Original research article

Impact of Stimulated Environmental Conditions on Downy mildew Disease Dynamics and in Ridge gourd

Abstract

Significant effect ofelevated CO_2 and temperatures levels was observed on downy mildew severity in ridge gourd wherein, highest disease severity of 52.68 PDI was observed in open reference plot followed by ambient CO_2@410 ±25 ppm with normal temperature (46.80 PDI). Meanwhile, the lowest disease intensity of 13.49 PDI was recorded in chambers with ambient CO_2 @410 ±25 ppm with 2^0C rise in temperature. Similarly, these weather variables significantly impacted the biochemical composition of ridge gourd leading to resistance/susceptible reaction against the disease. In respect of total sugar content, the treatment with elevated CO_2 @ 550 (±) 25 ppm with 2 °C rise in temperature recorded highest total sugar content of 17.89 mg g^{-1} followed by the treatment with elevated CO_2 @ 550 (±) 25 ppm with normal temperatures (14.28 mg g^{-1}). In a similar fashion, substantial effect on phenolic content was recorded wherein, highest total phenoloic content of 129.25 mg/100g was recorded for the treatment with ambient CO_2 @ 410(±)25 ppm with 2 °C rise in temperature. Meanwhile, significant lowest total phenoloic content (109.65 mg/100g) was recorded in for ridge gourd grown in open/reference plot.

Keywords: Climate Change, Downy mildew, Biochemical, Ridge gourd

Introduction

Among different cucurbits, ridge gourd which is popularly known as “Kalitori” or angled gourd belongs to the family cucurbitaceae and being cultivated in nations like India, Indonesia, Malaysia, Myanmar, Philippines, Sri Lanka and Taiwan.Ridge gourd is considered as “vegetables of diet food” because of high moisture per cent and low calorific value. However, this crop highly prone to pest and diseases. Array of pathogens such as fungi, bacteria, viruses and nematodes cause a significant losses in the sense of production and export. Among these, downy mildew caused an oomycete fungi *Pseudoperonospora cubensis* is one of the major foliar disease and its occurrence is highly influenced by the environmental conditions such as temperature, humidity, and rainfall which are increasingly affected by climate change.Downy mildew pathogen thrives in cool and humid conditions. There are several reports pointing that, climate change which leading to unpredictable shifts in weather patterns, that creating conditions which favorable for the spread of this disease. Increased periods of humidity and warmer nights promote the development of the pathogen and encourage its reproduction. Furthermore, fluctuations in day and night temperatures, often seen with climate change, create conducive environments for pathogen sporulation, increasing the risk of infection in ridge gourd.Further, response of plants to elevated CO_2 and temperature have been much studied in recent years but effects of climate change on pathological responses are largerly unknown. It is fact that, Climate change may affect the defense responses in plants by several ways. Elevated CO_2can alter the plant's metabolism, affecting the balance of carbon and nitrogen in the plant. This might reduce the concentration of defense-related compounds, weakening the plant's ability to fend off the pathogen. In the context of climate change, the biochemical interplay between ridge gourd plants and the downy mildew pathogen is likely to shift in ways that may increase the severity and frequency of infections. Rising temperatures, altered precipitation, and increased CO_2levels can enhance pathogen virulence, disrupt plant immune responses, and lead to oxidative stress, all of which make plants more vulnerable to downy mildew.Hence, an attempt has been made to understand the response of *P.cubensis*under different simulated climatic conditions.

MATERIALS AND METHODS

The relationship of elevated CO_2 and temperatures on downy mildew severity of ridge gourd, was systematically conducted under Open Top Chambers (OTCs) at Centre for Agroclimatic studies, UAS, Raichur during *kharif* 2022. The OTCs are a specialized facility, where a specific level of CO_2 is maintained through the emission of CO_2 from the cylinders when needed. Each chamber was constructed by iron structure, keeping the top or frustum open for free-air exchange in order to reduce temperature and humidity. Each open top chamber (OTC) is considered as one treatment with the adjustment of different levels of CO_2 and temperature whereas, open plot served as a reference for the study. Susceptible variety**,** NAGA F1was sown in five pots for each set and raised as per recommended practices except for downy mildew management. The seedlings in pots were subjected to five different set of treatments in OTCs as mentioned below. Twenty five days after sowing, artificial inoculation was done with freshly prepared sporangial suspension (1000 sporangia/ ml). The downy mildew intensity was recorded at weekly interval with the first observation of disease on referring 0-5 scale (Singh *et al.*, 1996) and PDI was calculated. The treatment details are furnished below.

