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EFFECT OF *CUSCUTA* INFESTATION ON GROWTH, PHYSIOLOGICAL AND BIOCHEMICAL ATTRIBUTES OF *CONOCARPUS ERECTUS* L. FROM DIVERSE HABITATS

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Abstract

Cuscuta is a parasitic plant that is increasingly becoming an ecological and management problem especially in urban and semi-arid areas. This paper examined the environmental factors and the host physiological reactions that resulted in *Cuscuta campestris* infestation in *Conocarpus erectus* in four ecologically different areas of Punjab, Pakistan (Faisalabad, Khanewal, Toba Tek Singh and Wah Cant). Incidence measurements in the field were correlated with laboratory analyses in order to measure growth, photosynthetic pigments, osmolytes, stress markers, and antioxidant enzymes between the infested and healthy hosts. Host-parasite-environmental interactions were deciphered using statistical modelling, such as multivariate analysis, principal component analysis (PCA) and multiple linear regressions (MLR). Findings showed that site-specific climatic conditions, especially humidity and temperatures, were highly affecting the *Cuscuta* infestation intensity, as well as the host physiological impairment. High infestation rates, oxidative stress and high antioxidant and phenolic defenses were observed in warm and moderately humid environments (Faisalabad, Khanewal) and low parasite success and milder host responses in mild or dry cooler sites (Wah Cant, Toba Tek Singh). PCA predicted 83.7 per cent of the total variance and set the difference between the stressed infested hosts that exhibited high activity of antioxidants and osmolytes and the healthy pigment rich individuals in cool and moist regions. The interactions of humidity the most important environments predicting *Cuscuta* spread and host mortality and the next important factor was temperature, whereas excessive rain and low temperatures decreased the potential infestation. These data support the idea that the *Cuscuta*-*Conocarpus* interactions are controlled by the synergistic climatic controls and host metabolic adaptations. The infestation severity depended on environmental gradients which also interfere with the distribution of host defense between the resistance to growth and resistance to stress.

Key words: *Cuscuta campestris*; Dodder; *Conocarpus erectus*; Green buttonwood; Host specificity; Oxidative stress; Humidity gradient, Parasitic ecology, Environmental drivers

Abbreviations: Infestation status: H: Healthy; I: Infested; Site: FS: Faisalabad; Kn: Khanewal; Tt: Toba Tek Singh; Ec: Wah Cant Environmental attributes: Ele: Elevation; MxT: Maximum Temperature; MiT: Minimum temperature; AAT: Average annual temperature; ARF: Annual Rainfall; MxH: Maximum humidity; MiH: Minimum humidity; ARH: Annual relative humidity; Infestation attributes: Cin: *Cuscuta* Incidence; InH: Numbered of infested hosts; HMr: Host mortality; HCA: Host canopy area covered; Hhh: Host health; Chh: *Cuscuta* health; Host attributes: PH: Plant height; SFW: Shoot fresh weight; SDW: Shoot dry weight; LFW: Leaf fresh weight; LDW: Leaf dry weight; LA: Leaf area; Chla: Chlorophyll a; Chlb: Chlorophyll b; Car: Carotenoids; TSS: Total soluble sugars; TFA: Total free amino acids; TSP: Total soluble proteins; Prot: Protease; βAmy: βAmylase; Prol: Proline; GB: Glycine betaine; ASA: Ascorbic acid; SOD: Superoxide dismutase, POD: Peroxidase; CAT: Catalase; MDA: Malondialdehyde dehydrogenase; H_2O_2: Hydrogen per-oxide; Phen: Phenolics; Fla: Flavonoids; Anth: Anthocyanin; APX: Ascorbate peroxidase

Introduction

Parasitic plants, particularly those in the genus *Cuscuta*, represent a major, but an understudied, biotic stress agent in ecological and agricultural systems (Press & Graves, 1995). *Cuscuta* is a holoparasite stem that establishes haustorial associations with its host plants to not only get water and inorganic nutrients but also organic carbon, thus creating several physiological burdens to the hosts (Kaiser *et al*., 2015). The results of this type of parasitism may be extensive in terms of decreased photosynthetic ability, changes in the pigment content and alteration of the water relations to significant alterations in antioxidant metabolism (Kaiser *et al*., 2015). The effects are usually similar or coincide with the effects of abiotic stresses like salinity, drought, or nutrient imbalance (Ashraf & Harris, 2013). The investigation of *Cuscuta* infestation in diverse habitats will give the chance to define the way of interaction between the biotic and the abiotic factors in the development of the host-parasite interaction, contributing to the general picture of plant adaptive to the combined situations (Phoenix & Press, 2005).

Conocarpus erectus (Buttonwood) is a particularly suitable model for this kind of investigation. It is a species native to coastal or saline environments but is also frequently planted across a range of habitats including urban, roadside, wasteland and agricultural environs in Pakistan and other subtropical regions (Hameed *et al*., 2012; Safwat *et al*., 2018; Khalil *et al*., 2020). Previous phytochemical and biological studies have established that *C. erectus* possesses a substantial complement of secondary metabolites i.e. phenolics, flavonoids, tannins

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and shows antioxidant and other bioactive properties (Abdel-Hameed, 2009). At the same time, some studies under abiotic stress (salinity, pollution, water deficit) have shown that *C. erectus* is capable of physiological and structural plasticity, adjusting ionic content, osmolyte concentrations, and antioxidant enzyme activities to cope with harsh conditions (Iqbal *et al.*, 2024). This background suggests that *Cuscuta* infestation on *C. erectus* may amplify or modify such stress responses, or perhaps trigger defense mechanisms usually associated with abiotic stress.

The understanding of how parasitic plants such as *Cuscuta* influence the physiological, biochemical, and ecological dynamics of their hosts, particularly woody ornamentals like *Conocarpus erectus* L. is very necessary from its management perspective (Mehmood *et al.*, 2024). Despite being considered aggressive generalist parasite and having broad host preferences (Dawson *et al.*, 1994; Press & Phoenix, 2005), the biology of *Cuscuta* spp. in relation to perennial woody species has not been studied in a variety of habitats. *Conocarpus erectus* is currently a widespread part of greening project both in urban and rural areas in Pakistan. Its environmental plasticity allows it to be used as a model in the study of host-parasitic interactions in the context of natural gradients of environmental stress. Combined biotic and abiotic stress-like effects are imposed by *Cuscuta* since it attaches itself to host tissues through haustoria and directly extracts photosynthates, water, and mineral nutrients (Kaiser *et al.*, 2015). Subsequently, the study of *C. erectus* adapting to its growth, osmotic potential, antioxidant metabolism, and ionic response to parasitism will be deeply informative of its defenses, resource distribution patterns and resilience limits.

The study integrates physiological (pigments, photosynthetic capacity), biochemical (osmolytes, antioxidant enzymes, phenolics, flavonoids), and ionic parameters to decipher the cascade of responses in *C. erectus* under *Cuscuta* infestation. The choice of these parameters was made on the basis of their proven use as parameters of stress tolerance and compatibility of host-parasite (Press & Graves, 1995; Chen *et al.*, 2025). As an example, osmoprotection and ROS scavenging through the accumulation of proline, glycine betaine, and soluble sugars has been implicated (Ahmad *et al.*, 2011), and antioxidant enzyme activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) alterations can be used to measure the extent of mitigating oxidative stress (Ashraf & Harris, 2013). Likewise, phenolic and flavonoid compounds are also multifunctional antioxidants and biochemical deterring agents affecting parasite adhesion and growth (Zaynab *et al.*, 2018). Using these characteristics in connection with field-obtained indices of infestation (e.g., *Cuscuta* coverage, shoot vigor, and canopy density, host mortality, *Cuscuta* incidence rates), the paper will uncover short-term physiological costs and long-term adaptive responses in the host.

The study hypothesis, based on the above mentioned objectives and expected results, was that the environmental factors and host plant properties affect *Cuscuta* infestation causing different degrees of infestation and influence on the health of a host plant. The research questions are: (1) how environmental factors affect the *Cuscuta* infestation rates and host health, (2) do host plant characteristics affect the infestation rates and severity, (3) how the infestation rates and host mortality can be predicted by the environmental and host characteristics. The potential results are to determine the main environmental determinants of *Cuscuta* infestation, the correlation between host characteristics and infestation levels and come up with predictive models of infestation and host plant death. The results of this research may serve as useful information on the ecological interactions of *Cuscuta-Conocarpus* and to come up with effective methods to manage *Cuscuta* populations.

Materials and Methods

Cuscuta is a common plant-plant parasite infesting many economic, ornamental and exotic/introduced plants. The objective of the present study was to determine the growth responses of *Conocarpus* to *Cuscuta* infestation. For this purpose, samples of *Cuscuta* infested *Conocarpus* plants were collected from for sites of Punjab Province i.e. Khanewal, Toba Tek Singh, Faisalabad and Wah Cant. These sites were selected on the basis of *Cuscuta* prevalence, host (*Conocarpus*) canopy area covered and geographic localities of the study sites. The environmental and geographic attributes of the study sites is presented in Table 1.

Host's infestation status: The host plants were surveyed in the study area. Representative photographs were taken (Figs. 1 and 2). The hosts and parasite were carefully evaluated for infestation attributes. The total number of infested host plants (n) was counted in each study area and degree of incidence was quantified as abundant, frequent and rare. The host mortality was counted as number (n) of dead plants. The host canopy area covered (%) by *Cuscuta* parasite was estimated. *Conocarpus* and *Cuscuta* health (%) was also measured as visual estimates (Table 2).

