as a fruits and vegetables in all over the word Berkeley and Curtis .(1868) Cohen.(1980) It has worldwide distribution and probably occurs wherever cucurbits are grown except unirrigated, very dry climates and especially prevalent in area with a warm, humid climates Most wild and cultivated plants as a fruits and vegetables in all over the word Sashadri (1986) Reddy (2002).In literature cited very little information was availed regarding the host range of the pathogen biochemical changes occur due to the pathogen and ecofriendly management of the disease.

Materials and Methods

Chlorophyll content-

Chlorophyll content from healthy and infected plant leaves were estimated by the method suggested by Aronon [1949],for this 2 gram of plant leaves were crushed in Mortar and pestle in 80% chilled action containing 4 ml of liquor ammonia per liter in dark code room. A pinch of Magnesium carbonate [MgCo3] was added during crushed. The extracts were filtrated trough Buchnners funnel using Whitman filter paper No.1 and volume of the filtrated was made up to 100 ml 80% with acetone 5 ml.of the extract was diluted up to 50 ml. with acetone in volumetric flask. In ordered to avoid destruction of chlorophyll by light, the flasks containing chlorophyll extracts were covered with black paper and stored at low temperature.

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How to cite this article:
Pawar, V. P. (2025). Bio mechanisms changes of Downy mildew fungi Pseudoperonospora cubensis on Cucurbits. Journal of Research and Development, 17(9), 22–25. https://doi.org/10.5281/zenodo.17357053

Quick Response Code:
Website: https://jrdrvb.org/
DOI: 10.5281/zenodo.17357053
OPEN ACCESS

Journal of Research and Development
Peer Reviewed International, Open Access Journal.
ISSN : 2230-9578 | Website: https://jrdrvb.org Volume-17, Issue-9| September 2025

The absorbance was measured at 645 nm and 663 nm double beam spectrophotometer. Chlorophyll [mg/g fresh weights]were calculated using formula given below.

Chlorophyll a=12.7 O.D663-2.69 O.D 645 D.F

Where 12.7 and 2.69 are constant

D.F= Dilution factor Chloropyll b= 22.9 O.D 645-4.68 O.D 663 D.F

Where 22.9 and 4.68 are constant

D.F.= Dilution factor Total Clorophhyll =20.2 O.D 645 -8.02 O.D 663 D.F.

Where 20.2 and 8.02 constant.

D.F=Dilution Factor.

Nitrogen content-

Estimations of nitrogen contents were made by Microkjeldahl method (A.O.A.C.,1970).For this 300mg dry sample of healthy and infected leaves were taken in Microkjeldhl flasks. A pinch of catalyst was added to it with the help of spatula 7.5 ml. of concentrated sulphuric acid(H2 So4) was added to flask. The flask were heated on a digestion stand unit (6 to 10 hr.). The mixture was clearing i.e. apple green in color or colorless. During digestion care was taken to avoid principles of indigested carbon sticking on the sides of the tube. 5 ml of the diluted material was introduced in Markham's distillation apparatus, through the side tube funnel to which glass stopper was fitted 50 ml conical flask containing 10 ml of 2% boric acid solution mixed with indicate or was at the delivery end of the condenser to collect the ammonium tetra borate((NH4)3 Bo3) .It was then titrated with 0.035 N HCl/toll the pink color obtained and titration values were recorded .Nitrogen content present in mg/g day sample was calculated by calculating the strength of NH3 in the distillate using equation. 1ml of 0.35 NHCL=0.5 of Nitrogen. From the above equation the amount of nitrogen for 5ml of sample was calculated, which will be equivalent to that of present in 300mg. of dry sample .It was recorded as percentage nitrogen of dry sample.

Estimation of crude protein

This was done by estimating N content in the sample with the help of Microkjeldahl technique(A.O.A.C.,1970) .The amount of N content was multiplied by 6.25 factor which gave crude protein content of the sample.300 mg seed powder were taken in Microkjeldahl flask along with 250 mg K2 So4 and 40 mg CuSo4 and kept overnight. This was digested till the mixture become white .After complete digestion the flasks were allowed to cool. The digest was processed for distillation with the help of Markham's distillation test .Digested was diluted to 50 ml. volumetric flask, 5 ml aliquots were taken and introduced in distillation unit through the side tube funnel .The glass stopper was immediately fitted .To this 10 ml 40/NaOH into the digest.NH3 is liberated into 10 ml 2/boric acid (with indicator) containing 50 ml conical flask. After distillation green colored ammonium borate was obtained (This gave 1 ml 0.035 NHCL =0.5 mg N/ crude protein=N/ (6.25) Crude protein of seed was calculated as percent nitrogen liberated 6.5.

Estimation of Reducing Sugar-

The sugar content in the plant material was estimated by the procedure recommended by Oster(1979) as follow 500 gm. of seed powder was taken in 50 ml. distilled water and boiled it, then filtered it and the filtrate is diluted up to 100ml.Three Following tube taken and added following manner(1) Blank-D.W. 2ml (2) 2ml.glucose C solution (3) 2 ml filtrate .In each tube 3 ml alkaline solution of copper was added then tube was boiled in boiling water bath for 8 minutes .Cooled the tube under tap water and add 2 ml of phosphomolybdic acid solution which gave blue colour .then this solution was diluted up to 25 ml distilled water and optical density determined at 420 nm and calculated the amount of reducing sugar present in seed powder.