T_1: Elevated CO_2 @ 550(±)25 ppm with normal temperature

T_2: Elevated CO_2 @ 550(± 25 ppm with 2 °C rise in temperature

T_3: Ambient CO_2 @ 410(±)25 ppm with 2 °C rise in temperature

T_4: Ambient CO_2 @410(±)25 ppm with normal temperature

T_5: Open plot (reference plot)

Further, the biochemical analysis was carried out to know the effect of elevated CO_2 and temperature levels on total sugars and total phenolic content in healthy and infected leaves of ridge gourd at seven days after inoculation. The detailed methodology is given hereunder.

Estimation of total sugars

The reducing sugar content (RSC) was determined using the 3,5-dinitrosalicylic acid (DNSA) method. The measurement was performed according to the procedure of Krivorotova and Sereikaite (2014) with little modification. DNSA reagent was prepared by dissolving 1 g of

DNSA and 30 g of sodium-potassium tartaric acid in 80 mL of 0.5 N NaOH at 45°C. After dissolution, the solution was cooled down to room temperature and diluted to 100 mL with the help of distilled water. For the measurement, 2 mL of DNSA reagent was pipetted into a test tube containing 1 mL of plant extract (1 mg/mL) and kept at 95°C for 5 min. After cooling, 7 mL of distilled water was added to the solution and the absorbance of the resulting solution was measured at 540 nm using a UV-VIS spectrophotometer. The reducing sugar content was calculated from the calibration curve of standard D-glucose (200-1000 mg/L), and the results were expressed as mg D-glucose equivalent (GE) per gram dry extract weight.

Estimation of total phenol

The total phenolic contents of the aqueous and ethanolic extracts of the samples were estimated using the Folin Ciocalteau reagent (FCR) as described by Singleton and Rossi (1965). The calibration curve was plotted by mixing 1 ml aliquots of 50, 100, 150, 200, 250, 300, 350, 400 and 450 µg/ml of Gallic acid solutions with 0.5 ml of Folin Ciocalteu reagent (diluted tenfold) and 2.0 ml of sodium carbonate solution. The absorbance was measured after 30 min at 650 nm. Total phenol content was then expressed as mg of GAE/gm of extract.

Results

Effect of elevated CO_2 and temperature on downy mildew disease progression

Effect of elevated CO_2 and temperatures on downy mildew severity in ridge gourd was studied during *kharif* 2022 at Centre for Agroclimatic studies, UAS, Raichur using three Open Top Chambers (OTCs) adjusted with different CO_2 and temperature levels. The observations on downy mildew disease progression was taken initially at 30 DAS and continued the recording upto physiological maturity (79 DAS). Among the different CO_2 and temperature levels, the significant highest disease severity of 52.68 PDI was noticed in open reference plot followed by ambient CO_2 at 410 ±25 ppm with normal temperature (46.80 PDI). Significantly, the moderate disease severity of 37.76 PDI was noticed in OTC's with elevated CO_2 at 550 ±25 ppm with normal temperature (28 ^{0}C). Meanwhile, the lowest disease intensity of 13.49 PDI was recorded in chambers with ambient CO_2 at 410 ±25 ppm with 2^{0}C rise in temperature followed by elevated CO_2 at 550±25 ppm with 2 ^{0}C rise in temperature (21.32 PDI) (Table. 1 & Fig. 1).

Effect of elevated CO_2 and temperature levels on biochemical composition of ridge gourd in relation to downy mildew

Healthy and infected leaf samples from ridge gourd were drawn from different OTC's with varied levels of CO_2 and temperatures and subjected to biochemical analysis for the estimation of total sugars and total phenolic content at different dates after inoculation. Assessment of these biochemical parameters gives an insights on factors responsible in imparting resistance against downy mildew over different simulated environmental conditions.

Among the different CO_2 and temperature levels, the treatment Elevated CO_2 at 550(±)25 ppm with 2 °C rise in temperature recorded highest total sugar content of 17.89 mg g^{-1}f.w in healthy leaves and after 14 days after post innoculation (DPI), there is decline in sugar content to 9.57 mg g^{-1}f.w. The next higher total sugar content of 14.28 mg g^{-1}f.w was noticed in the treatment Elevated CO_2 at 550(±)25 ppm with normal temperatures for healthy leaves. However as the disease progressed (14 DPI), there is a decline in the total sugar content in an infected leaves (9.43 mg g^{-1}f.w). Meanwhile, among the different treatments, the lowest total sugar content (9.15 mg g^{-1}f.w) was noticed in healthy leaves further, it is reduced to 7.04 mg g^{-1}f.w after 14 DPI. Similar trend was noticed for total phenolic content in ridge gourd at different dates after inoculation, wherein significantly highest total phenoloic content of 129.25 µg of GAE g^{-1} was recorded for the treatment ambient CO_2 at 410(±)25 ppm with 2 °C rise in temperature at 14 DPI followed by the treatment elevated CO_2 at 550(±)25 ppm with 2 °C rise in temperature with 120.73 µg of GAE g^{-1}. Meanwhile, significant lowest total phenoloic content (109.65 µg of GAE g^{-1}) after 14 DPI was recorded for the treatment Open/reference plot followed by the treatment ambient CO_2 at410(±)25 ppm with normal temperature with 111.32 µg of GAE g^{-1} at 14 days after inoculation (Table. 2).