Table 1. Environmental and geographic attributes of *Cuscuta* collection sites from four eco-regions of Punjab Province, Pakistan.

Sites →	Khanewal	Toba Tek Singh	Faisalabad	Wah Cant
District	Khanewal	Toba Tek Singh	Faisalabad	Rawalpindi
Elevation (m a.s.l.)	129	149	185	465
Latitude (N)	30° 19' 16"	30° 55' 31"	31° 26' 13"	33°47'15"
Longitude (E)	71° 58' 55"	72° 39' 34"	73° 4' 46"	72°45'05"
MxT (°C)	49	47	48	27
MinT (°C)	3	2	3	2
AAT °C	26.7	25.2	23.7	12.1
Rainfall (mm)	362	473	903	1410
MxH (%)	58	68	72	72
MiH (%)	21	24	43	36
ARH (%)	29	32	58	42
Site description	Barren plains	Irrigated land	Wasteland	Humid, cold, mountainous

Table 2. *Cuscuta* infestation and host health attributes from four eco-regions of Punjab Province, Pakistan.

Infestation attribute	Khanewal	Toba Tek Singh	Faisalabad	Wah Cant
Incidence	Abundant	Abundant	Abundant	Frequent
Infested host plants (n)	35	31	42	18
Host mortality (n)	4	6	8	2
Host canopy area covered (%)	72	78	82	45
Conocarpus health (%)	65	62	73	89
Cuscuta health (%)	85	73	94	69

Infested and healthy host (*Conocarpus*) attributes

Growth attributes: The plant height (m) of host plant was measured from soil to the top of a standing tree. The shoot (g m^{-1} branch) and leaf fresh weight (g m^{-1} branch) was measured. These plant materials were wrapped in paper bags, dried in an oven at 65ºC to a constant dry weight and their dry weights determined with an analytical balance. For determination of leaf area, the lengths and widths of six fully mature leaves were measured. The formula proposed by Polster & Reichenbach (1958) was employed to calculate the leaf area (cm m^{-1} branch):

Leaf area = Maximum length x Maximum width x 0.857

Concentration of photosynthetic pigments: The concentration of photosynthetic pigments in host plant was determined spectrophotometerically following the methods of Arnon (1949) for chlorophylls and Davies (1965) for carotenoids. These photosynthetic pigments (Chl *a*, Chl *b*, Car) were determined from the fresh leaf sample (0.1g) and extracted in 80% acetone and expressed as mg g^{-1} f. wt.

Organic osmolytes: The concentration of organic osmolytes was determined in fresh samples of *Cuscuta* infested and healthy host plants. The concentration of total soluble sugars (TSS) was determined following the method of Yemm and Willis (1954) and expressed as (µmol g^{-1} f. wt.). Total free amino acids was determined following the method of Hamilton and Van Slyke (1943) and expressed as (µmol g^{-1} f. wt.). Bradford (1976) method was used to determine total soluble proteins (µmol g^{-1} f. wt.).

Protein and starch degrading enzymes: Proteases and beta-amylases are enzymes that play important roles in plant defense and nutrient mobilization. The manipulation of proteases and beta-amylases in host plants by *Cuscuta* could be a strategy to acquire essential nutrients, such as nitrogen and carbon, to support the parasite's growth and development. Protease was extracted following the method of Ainouz *et al*., (1972) while enzyme quantification was done following Lowry *et al*., (1951). The final concentration of enzyme was expressed as units mg^{-1} protein). The activity of βAmylase was quantified using Bernfeld (1955) method and expressed as units mg^{-1} protein.

Osmoprotectants: Proline and GB are osmoprotectants that assist plants to survive under abiotic stress such as drought, salinity and extreme temperature. The compounds are also used in the defense mechanism of plants against pathogens and pests. Proline concentration was ascertained in fresh leaf samples of the healthy and infested host plants when applying the methods of Bates *et al*., (1973) of ninhydrin and was represented as mmol g^{-1} FW. Glycinebetaine concentration was measured using the same procedure used by Grieve and Grattan (1983) and given as mmol/g^{-1} FW.

Oxidative stress parameters: The H_2O_2 (Hydrogen peroxide) and malondialdehyde dehydrogenase (MDA) are all signs of oxidative stress. They give an approximation of the damage to membranes and serve in various functions of plant defense against pathogens. The dehydrogenase of malondialdehyde was calculated according to Carmak & Horst (1991) and measured in nmol/g/fresh weight. The determination of hydrogen per-oxide was done as described by Velikova *et al*., (2000) and was expressed as mmol/g f. wt.

Anti-oxidative enzymes: The enzymes involved in the scavenging of reactive oxygen species (ROS) and cellular homeostasis against stress are called anti-oxidative enzymes. Their activity can be analyzed to understand the way the host plants react on *Cuscuta* infestation and determine what possible tolerance mechanisms may be used. The amount of ascorbic Acid (ASA) was measured using method of Mukherjee & Choudhri (1983) and was measured as mg g^{-1} FW. The method used to detect SOD was that of Giannopolitis & Ries (1977). The peroxidase (POD) and catalase (CAT) were established in line with the approaches of Chance & Maehly (1955). SOD, POD and CAT activity was in units of enzyme mg^{-1} protein. Activities of the ascorbate peroxidase (APX) were ascertained based on the method of Nakano & Asada (1981) and in mmol min^{-1} mg^{-1} protein.

Shoot ionic contents: The effects of *Cuscuta* parasitism on the nutrient balance and the ion homeostasis of the host plant were determined. The flame photometric determination of the shoot sodium (Na), potassium (K) and calcium (Ca) was done in the acid digested material in the method described by Wolf (1982). Their concentration was measured in mg g^{-1} d.wt.

Metabolites defense: Secondary metabolites (phenolics, flavonoids, and anthocyanins) are central in the plant defense against pathogens and pests. Phenolics (Phen) and Flavonoids (Fla) were determined by method as described in a study by Singleton & Rossi (1965) and Zhishen *et al*., (1999) respectively and represented as (mg gallic acid equivalent g^{-1} DW) and (mg quercetin equivalent g^{-1} DW); respectively. The amount of anthocyanin (Anth) was ascertained using the procedure of Giusti and Wrolstad (2001) and expressed in the form of mg Cyanidin-3-Glucoside Equivalents g^{-1} DW.

Statistical Analysis

The comparison of the infestation rates, host plant health, and environmental variables across habitats was done through **ANOVA**. The significance of habitat means was tested through least significant difference (**LSD @5%** confidence level) test. **Heatmap clustering** analysis was run to examine relationships between environmental variables, host plant characteristics, and infestation rates. **Multiple Linear Regression (MLR)** analysis was used to model the relationship between host-parasite attributes (e.g., infestation rate, host plant health) and one or more predictor (environmental) variables. **Principal Component Analysis** (PCA) was used to identify patterns in environmental variables and their relationship with infestation rates. All these analysis were performed in CoStat (version 6.303, Cohort software, USA), CanoCo (version 4.5, Biometrics - Plant Research International, Wageningen), and R (R studios Version 1.1.463, R version 4.0.3).

Results

***Cuscuta* incidence rates and host health:** *Cuscuta* infestation intensity varied noticeably across the four surveyed sites. The highest levels generally observed in Faisalabad and the lowest in Wah Cant. Incidence was recorded as abundant in Khanewal, Toba Tek Singh, and Faisalabad sites, while it was only frequent in Wah Cant. The highest number of infested host plants was recorded in Faisalabad (42) and Khanewal (35) and Toba Tek Singh (31), but Wah Cant had a minimum infestation (18). In line with this, the mortality of hosts varied between 8 individuals in Faisalabad and 2 in Wah Cant which indicates that there was a positive relationship between intensity of infestation and host mortality. Faisalabad was also highest in the host canopy area covered by *Cuscuta* (82%), then Toba Tek Singh (78%), and Khanewal (72%), but fell drastically to 45% in Wah Cant, indicating the weaker parasitic pressure in the cool and humid climate of the northern area. The opposite trend was observed in *Conocarpus* health where the lowest was at Toba Tek Singh (62%), followed by Khanewal (65%), Faisalabad (73%), and Wah Cant (89%). Conversely, *Cuscuta* health grew best in Faisalabad (94%) which is an indicator of the favorable conditions under warm, but not excessive, humidity. It reduced in Wah Cant (69%), which is indicative that the intensity of the parasite is not high in cool and high altitude environments (Table 2).

Host growth attributes: The plant height (PH) of infested hosts declined by 11-18%, being the least affected in Khanewal (3.6 to 3.2 m) and the most in Wah Cant (3.3 to 2.7 m). Shoot fresh and dry weights (SFW, SDW) decreased by 5-15%, with the greatest losses observed in Faisalabad (SFW: 9.31 to 8.9 g m^{-1}; SDW: 2.81 to 2.16 g m^{-1}), reflecting stronger parasitic stress under warm, semi-arid conditions. Similarly, leaf fresh and dry weights (LFW, LDW) declined notably in all ecotypes, averaging 10-15% reduction. The leaf area (LA) decreased by 8-15%, with the maximum noted in Wah Cant (12.9 to 10.9 cm m^{-1}). Among sites, Toba Tek Singh and Khanewal hosts maintained relatively better growth, likely due to higher soil moisture and irrigation availability, whereas the hot plains of Faisalabad and the cooler, humid zone of Wah Cant exhibited stronger biomass suppression (Table 3).