Estimation of starch-

Starch contents of healthy and downy mildew infected plant sample were estimated by Anthron Method .For this 0.5 g of the sample was homogenized with hot 80% ethanol and sugars were removed. It was then centrifuged, the residue was dried well over a water bath.0.5 ml of distilled water and 6.5 ml of 52% perchloric acid were added to the residue. It was then centrifuged [after 20 minutes of addition] and the supernatant was collected. The extraction was repeated by using fresh perchloric acid, it was again centrifuged and supernatant was adjusted up to 100 ml with distilled water,0.2 ml of the supernatant was pipetted out and to it 0.8 ml distilled water was added. The standard was prepared by taking 0.2 ml,0.4 ml, 0.6 ml 0.8 ml and 1 ml of the working standard in test tubes. The volume of each tube was made up to 1 ml with distilled water. To each tube 4 ml of Anthron regent was added .It was then heated for 8 minutes in a boiling water bath. The tubes were cooled rapidly in tap water and intensity of green to dark green colour was measured at 630 nm on spectrophotometer.

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Result and Disccussion

It is clear from the (Table-1) that chlorophyll a and chlorophyll b pigment of the infected leaf were found to be decreased due to infection of downy mildew. Nitrogen and protein content of leaves were also depleted to downy mildew .However maximum loss in protein content was reported in the leaves *Cucumis melon* (Table-2) Reducing sugars were founded decreased in the infected host except *Citrullus lanatus* and *Cucurbita pepo* (Table-3)

TableNo-1 Effect of Downy Mildew on Chlorophyll content of cucurbits leaves

Name of crop	Common Name	Name of pathogen	Chlorophyll a		Chlorophyll b		Chlorophyll a/b	
			Healthy	Infected	Healthy	Infected	Healthy	Infected
Citrullus lanatus	Water melon	*Pseudoperonospora cubensis*	0.56	0.23	1.03	0.40	0.54	0.57
Cucumis sativus	Cucumber		0.55	0.29	1.13	0.50	0.48	0.58
Cucumis melon	Musk melon		0.33	0.18	0.64	0.40	0.51	0.45
Cucurbita pepo	Red pumpkin		0.55	0.27	1.07	0.48	0.51	0.56
Lagenaria siceraria	Bottle gourd		0.82	0.41	0.52	0.24	1.67	1.70
Luffa actangula	Ridge gourd		0.93	0.36	0.87	0.29	1.06	1.24
Luffa cylindria	Sponge gourd		0.76	0.32	0.56	0.19	1.35	1.68

TableNo-2 Effect of Downy Mildew on Nitrogen and Protein content of Cucurbits

Name of crop	Common Name	Name of pathogen	% Nitrogen		% Protein	
			Healthy	Infected	Healthy	Infected
Citrullus lanatus	Water melon	*Pseudoperonospora cubensis*	3.24	2.67	20.00	16.68
Cucumis sativus	Cucumber		2.89	2.43	18.06	15.18
Cucumis melon	Musk melon		3.14	1.83	19.60	11.43
Cucurbita pepo	Red pumpkin		3.07	2.41	19.18	15.06
Lagenaria siceraria	Bottle gourd		2.81	1.82	17.56	11.37
Luffa actangula	Ridge gourd		3.17	2.18	19.81	13.62
Luffa cylindria	Sponge gourd		3.03	2.02	18.93	12.62

Table No-3 Effect of Downy Mildew on Reducing Sugar content of Cucurbits leaves

Name of crop	Common Name	Name of pathogen	Reducing sugar (mg/g)		Non-reducing sugar (mg/g)		Total sugar (mg/g)	
			Healthy	Infected	Healthy	Infected	Healthy	Infected
Citrullus lanatus	Water melon	*Pseudoperonospora cubensis*	1.01	0.14	0.34	1.42	1.35	1.56
Cucumis sativus	Cucumber		0.96	0.12	0.03	0.98	0.99	1.11
Cucumis melon	Musk melon		0.98	0.34	0.02	0.67	1.00	1.01
Cucurbita pepo	Red pumpkin		0.64	0.28	0.25	0.71	0.90	0.99
Lagenaria siceraria	Bottle gourd		0.84	0.33	0.13	0.69	0.97	1.02
Luffa actangula	Ridge gourd		0.71	0.16	0.26	0.87	0.98	1.04
Luffa cylindria	Sponge gourd		0.82	0.28	0.16	0.77	0.98	1.05

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TableNo-4Effect of Downy Mildew on composition of Phenol and Starch in Cucurbits

Name of crop	Common Name	Name of pathogen	Phenol		Starch	
			Healthy	Infected	Healthy	Infected
Citrullus lanatus	Water melon	*Pseudoperonospora cubensis*	0.51	0.74	1.02	0.12
Cucumis sativus	Cucumber		0.47	0.69	1.09	0.12
Cucumis melon	Musk melon		0.48	0.62	1.08	0.12
Cucurbita pepo	Red pumpkin		0.46	0.70	1.09	0.12
Lagenaria siceraria	Bottle gourd		0.49	0.69	1.14	0.13
Luffa actangula	Ridge gourd		0.54	0.75	0.94	0.12
Luffa cylindria	Sponge gourd		0.50	0.71	0.99	0.10

It is clear from the result summarized in table .4 that phenols were found to be increased significantly due to infection in all the cucurbits plant .the increase was maximum in case of with *Citrullus lanatus infected Cucumis melon* and *Luffa actangula infected with Lagenaria siceraria.*It was surprising to note that there was no decrease in starch content due to infection of any species of *Pseudoperonospora cubensis* however there was slight increase in starch contents due to infection in all the cucurbits

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