Discussion

Very few empherical studies were conducted on climate change effects on downy mildew disease progression in limited crops. Both elavated CO_2 and temperature levels significantly impacted the progression of downy mildew disease in ridge gourd (Fig. 2). A 2 ^{0}C rise in temperature levels resulted in decline of disease severity in comparision with increased CO_2 levels with normal temperature, wherein the disease severity get shoots up due to the increase in the biomass of plant as CO_2 gets accumulated in the plant tissues. Elevated carbon dioxide (CO_2) resulted in an increase in photosynthesis leading to plant growth, biomass and yield (Rai *et al.* 2016; Ainsworth and Long 2005). On the other hand, an increase in temperature alone did not affect the chlorophyll content (Pugliese *et al.*, 2010). However, when there is an ambient CO_2 without rise in temperature showed high disease intensity as temperature playing significant role in disease development. Gilardi *et al.* (2016) reported that, there is a significant increase in disease incidence of downy mildew of basil incited by *Peronospora belbahrii* when subjected at double level of carbon dioxide (850 ppm) with moderate temperatures ranging from 18-26 °C. However, the increase in CO_2 levels did not determine the increase in disease incidence at the maximum tested temperatures (26-30 °C), which are not favourable for the development of downy mildew. Similarly, in bean rust causing pathosystems (*Uromyces appendiculatus*/*Phaseolus vulgaris*), temperature, CO_2 levels and their interaction significantly affected disease severity in both the pathosystems. Higher carbon dioxide levels significantly increased rust severity, in particular at the lowest tested temperatures (14-22 °C), while the highest tested temperatures inhibited rust development (Gilardi *et al.*, 2016).

Corrspondingly, there is a significant effect of elevated CO_2 and temperature levels on the biochemical composition in ridge gourd before and after the inoculation. Increased levels of both temperature and CO_2 significantly increased the total sugar content in healthy leaves when compared with ambient levels of temperature and CO_2. This is because, as higher CO_2 levels results in greater photosynthetic rates initially but often photosynthetic acclimation, *i.e.*, down regulation of net photosynthetic rates in the long term (Long *et al.*, 2004; Kirschbaum, 2011), which indicates that, increase in CO_2 concentration can substantially affect the biochemical composition of leaves. From the previous research findings it can be concluded that, elevated CO_2 and temperature levels significantly enhance the total sugar content in major crop species. A meta-analysis report showed that, the elevated CO_2 increased the concentrations of glucose by 13.2 %, fructose by 14.2 %, sucrose by 3.7 % and total soluble sugars by 17.5% in all the vegetables (Dong *et al*., 2018). Similarly, the plants exposed to higher temperature levels shows slight increase in total sugar content (TS) in major crops. At an higher temperature conditions, the decomposition rates of glucose or oligomers (cellotetraose, cellotriose, and cel- lobiose) are faster than the regular cellulose hydrolysis rate that occurs in ambient conditions (Zhao *et al.*, 2011).However, there is a sharp decline in total sugars upon inoculation with *P. cubensis* among the treatments. The decrease in sugar synthesis is due to necrotic like symptoms on leaf surface at later stages, which hinders the photosynthesis and lead to less sugar synthesis. Murria *et al*. (2022) reported the significant reduction in photosynthetic rate due to infection by *sclerospora graminicola* on pearl millet. However, the levels of total phenols, peroxidase and polyphenol oxidase (PPO) were increased as a results of secondary defence response.

Conclusion

From the above results it can be concluded that, the pathogen,*P. cubensis*exhibitted a significant variations in disease severity under stimulated envirnomental conditions with different CO2 and temperature levels. Increase in temperature is certainly not favourable for the pathogen to its propogation. However, accumulation of excess CO_2 resulted in moderate severity due to conducive biochemical composition of leaves. Treatments with elevated CO_2 and temperature showed a decline in total sugars as the disease progressed, whereas treatments with ambient CO_2 and elevated temperatures led to increased phenolic content, suggesting a potential mechanism for enhanced disease resistance. These findings highlight the importance of understanding plant-pathogen interactions under changing environmental conditions and may inform future management strategies for downy mildew in the context of climate change.