Concentration of photosynthetic pigments in host plants: Chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*) declined by 20-35% in infested plants, with the highest reductions recorded in Faisalabad (Chl *a*: 1.92 to 1.43 mg g^{-1} FW; Chl *b*: 0.93 to 0.63 mg g^{-1} FW) and Khanewal (1.72 to 1.29 mg g^{-1} FW), corresponding to greater *Cuscuta* vigor in these warmer regions. Toba Tek Singh hosts maintained slightly higher pigment stability (Chl *a*: 1.84 to 1.39 mg g^{-1} FW), likely due to moderate rainfall and temperature, while Wah Cant, despite its cool and humid climate, showed the lowest pigment levels (Chl *a*: 1.69 to 1.04 mg g^{-1} FW; 0.89 to 0.67 mg g^{-1} FW) with comparable proportional losses. Total chlorophyll (T Chl) and carotenoids (Car) followed similar trends, with mean decreases of 25-30%, indicating reduced photosynthetic efficiency and weakened photo-protection under parasitic stress. The highest decline in carotenoids was observed in Toba Tek Singh (1.29 to 1.09 mg g^{-1} FW) and Faisalabad (1.21 to 1.08 mg g^{-1} FW), suggesting enhanced oxidative degradation in the hot plains (Table 3).

Organic osmolytes: Significant variation was observed in the levels of organic osmolytes among the *Cuscuta*-infested and healthy hosts of *Conocarpus* plants. The concentration of total soluble sugars (TSS) showed a consistent decline under infestation in all hosts compared with their respective healthy controls. Among healthy plants, the highest TSS content (0.92 µmol g^{-1} f. wt.) was recorded in the Khanewal, followed by Toba Tek Singh (0.89 µmol g^{-1} f. wt.) and Wah Cant (0.88 µmol g^{-1} f. wt.) hosts. The lowest was found in Faisalabad (0.85 µmol g^{-1} f. wt.) ecotype. When *Cuscuta* was infested, the TSS levels dropped significantly, with the Wah Cant hosts retaining comparatively higher levels of sugar concentrations (0.66 µmol g^{-1} f. wt.) than Toba (0.59 µmol g^{-1} f. wt.) and Faisalabad (0.61 µmol g^{-1} f. wt.) hosts, which showed a relative physiological reaction of the host populations. On the same note, the total free amino acids (TFA) also showed a significant decrease in infestation stress. The TFA contents of healthy *Conocarpus* hosts were between 0.66 and 0.83 µmol g^{-1} f. wt., with the highest and the lowest rates of 0.83 and 0.66 respectively in Khanewal and Toba Tek Singh. Infection by *Cuscuta* led to a drastic reduction in TFA (0.29 µmol g^{-1} f. wt.) in Khanewal (0.29 µmol g^{-1} f. wt.) and Wah Cant (0.30 µmol g^{-1} f. wt.) which may indicate either increased use of amino acids or compromised synthesis by the parasitism. The minimal values of TFA as detected in infested tissues suggest the potential interference with the nitrogen metabolism related to the parasitic attachment. On the contrary, the total soluble proteins (TSP) also declined drastically during infestation. Protein level was observed to be highest in Wah Cant (4.09 µmol g^{-1} f. wt.), then Faisalabad (3.91 µmol g^{-1} f. wt.), and Khanewal hosts had the least protein level (3.45 µmol g^{-1} f. wt.). Hosts infested had a significant reduction in TSP, and Wah Cant hosts had relatively high protein levels (2.97 µmol g^{-1} f. wt.), indicating partial tolerance.

Fig. 1 Heavy *Cuscuta* infestation on *Conocarpus eructs* (a: Khanewal, b: Toba Tek Singh, c) Faisalabad; d: Wah Cant.

Reserve metabolizing enzymes: The reserve metabolizing enzyme activity was highly changed in *Conocarpus* hosts infested with *Cuscuta* (Table 3). On the whole, a significant increase in the activity of the enzymes was observed in the conditions of *Cuscuta* infestation and it shows the violation of the stability of reserve metabolites and the activation of the degradation processes in the host tissues. Protease activity differed greatly among the hosts both in a healthy state and an infested state. The highest protease activity (97.1 units mg^{-1} protein) was found in Toba Tek Singh ecotype, with Faisalabad (92.8 units mg^{-1} protein) and Wah Cant (90.7 units mg^{-1} protein) following the Toba Tek Singh ecotype. The minimal activity was observed in Khanewal (86.4 units mg^{-1} protein) ecotype. Protease activity dropped significantly in all ecotypes on *Cuscuta* infestation, indicating an inhibition of protein degradation or loss of substrate proteins by the interaction of parasites. Although this was lesser, Toba Tek Singh hosts continued to have relatively higher protease activity (78.2 units mg^{-1} protein) indicating a more efficient protein turnover or partial tolerance mechanism than Khanewal with the lowest activity (68.7 units mg^{-1} protein). Conversely, the β-amylase activity had a reverse pattern with a big rise in all those infested hosts compared to their respective healthy controls. The β-amylase activity in the healthy plants was in the range of 20.8 to 22.7 units per mg^{-1} protein, with the highest value being observed in Toba Tek Singh and Faisalabad, and the lowest in the Wah Cant. After infestation, there was significant increment in the β-amylase activity with Khanewal recording highest (32.5 units mg^{-1} protein) followed by Wah Cant (31.5 units mg^{-1} protein) and Toba Tek Singh (30.7 units mg^{-1} protein). The rise in β-amylase activity during infestation was an indication of fast rate of hydrolysis of starch, which could have been essential to meet the high rate of energy requirement due to parasitic stress.

Compatible osmolytes: There were highly significant differences in the accumulation of suitable osmolytes, proline (Prol) and glycine betaine (GB) in the *Conocarpus* host plants in response to *Cuscuta* infestation (Table 3). These are major stress tolerance and osmotic adjustment indicators in plants. Proline varied widely in the healthy plants with values ranging between 19.7 and 23.3 µmol g^{-1} fresh weight (FW). The highest proline level was in Toba Tek Singh ecotype (Toba Tek Singh) with 23.3 µmol g^{-1} FW, and the value was lower at Khanewal (21.8 µmol g^{-1} FW) and Faisalabad (20.4 µmol g^{-1} FW). When infested with *Cuscuta*, there was a decrease in proline concentrations across all ecotypes indicating a decrease in synthesis or an increase in utilization by the stress of parasitism. Although this decrease, youngest infested plants (Toba Tek Singh) had the highest proline level (20.7 µmol g^{-1} FW) indicating relatively constant osmoprotective process whereas Khanewal had the lowest (17.6 µmol g^{-1} FW). The general decrease of proline concentration showed the parasitic interference of natural metabolic order that is connected with stress adaptation. On the same note, the levels of glycine betaine reduced significantly in the infested plants as opposed to their healthy counterparts. GB was found in the healthy tissues in the range of 18.2 to 21.8 µmol g^{-1} FW with the Wah Cant having the highest content with Faisalabad and Toba Tek Singh ecotypes having lower contents respectively. During infestation, all the ecotypes decreased dramatically, with the lowest levels (13.7 µmol g^{-1} FW) in Khanewal ecotype and quite high levels (17.2 µmol g^{-1} FW) in Faisalabad. The gradual decrease in GB indicates that the presence of *Cuscuta* parasitism inhibits the production or storage of quaternary ammonium compound in maintaining the osmotic pressure and safeguarding cellular organization during stress.

Oxidative stress markers: Table 3 demonstrates significant differences in levels of oxidative stress biomarkers, malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) between *Conocarpus* hosts under healthy and *Cuscuta*-infested conditions. Both parameters were significantly elevated on infestation, which is a sign of oxidative damages by the accumulation of oxidative stressors, which is reactive oxygen species (ROS). One of the indicators of lipid peroxidation, the MDA content, differed significantly among the ecotypes. Healthy plants had values of 0.49-0.62 nmol g^{-1} fresh weight (FW) and the highest and the lowest values were observed in Khanewal and Toba Tek Singh respectively. When *Cuscuta* was infested, MDA level rose significantly in all the ecotypes, an indicator of lipid destruction of the membrane as a result of oxidative stress. The highest concentration of MDA (0.99 nmol g^{-1} FW) was found in the Wah Cant ecotype (Wah Cant) and next were Khanewal (0.91 nmol g^{-1} FW), Faisalabad (0.82 nmol g^{-1} FW) and Toba Tek Singh (0.86 nmol g^{-1} FW), respectively. Such an acute rise of MDA indicates more intense peroxidative damage of cellular membranes, which probably happens because of an overproduction of ROS and a deficiency in antioxidant responses in the infested tissues. Equally, the level of hydrogen peroxide (H_2O_2) also experienced a remarkable increase of infestation among all ecotypes. H_2O_2 content in healthy plants was between 0.07 and 0.12 µmol g^{-1} FW with the highest and lowest value of 0.12 and 0.07 µmol g^{-1} FW in Wah Cant and Faisalabad respectively. The accumulation of H_2O_2 was also observed with enormously increase in infested samples with Toba Tek Singh recording highest level (0.38 µmol g^{-1} FW) followed by Wah Cant (0.34 µmol g^{-1} FW) and Faisalabad (0.33 µmol g^{-1} FW). Khanewal (0.29 µmol g^{-1} FW) had the least rise. The increased levels of MDA and H_2O_2 in infected hosts, in general, are the demonstrations of the critical oxidative stress and cell damage caused by *Cuscuta* oocytes parasitism. Wah Cant and Toba Tek Singh exhibited a relatively higher oxidative stress indices, indicating that they are more sensitive whereas Faisalabad revealed a relatively moderate index, indicating that it has a certain level of oxidative resilience during parasitic attack.

Fig. 2. Dead *Conocarpus* plants resulting from heavy *Cuscuta* infestation (a, b, c, d) and green *Conocarpus* plants with dead *Cuscuta* patches (e, f, g, h) suggesting that some plants of the same host species might have developed resistance mechanisms to override *Cuscuta* infestation (photo taken at Faisalabad).

Table 3. Growth, Physiological and biochemical attributes of healthy and *Cuscuta* infested host plants collected from diverse habitats.

Ecotypes	PH	Infested	SFW	Infested	SDW	Infested	LFW	Infested	LDW	Infested
	Healthy		Healthy		Healthy		Healthy		Healthy	
Ce-Fs	3.4±0.26b	2.9±0.18b	9.31±0.97c	8.9±0.82c	2.81±0.65d	2.16±0.38c	1.12±0.17c	0.98±0.14c	0.24±0.09c	0.15±0.04c
Ce-Kn	3.6±0.31a	3.2±0.24a	10.1±0.82b	9.1±0.76b	3.13±0.48c	2.79±0.46b	1.19±0.19b	1.08±0.11a	0.28±0.07a	0.11±0.01d
Ce-Tt	2.9±0.19c	2.8±0.21c	11.4±0.73a	10.7±0.91a	3.79±0.57b	3.17±0.51a	1.21±0.21a	1.04±0.09b	0.23±0.11c	0.14±0.03b
Ce-Wc	3.3±0.27b	2.7±0.15c	11.0±0.92b	10.8±0.73a	3.82±0.47a	3.28±0.47a	1.11±0.11c	0.91±0.12c	0.26±0.06b	0.16±0.04a
LSD(5%)	**0.21**	**0.28**	**0.21**	**0.17**	**0.19**	**0.21**	**0.033**	**0.019**	**0.017**	**0.009**
	LA	**Infested**	**Chla**	**Infested**	**Chlb**	**Infested**	**TChl**	**Infested**	**Car**	**Infested**
	Healthy		**Healthy**		**Healthy**		**Healthy**		**Healthy**	
Ce-Fs	14.3±1.1b	12.7±1.2a	1.92±0.21a	1.43±0.19a	0.93±0.04a	0.63±0.01a	2.85±0.12a	2.06±0.21a	1.21±0.09c	1.08±0.11b
Ce-Kn	15.8±1.3a	11.8±0.8b	1.72±0.19c	1.29±0.11b	0.86±0.02b	0.61±0.04b	2.58±0.17c	1.91±0.18b	1.34±0.11a	1.12±0.14a
Ce-Tt	13.5±0.9c	11.4±1.0c	1.84±0.27b	1.39±0.16b	0.98±0.06a	0.58±0.03c	2.82±0.09a	1.97±0.14b	1.29±0.14b	1.09±0.13b
Ce-Wc	12.9±1.2d	10.9±1.3d	1.69±0.20d	1.04±0.17c	0.89±0.05b	0.67±0.05a	2.78±0.14b	2.01±0.17a	1.26±0.13b	1.11±0.09a
LSD(5%)	**0.69**	**0.38**	**0.19**	**0.17**	**0.09**	**0.04**	**0.11**	**0.13**	**0.08**	**0.03**
	TSS	**Infested**	**TFA**	**Infested**	**TSP**	**Infested**	**Prot**	**Infested**	**βAmy**	**Infested**
	Healthy		**Healthy**		**Healthy**		**Healthy**		**Healthy**	
Ce-Fs	0.85±0.05c	0.61±0.08b	0.79±0.06b	0.34±0.02a	3.91±0.23b	2.56±0.18b	92.8±2.37b	71.4±3.02c	22.3±1.08a	29.1±0.27c
Ce-Kn	0.92±0.09a	0.62±0.09b	0.83±0.02a	0.29±0.04b	3.45±0.19d	2.69±0.14b	86.4±3.04d	68.7±2.75d	21.9±0.92b	32.5±0.13b
Ce-Tt	0.89±0.04b	0.59±0.06c	0.66±0.09c	0.37±0.01a	3.67±0.27c	2.27±0.19c	97.1±2.09a	78.2±2.49a	22.7±0.87a	30.7±0.29c
Ce-Wc	0.88±0.10b	0.66±0.07a	0.71±0.08b	0.30±0.04b	4.09±0.21a	2.97±0.22a	90.7±2.43c	75.2±2.92b	20.8±1.11b	31.5±0.18a
LSD(5%)	**0.011**	**0.019**	**0.034**	**0.032**	**0.134**	**0.201**	**3.71**	**2.14**	**0.34**	**0.97**
	Prol	**Infested**	**GB**	**Infested**	**MDA**	**Infested**	**H_2O_2**	**Infested**	**ASA**	**Infested**
	Healthy		**Healthy**		**Healthy**		**Healthy**		**Healthy**	
Ce-Fs	20.4±1.21c	18.5±0.87c	19.8±1.13b	17.2±0.47a	0.51±0.07c	0.82±0.06b	0.07±0.01c	0.33±0.02b	18.7±0.76b	21.3±0.57b
Ce-Kn	21.8±0.98b	17.6±0.93d	18.2±0.08c	13.7±0.86d	0.62±0.03a	0.91±0.07c	0.09±0.02b	0.29±0.01c	19.2±0.43a	22.7±0.25a
Ce-Tt	23.3±1.13a	20.7±0.89a	20.1±1.07b	15.5±0.92c	0.49±0.05d	0.86±0.04b	0.08±0.02c	0.38±0.03a	19.6±0.54a	20.6±0.37c
Ce-Wc	19.7±1.18c	19.3±1.17b	21.8±0.98a	16.4±0.74b	0.58±0.02b	0.99±0.03a	0.12±0.01a	0.34±0.04b	17.4±0.97c	21.7±0.53b
LSD(5%)	**1.43**	**1.42**	**1.17**	**1.03**	**0.37**	**0.41**	**0.02**	**0.07**	**0.59**	**1.17**
	SOD		**POD**		**CAT**		**APX**		**Na**	
	Healthy	**Infested**	**Healthy**	**Infested**	**Healthy**	**Infested**	**Healthy**	**Infested**	**Healthy**	**Infested**
Ce-Fs	4.6±0.34c	9.2±0.54b	16.2±0.56a	29.1±0.27b	30.2±1.21a	64.4±1.67a	0.08±0.02b	0.26±0.06b	32.5±0.58a	12.5±0.27c
Ce-Kn	5.1±0.29a	8.7±0.37c	15.1±0.48b	32.4±0.39a	21.5±1.49d	58.2±1.18c	0.09±0.01a	0.31±0.04a	29.1±0.49b	19.1±0.34a
Ce-Tt	4.9±0.33b	7.4±0.42d	14.9±0.39b	24.6±0.42d	24.6±1.09c	61.7±1.34b	0.06±0.03c	0.21±0.09c	21.7±0.67d	16.7±0.19b
Ce-Wc	4.8±0.31b	9.9±0.45a	15.8±0.62a	27.5±0.31c	28.7±1.18b	54.3±1.49d	0.08±0.01b	0.27±0.07b	24.3±0.32c	13.8±0.28c
LSD(5%)	**0.22**	**0.24**	**0.87**	**0.62**	**0.67**	**1.34**	**0.012**	**0.014**	**1.37**	**1.02**
	K	**Infested**	**Ca**	**Infested**	**Phen**	**Infested**	**Fla**	**Infested**	**Anth**	**Infested**
	Healthy		**Healthy**		**Healthy**		**Healthy**		**Healthy**	
Ce-Fs	10.4±0.11d	6.5±0.05d	4.58±0.28a	2.29±0.17a	23.5±0.17a	76.2±2.8b	11.7±2.1b	31.5±1.2d	0.21±0.01c	0.51±0.04b
Ce-Kn	11.2±0.19b	8.7±0.11a	3.61±0.17d	1.21±0.29d	21.6±0.23c	81.7±3.2a	10.6±1.8d	39.2±2.4a	0.28±0.05a	0.69±0.03a
Ce-Tt	10.8±0.08c	7.2±0.12c	4.19±0.35b	1.96±0.32b	20.7±0.15d	71.4±1.7d	12.7±1.4a	34.6±1.8b	0.23±0.03b	0.47±0.06c
Ce-Wc	11.5±0.13a	8.1±0.09b	3.92±0.26d	1.78±0.12c	22.9±0.31b	74.3±2.3c	11.1±1.9c	32.7±1.7c	0.27±0.05a	0.57±0.01b
LSD(5%)	**0.31**	**0.25**	**0.27**	**0.31**	**1.12**	**1.59**	**0.57**	**1.29**	**0.015**	**0.028**

Ecotypes: Ce-Fs: Faisalabad; **Ce-Kn:** Khanewal; **Ce-Tt:** Toba Tek Singh; **Ce-Wc:** Wah Cantt