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Table 1. Effect of elevated CO2 and temperature on downy mildew disease progression in ridge gourd

Treatment details	**Standard Meterological Week**							
	32	**33**	**34**	**35**	**36**	**37**	**38**	**39**
	Per cent Disease Index (PDI)							
	10th Aug (30 DAS)	**17TH Aug (37 DAS)**	**24th Aug (44 DAS)**	**31st Aug (51 DAS)**	**7th Sept (58 DAS)**	**14th Sept (65 DAS)**	**21st Sept (72 DAS)**	**28th Sep (79 DAS)**
T_1: Elevated CO_2 @ 550(±)25 ppm with normal temperature	9.66 (18.10)*	14.62 (22.47)	16.59 (24.03)	21.14 (27.37)	24.81 (29.87)	30.04 (33.23)	33.64 (35.44)	37.76 (37.91)
T_2: Elevated CO_2 @ 550(±)25 ppm with 2 °C rise in temperature	6.46 (14.69)	7.82 (16.23)	10.01 (18.43)	12.82 (20.98)	14.69 (22.52)	16.46 (23.93)	18.06 (25.14)	21.32 (27.49)
T_3: Ambient CO_2 @ 410(±)25 ppm with 2 °C rise in temperature	2.66 (9.32)	3.87 (11.33)	5.40 (13.42)	7.19 (15.55)	9.01 (17.46)	11.07 (19.43)	12.50 (20.70)	13.49 (21.55)
T_4: Ambient CO_2 @410(±)25 ppm with normal temperature	10.63 (19.02)	18.20 (25.25)	21.94 (27.92)	29.87 (33.12)	38.77 (38.50)	40.33 (39.42)	42.30 (40.56)	46.80 (43.15)
T_5: Open plot (reference plot)	9.10 (17.56)	11.63 (19.93)	18.18 (25.23)	24.29 (29.52)	30.60 (33.58)	38.52 (38.36)	45.18 (42.22)	52.68 (46.53)
S.Em(±)	**0.30**	**0.25**	**0.30**	**0.22**	**0.34**	**0.30**	**0.26**	**0.23**
C.D. @ 1 %	**1.26**	**1.07**	**1.27**	**0.94**	**1.42**	**1.26**	**1.09**	**0.96**

* Figures in the parentheses are arc sine transformed values

Table 2. Influence of elevated CO_2 and temperatures on biochemical composition of ridge gourd in healthy and infected leaves at different dates after inoculation.

Treatment details	**Reducing sugar (mg/g of fresh weight)**			**Non-reducing sugar (mg/g of fresh weight)**			**Total sugar (mg/g of fresh weight)**			**Total phenols(µg of GAE g^{-1})**		
	H	**7 DPI**	**14 DPI**	**H**	**7 DPI**	**14 DPI**	**H**	**7 DPI**	**14 DPI**	**H**	**7 DPI**	**14 DPI**
T_1: Elevated CO_2 @ 550(±)25 ppm with normal temperature	11.22	9.79	7.09	3.16	2.74	2.34	14.28	12.53	9.43	106.30	110.40	115.36
T_2: Elevated CO_2 @ 550(±)25 ppm with 2 °C rise in temperature	12.58	10.82	7.78	5.30	3.10	1.78	17.89	13.93	9.57	107.78	112.64	120.73
T_3: Ambient CO_2 @ 410(±)25 ppm with 2 °C rise in temperature	9.17	8.22	6.30	2.90	2.28	2.21	12.07	10.50	8.51	105.52	114.66	129.25
T_4: Ambient CO_2 @410(±)25 ppm with normal temperature	7.98	7.58	6.29	2.48	2.17	2.04	10.47	9.75	8.34	107.18	108.50	111.32
T_5: Open plot (reference plot)	6.96	5.68	5.52	2.19	1.87	1.52	9.15	7.55	7.04	106.29	108.33	109.65
S.Em(±)	**0.20**	**0.14**	**0.18**	**0.16**	**0.11**	**0.12**	**0.29**	**0.19**	**0.18**	**0.17**	**0.27**	**0.29**
C.D. @ 1 %	**0.82**	**0.61**	**0.75**	**0.68**	**0.45**	**0.51**	**1.23**	**0.82**	**0.76**	**0.74**	**1.14**	**1.23**