Attributes: PH: Plant height (m); **SFW:** Shoot fresh weight (g m^{-1} branch); **SDW:** Shoot dry weight (g m^{-1} branch); **LFW:** Leaf fresh weight (g m^{-1} branch); **LDW:** Leaf dry weight (g m^{-1} branch); **LA:** Leaf area (cm m^{-1} branch); **Chla:** Chlorophyll a (mg g^{-1} f. wt.); **Chlb:** Chlorophyll b (mg g^{-1} f. wt.); **Car:** Carotenoids (mg g^{-1} f. wt.); **TSS:** Total soluble sugars (µmol g^{-1} f. wt.); **TFA:** Total free amino acids (µmol g^{-1} f. wt.); **TSP:** Total soluble proteins (µmol g^{-1} f. wt.); **Prot:** Protease (units mg^{-1} protein), **βAmy:** βAmylase (units mg^{-1} protein); **Prol:** Proline (µmol g^{-1} FW); **GB:** Glycine betaine (µmol g^{-1} FW); **ASA:** Ascorbic acid (µg g^{-1} FW); **SOD:** (Units mg^{-1} protein), **POD:** Peroxidase (Units mg^{-1} protein); **CAT:** Catalase (Units mg^{-1} protein); **MDA:** Malondialdehyde dehydrogenase (nmol g^{-1} fresh weight); **H_2O_2:** Hydrogen per-oxide (µmol/g f. wt.); **Host biochemical attributes**: **Phen:** Phenolics (mg gallic acid equivalents g^{-1} DW); **Fla:** Flavonoids (mg quercetin equivalents g^{-1} DW) ; **Anth:** Anthocyanin (mg Cyanidin-3-Glucoside Equivalents g^{-1} DW); **APX:** Ascorbate peroxidase (µmol min^{-1} mg^{-1} protein)

Non-enzymatic and enzymatic antioxidants: Non-enzymatic and enzymatic antioxidant concentrations showed great variance with regards to *Conocarpus* ecotypes in *Cuscuta* infestation (Table 3). There was a significant increase in the level of ascorbic acid (ASA), which is a major non-enzymatic antioxidant in all hosts after *Cuscuta* infestation. The ASA concentration levels were 17.4-19.6 µg g^{-1} fresh weight (FW), with the highest level in Toba Tek Singh and lowest level in Wah Cant. Infected plants indicated a uniform increase and showed that the antioxidant metabolism had been triggered by the stress. Khanewal had the highest ASA level (22.7 µg g^{-1} FW) followed by Wah Cant (21.7 µg g^{-1} FW) and Faisalabad (21.3 µg g^{-1} FW). The primary defense enzyme against superoxide radicals, which is the SOD, increased significantly under infestation compared to each ecotype. The SOD activity in healthy plants was 4.6-5.1 unit mg^{-1} protein with the highest activity recorded in Khanewal ecotype. When infested, the activity of SOD rose significantly with the highest point in Wah Cant (9.9 units mg^{-1} protein) and Faisalabad (9.2 units mg^{-1} protein). This rapid rise showed that there was an adaptive mechanism to de-toxify the excess superoxide radicals that were generated during parasitic stress. The same pattern was observed in peroxidase (POD) activity. In healthy plants, the range of POD was between 14.9 and 16.2 units mg^{-1} protein whereas the infested plants showed high levels of POD which attained 32.4 units mg^{-1} protein in the Khanewal. There was significant growth also in Faisalabad and Wah Cant (29.1 and 27.5 units mg^{-}

^{1} protein each). CAT activities of the healthy *Conocarpus* plants were found to be 21.5-30.2 units of mg^{-1} protein with the highest and lowest Faisalabad and Khanewal respectively. After infestation, all hosts showed a sharp rise with Faisalabad being the most active (64.4 units mg^{-1} protein) and Toba Tek Singh was the next (61.7 units mg^{-1} protein) host. Plants in the healthy group had a total APX of 0.06-0.09 µmol min^{-1} mg^{-1} protein, contrasted with the infested ecotypes of 2-3x more. The best APX activity (0.31 µmol min^{-1} mg^{-1} protein) was observed at Khanewal, then Wah Cant and Faisalabad. An increase in APX activity was an indication of the importance of the enzyme in the maintenance of intracellular ROS homeostasis during parasitic stress.

Shoot ionic contents: The infestation of *Cuscuta* caused considerable changes in the mineral ion composition of the hosts *Conocarpus* (Table 3). Na^{+} content was significantly decreased in all the infested plants of *Conocarpus* as compared to their corresponding healthy plants. Healthy plants were found to have Na^{+} values between 21.7 and 32.5 mg g^{-1} dry weight (DW) with the highest being in plants of Faisalabad and lowest in those of Toba Tek Singh. Na^{+} concentration dropped drastically on infestation, especially in the Faisalabad (12.5 mg g^{-1} DW) and Wah Cant (13.8mg g^{-1} DW) plants. Interestingly, Na^{+} levels (19.1 mg g^{-1} DW) of the plants retained by Khanewal were relatively higher under the infested influence indicating partial maintenance of ions under parasitic pressure. The significant decrease in Na^{+} in all of the sites suggests inhibition of ion uptake or a high efflux by *Cuscuta* vascular disruption. K^{+} concentration also reduced drastically with infestation. The K^{+} was found to be 10.4-11.5 mg g^{-1} DW in healthy ecotypes and 30-40% lower in infested plants. K^{+} (8.7mg g^{-1} DW) was highest in *Conocarpus* which were growing at Khanewal, then Wah Cant (8.1mg g^{-1} DW) and finally Faisalabad (6.5mg g^{-1} DW). This loss of K^{+} reflected dysfunctional osmotic regulation and inhibited carbohydrate metabolism because of parasitic interference. Infested plants also experienced a significant reduction in the level of Ca^{2+}. The values of healthy hosts were within the range of 3.61 to 4.58 mg g^{-1} DW and on infested plants 50% of the values were reduced. Faisalabad had the highest Ca^{2+} concentration (2.29 mg g^{-1} DW) post-infestation and the lowest recorded in Khanewal hosts (1.21 mg g^{-1} DW).

Defense-related metabolites: The phenolic contents of healthy plants were between 20.7 and 23.5 mg gallic acid equivalents g^{-1} DW with a maximum of 23.5 in Khanewal hosts and the minimum in Wah Cant (Table 3). After infestation by *Cuscuta*, the phenolic content increased sharply between 71.4 and 81.7 mg g^{-1} DW. The highest increase was found in Toba Tek Singh (81.7 mg g^{-1} DW), then Khanewal (76.2 mg g^{-1} DW), and Wah Cant hosts had the lowest increase (71.4 mg g^{-1} DW). The significant increase in the number of phenolics in infestation showed the increased activity of phenylpropanoid pathway and the deposition of lignin as part of the structural reinforcement and ROS scavenging. The levels of flavonoids in healthy plants were 10.6-12.7 mg quercetin equivalents per g^{-1} DW with an increase of 2.504 in infested plants. The optimum flavonoid content was recorded in Toba Tek Singh (39.2 mg g^{-1} DW), Wah Cant (34.6 mg g^{-1} DW) and Kuhanewal (31.5 mg g^{-1} DW) ecotypes. The rise in production of flavonoid during the stress of parasitism indicates that antioxidant and signaling pathway is activated to alleviate oxidative stress and control the expression of defense-related genes. Healthy plants had anthocyanin values of 0.21 to 0.28 mg cyanidin-3-glucoside equivalent per g^{-1} DW with the highest value recorded at Toba Tek Singh. The anthocyanin level almost doubled after infestation in all the ecotypes, with the highest level being 0.69 mg g^{-1} DW in Toba Tek Singh, 0.57 mg g^{-1} DW in Faisalabad and 0.51 mg g^{-1} DW in Khanewal. The increased anthocyanin level was an indication of greater pigment-based antioxidant coverage and stress signaling in infested tissues.

Heatmap clustering of environmental, morpho-physiological, and biochemical attributes: Figure 3 (heatmap) shows that the standardized correlation coefficients of the environmental, physiological, and biochemical characteristics of *Conocarpus erectus* plants were hierarchically clustered. It was a comparison of healthy (Ce-H) vs *Cuscuta*-infested (Ce-I) people of four contrasting eco-regions of Punjab (Faisalabad, Khanewal, Toba Tek Singh and Wah Cant). The observation of specific clustering patterns indicate that there were strong site-specific reactions and that there was a distinct separation between healthy and infested hosts throughout the climatic gradient that initially started at the lowland plains (Faisalabad, Khanewal) and the final one at the humid and high-altitude area (Wah Cant). There were high positive correlations between oxidative stress indicators (H_2O_2, MDA), antioxidant enzymes (SOD, POD, CAT, APX), and secondary metabolites (Phen, Fla, Anth) that defined infested ecotypes across the matrix in a cluster of stress-response. These correlations propose a greater redox imbalance and induced metabolism of defense after parasitism. The darkest red colors of these parameters especially in infested *Conocarpus* ecotypes at Faisalabad and Khanewal were the signs of deep activity of stress in hot locations with highest temperatures (MxT = 48-49 °C) and lowest rainfall (362-473 mm). On the other hand, the infested hosts of Wah Cant, which were gathered at the cooler and more humid upland location (AAT 12 °C, ARF = 1410 mm), showed moderate oxidative and enzymatic activity and this suggested climatic moderation of severity of the infestation. Clusters of healthy hosts are individual and form a block related to growth and photosynthetic parameters (PH, SFW, SDW, LFW, LDW, LA, Chla, Chlb, TChl, Car), which show significant positive inter-correlations. Among this healthy community, the positions of Wah Cant and Toba Tek Singh were at upper clusters, where the pigment and biomass-related values were the best, and this indicated high levels of hydration and reduction of thermal strain in their natural habitats. The healthy *Conocarpus* plants in Faisalabad and Khanewal, though sharing the same growth attributes, were clustered into a subcluster, which exhibited lower photosynthetic pigment correlations and weaker associations with LA and biomass characteristics, which is characteristic of low-rainfall plains.

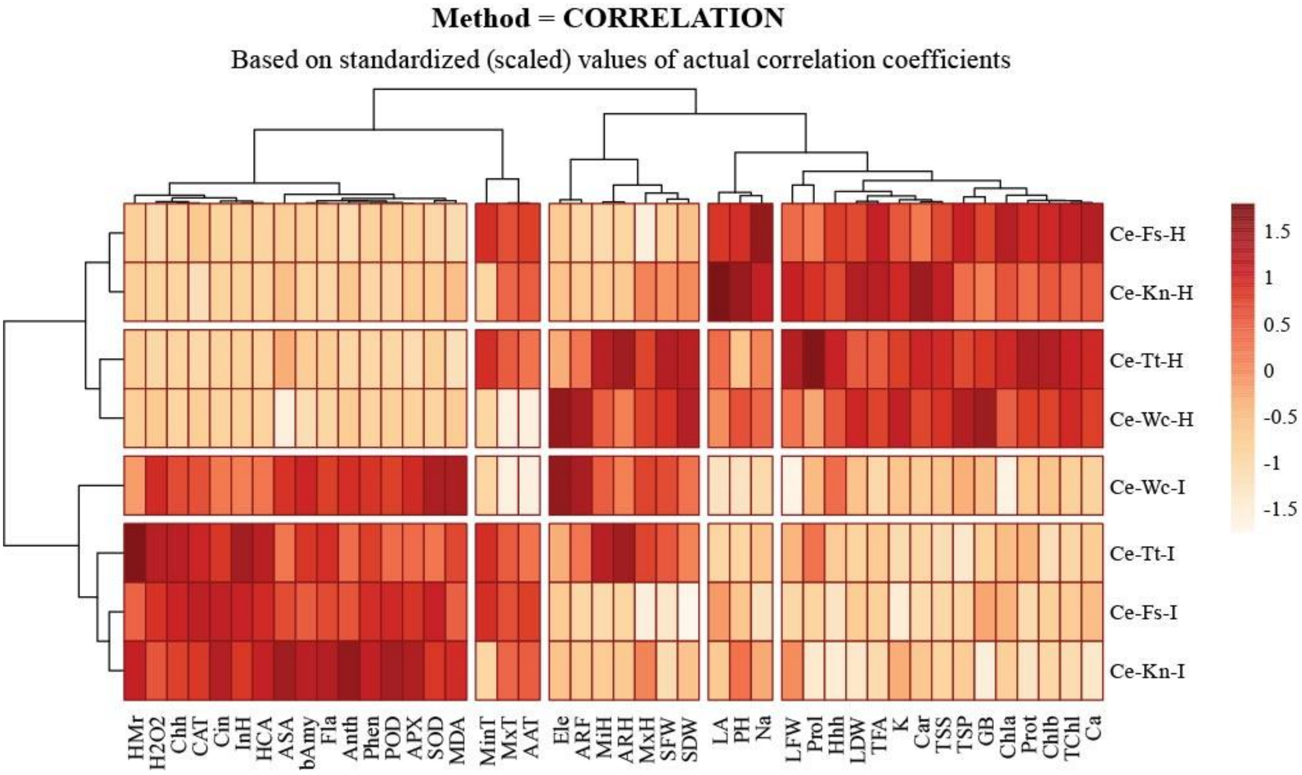

Fig. 3. Heatmap clustering of the healthy and *Cuscuta* infested *Conocarpus* host plants collected from four study sites. The heatmap is based on standardized (scaled) values of actual correlation coefficients. Abbreviations are given at start of manuscript.

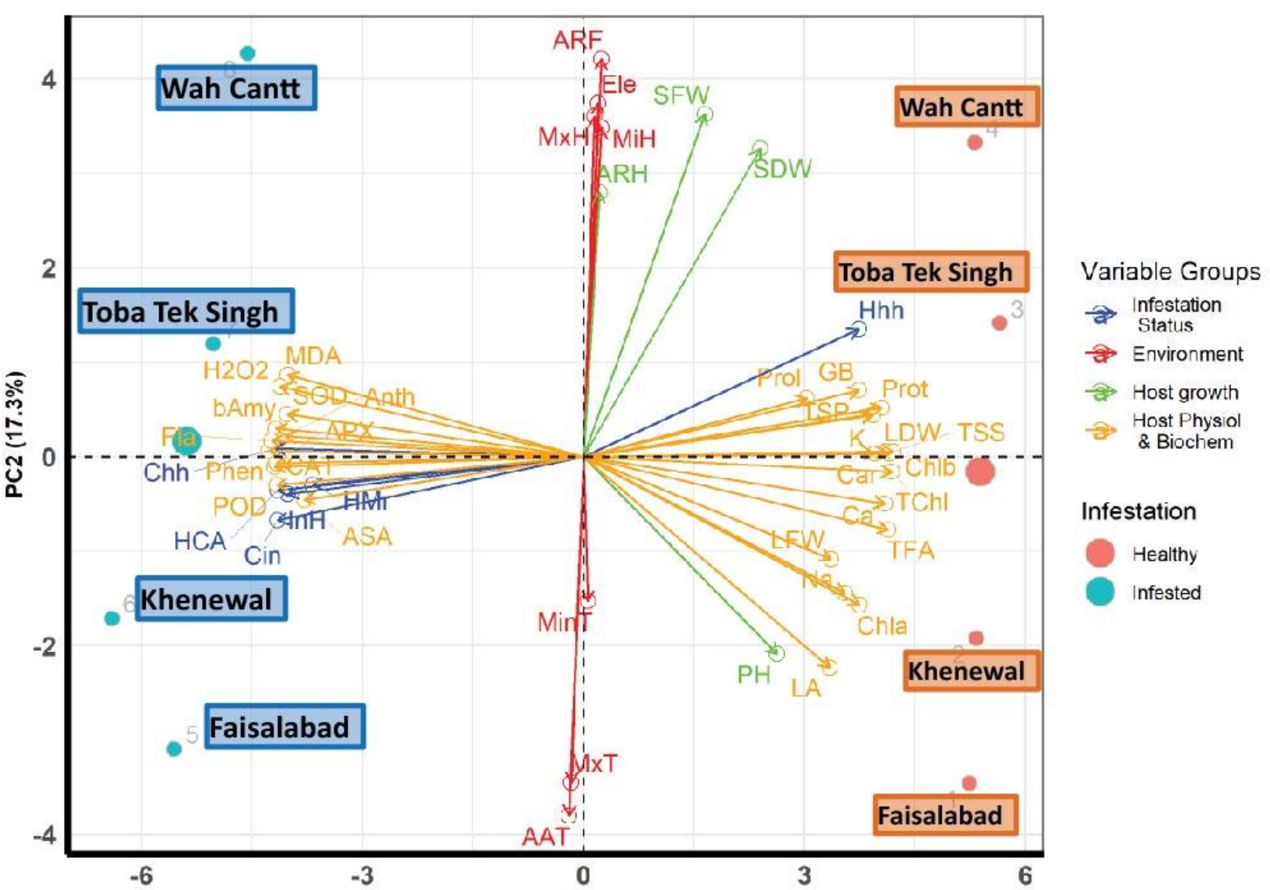

Fig. 4. Principal Component Analysis (PCA) biplot of the healthy and *Cuscuta* infested *Conocarpus* host plants collected from four study sites. Abbreviations are given at start of manuscript.