H: Healthy seedlings; DPI: Days after post inoculation

		
Fig. 1aElevated CO2 & temperature levels	**Fig. 1bElevated CO_2 at 550±25 ppm with normal temperature**	**Fig. 1c Ambient CO_2 at 410±25 ppm with 2 ^{0}C rise in temperature**

Fig.1 Experimental setup for climate change studies on downy mildew disease progression on ridge gourd (Fig. 1a); Effect of different levels of CO_2 and temperature on downy mildew disease progression in ridge gourd (1b & 1c)

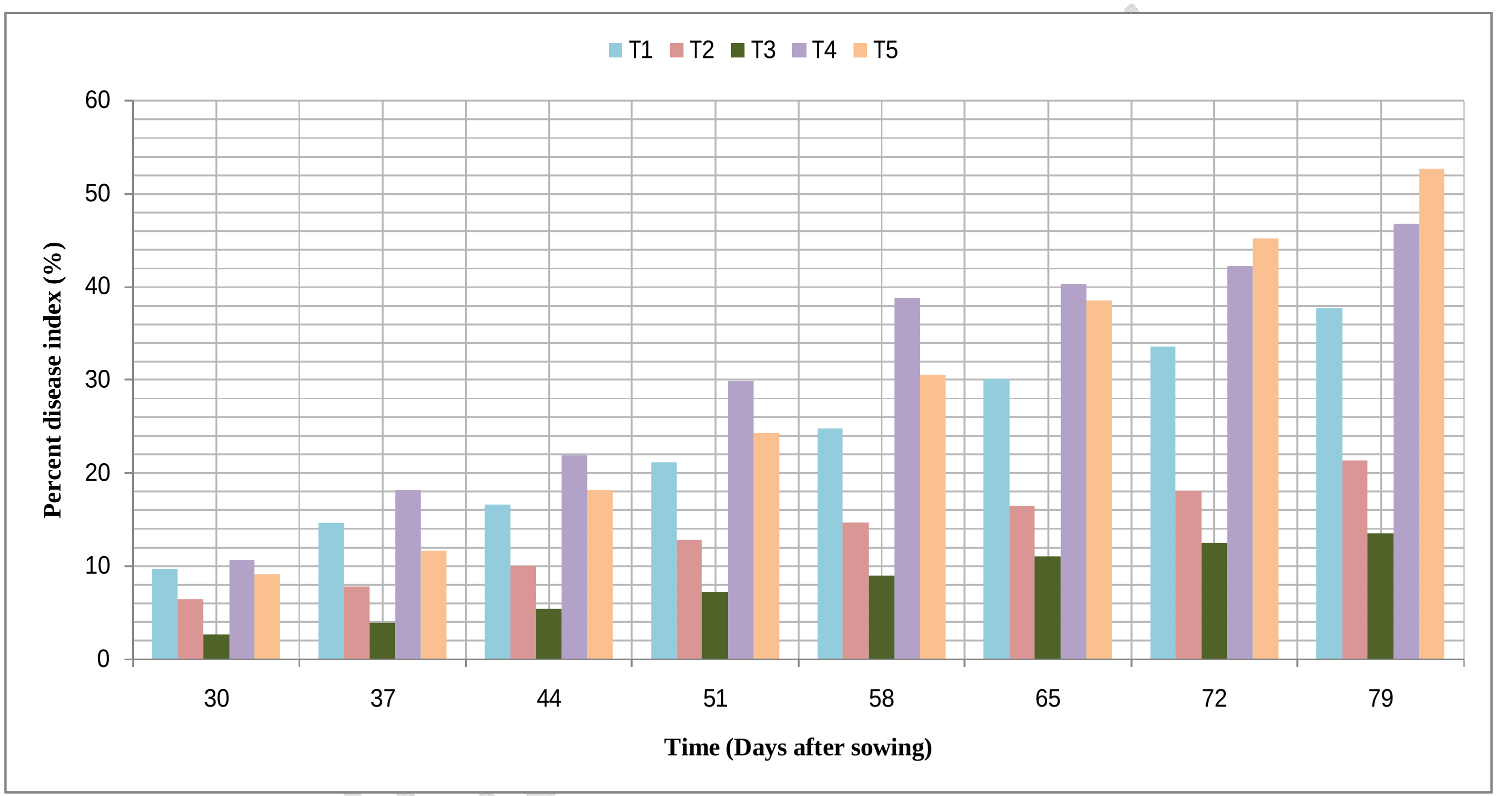

Fig. 2Effect of elevated CO2 and temperature on downy mildew disease progression in ridge gourd