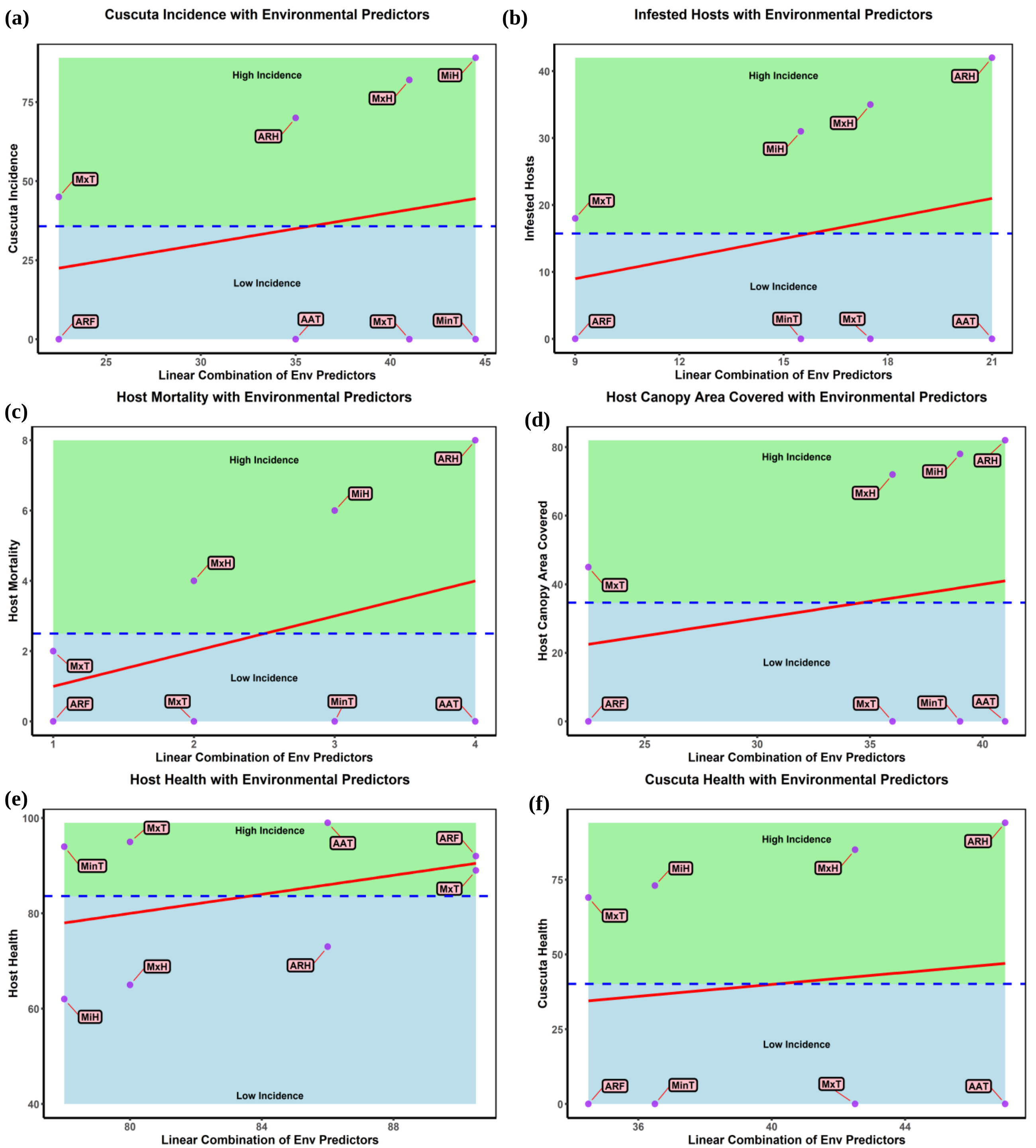

Fig. 5. Multiple linear regression (MLR) models constructed for *Cuscuta* infestation (Cin, Chh) and host health (InH, HMr, HCA, Hhh) attributes plotted with a liner combination of environmental predictors (Ele + MxT + MinT + AAT + ARF + MxH + MiH + ARH). Abbreviations are given at start of manuscript.

Principal component analysis of host-parasite-environment relationships: The PCA ordination (Fig. 4) describes a total variance of 83.7, PC1 (66.4) and PC2 (17.3) here have a clear separation between sites and the state of infestation. The main component of the first axis (PC1) was a physiological continuum between high metabolic stress and antioxidant activity (left side) and intense growth and pigment concentration (right side). The MDA, H_2O_2, SOD, POD, CAT, APX, and phenolics were negatively clustering around PC1, and were closely linked with infested samples of Faisalabad and Khanewal. This implied that drier and hotter conditions promote oxidative stress and oxidative defense metabolism in *Conocarpus erectus* that is a prey to *Cuscuta*. On the other hand, growth and pigment parameters (LA, PH, Chla, TChl, TFA, LFW, SDW) which are associated with healthy hosts of Wah Cant, a fairly humid, temperate environment dominated the positive PC1 loadings. MRs (MxT, AAT, ARF, MiT, and MiH) fell on PC1 and PC2, with a positive correlation indicated, showing that the more the temperature and humidity were lower, the greater the intensity of the infestation and biochemical stress. The infestation status vectors (Host health - Hhh) segregated along PC2 which means that it is highly attached to the host growth and physiological characteristics across sites. The intermediate results of Toba Tek Singh samples were a transitional response with intermediate stress but a slight retention of growth characteristics. Oxidative stress markers, host mobilizing enzymes (protease and beta-amylase) and protective secondary metabolites (Flavonoids, Phenolics and Anthocyanins) were tightly loaded into all the other infestation status attributes such as *Cuscuta* incidence and health, number of infested hosts, host mortality and host canopy area covered. The close proximity of the stress-reactive metabolites (antioxidants, phenols, anthocyanins, β-amylase) to chlorophylls and carbohydrates shows the antagonism between the growth and defense metabolism by the parasite pressure. Moreover, ionic variables (Na, K, Ca) were oriented to the growth group of the healthy plant and moved towards stress markers of the infested hosts, which proves an ionic imbalance as one of the main factors of the *Cuscuta*-induced physiological disturbance. The gradients of *Cuscuta* infestation reflected the underlying conditions of the environment with Faisalabad and Khanewal occupied at one end of the distribution with Wah Cant at the other end.

Multiple linear regression model for environmental drivers of *Cuscuta* incidence and impact: Several linear regression models (MLR) were estimated to demonstrate how *Cuscuta* characteristics connected to host infestation are associated with a linear interaction between environmental predictors. The x-axis was a mixture of various environmental factors, such as the elevation (Ele), the maximum temperature (MxT), minimum temperature (MinT), average annual temperature (AAT), annual rainfall (ARF), maximum humidity (MxH), minimum humidity (MiH), and annual relative humidity (ARH). The *Cuscuta* infestation and host health attributes (*Cuscuta* incidence rate, number of infested hosts, host health, *Cuscuta* health, host mortality, host canopy area covered etc.) was used as the dependent variable on the y-axis which indicated the intensity of infestation. The scatter points, denoted in purple, are associated with various environmental attributes, which are denoted as well. The regression line (in red) shows the general direction and explains a positive correlation existing between the linear combination of the predictors and host infestation. The low and high incidence zones are separated by a horizontal blue line with dots, the zones of low incidence (below the line, in blue color) and high incidence (above the line, in green color). Such demarcation indicates that response variables (e.g. levels of infestations) grow when predictor values exceed some level.

The multiple linear regression (MLR) analyses (Fig. 5a-f) revealed that the temperature and humidity variables were the major factors that determined *Cuscuta* incidence and outcomes on its host plants. Higher maximum and minimum humidity (MxH, MiH) and high maximum temperature (MxT) were always related to the increased intensity of infestation, coverage of canopy, mortality of the hosts, and intensity of the parasites. On the other hand, conditions with less heat (ARF) and more precipitation (ARF), less minimum and average annual temperature (MinT, AAT) were also associated with lower incidence and host damage. Warm and fairly moist areas including Faisalabad and Khanewal matched with the upper limit of infestation projections, and cooler and damper areas such as Wah Cant had little *Cuscuta* activity. In all the sub-models, humidity was the most significant environmental factor that promoted parasite growth and canopy colonization. Temperature had a secondary but a supporting effect as it favored infestation of hosts and impaired host health due to high temperatures. Conversely, heavy rains or too low temperatures slowed the growth of *Cuscuta*, and this showed the favorable condition of the parasite in warm, humid conditions. Generally, the MLR model forecasts the synergistic effect of the heat and humidity in the atmosphere as significant factors of determination of the *Cuscuta* incidence, spread, and host effect across locations.

Discussion

The level of infestation was significantly different across sites with Faisalabad having the highest intensity and Wah Cant having the lowest. The environmental data indicate that the warm climate of Faisalabad (mean temperature 23.7ºC; rainfall 903 mm; RH 58) was good to ensure *Cuscuta* attachment, biomass development and canopy expansion. Conversely, the cooler, high-altitude and moist climate of Wah Cant (12.1ºC mean and 1410 mm rainfall per year) does not seem to be conducive to the growth of *Cuscuta* as indicated by a lower prevalence rate and slow parasite activity. Such tendencies prove that *Cuscuta* prospers in warm and moderately humid regions that promote a high metabolic and haustorial rate (Ren *et al.*, 2020). Its efficiency was found to lower when it was subjected to high humidity or low temperature that restricts vascular penetration and flow of nutrients (Landi *et al.*, 2022). The mortality of the host was also proportional to the environmental gradient with the highest occurring in Faisalabad and the lowest in Wah Cant, indicating that the effect of the parasite on the vitality of the host depends on their environmental performance. This is due to the inverse correlation between host health and *Cuscuta* abundance, which is what is referred to as environment mediated host-parasite balance (He *et al.*, 2024). The demonstration by the intermediate infestation and host decline in Khanewal and Toba Tek Singh is that irrigation and moderate rainfall in the areas support the intermediate infestation and host decline, establishing dynamic balance but not complete dominance (Nagao *et al.*, 2025).

In all ecotypes, the *Cuscuta* infestation had a strong negative impact on the plant height and the biomass accumulation, which confirms that diversion of resources to the parasite inhibits host growth. The extent of the reduction was varied in different sites where Faisalabad hosts incurred maximum losses in shoot and leaf biomass with a partial tolerance in Toba Tek Singh and Khanewal hosts. These disparities can be explained by the fact that environmental modulation of host physiological capacity occurs (Bennett & Groten, 2022). Warm regions such as Faisalabad have the potential to amplify the metabolic load of the parasite by the higher level of transpiration, and absorb withdrawal, which causes faster carbohydrate depletion (Ni *et al.*, 2021; Chen *et al.*, 2025). Conversely, moist and irrigated conditions, in the case of Toba Tek Singh, maintain host hydration and to some extent counteract the losses in growth. The comparatively stronger inhibition of growth in Wah Cant even with the lower infestation rate could be as a result of a combination of parasitic and low-temperature stress which synergistically inhibits photosynthesis and carbon assimilation. This very fact highlights the fact that the impact of infestations is contextual and depends on the interplay between the level of parasitism and background stressors related to climate (Bennett & Groten, 2022).

A significant impairment of photosynthetic machinery was proved by reductions in chlorophyll and carotenoid contents of infested plants. Maximum pigment loss was also found in Faisalabad and Khanewal where *Cuscuta* vigor and canopy sectors were the highest. The degradation of chlorophyll is probably an indicator of increased oxidative stress, the loss of the nutrient under the influence of high-density twining stems of this parasite (Saric-Krsmanovic *et al.*, 2018). The intense reduction in carotenoids, particularly in Faisalabad, is a sign of compromised photoprotective ability, which makes them more vulnerable to photo-oxidative stress (Riaz *et al.*, 2023). This observation is in line with the earlier studies that the *Cuscuta* parasitism interferes with the stability of chloroplasts and pigment synthesis in the host tissues (Furuhashi *et al.*, 2011; Ayvaci *et al.*, 2025). Interestingly, both low absolute pigment values and similar proportional losses were also observed in Wah Cant, which indicated that the pigment production was suppressed in all healthy hosts despite the parasitic influence exerted by the environment, which was more pronounced in cooler environment of the sites (Akula & Ravishankar, 2011). Accordingly, environmental modulation of the pigment processes reflects direct climatic effect and indirect parasitic effect.

Infestation led to a consistent reduction of organic osmolytes, including soluble sugars, free amino acids, and proteins, which is an indication of impaired osmotic control and carbon- nitrogen metabolism. These osmolytes are usually stress alleviators that ensure the cell turgor and metabolic balance (Kumar *et al.*, 2018). Their depletion in case of infestation is indicative of *Cuscuta* apprehending photoassimilates and nitrogenous substances on the host therefore, diminishes osmolyte pools (Ni *et al.*, 2021). This deterioration was intense especially in Khanewal and Faisalabad which matched high parasite vigor and high assimilate extractions. The comparatively greater preservation of soluble sugars and proteins in Wah Cant hosts can be taken to mean a weak parasitic sink in cool and humid environments (Yuan *et al.*, 2023). Moreover, decreased levels of free amino acids suggest a suppressed production or an increase in defense and repair processes because the parasites tend to change the host nitrogen metabolite to their benefit (Irving & Cameron, 2016). These reactions are all indications of the metabolic price of maintaining the parasite in order to adapt to both environmental and oxidative stress (Foyer & Noctor, 2005).

Protease and β-amylase activities had opposing patterns during infestation, which demonstrates a reprogramming of reserve mobilization. Inhibition of protease implied the loss of protein substrates or inhibition of proteolysis in case of nutrient diversion. On the other hand, the induction of β-amylase activity indicated a fast-moving degradation of starch, which was likely to cover the energy gap due to parasitic siphoning (Ayvaci *et al.*, 2025). This trend reflects stress-induced reallocation of the metabolism in other parasitic relationships, as host tissues use carbohydrate reserves to maintain low physiological activity (Gonella *et al.*, 2019). The enhanced β-amylase activity in Khanewal and Wah Cant hosts revealed more intense metabolic stimulation, which may have been an element of compensatory responses to compounded environmental and parasitic stress (Savi *et al.*, 2019).

The *Cuscuta*-infested hosts also had a significant drop in both metabolites, contrary to the common abiotic stress responses where proline and glycine betaine have accumulated. This inconsistency indicates that parasitism deprives their production or increases catabolism as a result of different metabolisms of nitrogen and methyl group (Khaliq *et al.*, 2025). The intense decrease in proline in Khanewal and Faisalabad confirms the hypothesis of parasitic interference with the pathways of regulating stress, primarily those with the mediation of the synthesis of D1-pyrroline-5-carboxylate. Partially retained proline and GB in Toba Tek Singh suggests more equal osmotic regulation, which may be enabled by more favorable soil moisture conditions (Sharma *et al.*, 2012). The net loss of compatible osmolytes is what explains why the parasite is able to impair host defensive response to stress making it more vulnerable (Jeschke & Hilpert, 1997).

Biochemical evidence of the oxidative damage caused by the parasitism was noted as the high concentration of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) in the infested tissue. The accumulation of MDA means increased lipid peroxidation and membrane damage, and increasing H_2O_2 means excessive production of reactive oxygen species (ROS) (Sharma *et al.*, 2012). The max MDA of Wah Cant and Khanewal indicates high oxidative pressure in moderately cooler environment of Wah Cant and heat in Khanewal. Such observations agree with the idea that environmental stress increases the effect of parasitism on the production of ROS, which results in cumulative cell damage (Li *et al.* 2024). As a result, antioxidant systems were up-regulated. Non-enzymatic (ascorbic acid) as well as enzymatic antioxidants (SOD, POD, CAT and APX) exhibited acute increases in the conditions of infestation, indicating the mobilization of the defensive metabolism. Improved SOD and POD activities imply effective scavenging of superoxide radicals and hydrogen peroxide respectively, whereas an increase in CAT and APX increased detoxification of H_2O_2 (Gill & Tuteja, 2010). Faisalabad and Wah Cant had the best antioxidant response, which is equivalent to the high oxidative load (Kaur *et al.*, 2025). Nonetheless, the constant high level of ROS markers despite the enhanced levels of antioxidants activity indicated that the defense mechanisms despite its activation were not adequate to counteract oxidative damage (Hasanuzzaman *et al.*, 2020). Such imbalance represents the exhaustion of the metabolism that follows a long-term parasitic stress, in which antioxidants activation is not defensive but compensatory (Mittler, 2017).

After infestation, host ionic profiles were profoundly changed, with a decrease in the amount of Na^+, K^+, and Ca^{2+} in shoots. This is probably caused by broken xylem-phloem continuity and hindrance of ion uptake through haustorial invasion (Dawson *et al.*, 1994). The highest depletion of Na^+ and K^+ in Faisalabad hosts indicated intense interference with the vascular and osmotic imbalance in dry conditions. Specifically, K^+ deficit has significant ramifications of stomatal control, protein synthesis, and stress signaling (Wang and Wu, 2013). Its better ion retention that is relatively higher than that of Khanewal suggests that there is partial ionic homeostasis which may be attributed to better soil texture and irrigation. The reduction in Ca^{2+} as one of the most important signaling ions presupposes poor structural stability and deteriorated membrane integrity (Zhuang *et al.*, 2018). These ionic shifts collectively indicate that *Cuscuta* parasitism disturbs the mineral nutrition, where environmental stresses on soil water and nutrient availability enhance the degree of distress (Irving & Cameron, 2016).

Phenolics, flavonoids and anthocyanins were significantly accumulated during infestation, which indicates the defense and antioxidant signaling pathways. The extreme phenomenon of phenolic compounds, especially in Toba Tek Singh, is evidence of an increase in phenylpropanoid metabolism and lignin deposition, which probably increases cell walls against haustorial intrusion (Ayvaci *et al.*, 2025). Upregulation of flavonoid and anthocyanin functions is an indication of augmented redox buffering and signaling actions when the plants are exposed to a combination of parasitic and oxidative stress (Agati *et al.*, 2012). These are the secondary metabolites that play a central role in the ROS scavenging and the control of the expression of defense-related genes (Sharma *et al.*, 2019). The comparatively greater phenolic and flavonoid activation in irrigated moderate humid habitats (Toba Tek Singh, Khanewal) than in dry Faisalabad or colder Wah Cant may indicate that defense activation is optimal in moderate environmental stress, but excessive stress may repress metabolic reprogramming (Taiz & Zeiger, 2015).

The heatmap analysis (Fig. 3) showed that the morpho-physiological and biochemical reactions of *Conocarpus erectus* to *Cuscuta* infestation also varied by the regional climatic variation between various study sites. Evidence of a clear distinction between healthy and infest ecotypes was a conspicuous metabolic change between a growth-oriented mode of functioning to a defense dominance mode of operation in the face of parasitic pressures. Plants that were healthy had high clustering in features such as morphological and pigment characteristics such as biomass, leaf area, and chlorophyll and carotenoid concentration which showed co-ordinated growth and photosynthetic performance. This trend was also most pronounced in Wah Cant and Toba Tek Sing where the conditions were relatively warmer and more humid which helped in maintaining pigment stability and biomass growth (Akula & Ravishankar, 2011; Sharma *et al.,* 2012). Conversely, healthy plants in Faisalabad and Khanewal exhibited weaker pigment associations, and it indicated that the heat and moisture constraint caused the plants to experience baseline stress even in an uninfested condition (Li *et al.*, 2024). The infested plants exhibited a different oxidative-defense enclave with a high level of ROS, lipid peroxidation, antioxidant enzyme activity, and high level of phenolic and flavonoid production (Agati *et al.*, 2012; Sharma *et al.*, 2012). The strongest response was observed in Faisalabad and Khanewal, where the effects of climatic stress increased the effects of parasites, whereas the oxidative activation of Wah Cant and the intermediate response of Toba Tek Singh were moderate. The rearrangement of ionic characteristics among growth-related groups to stress related groups also explained the diversion of nutrient and ionic disturbance by parasitic interference (Irving & Cameron, 2016).

The PCA results (Fig. 4) offered a combined perspective of interaction among climate, intensity of infestation and their interaction with host physiology under ecological conditions. The two initial elements accounted 83.7 per cent of the overall variance, with PC1, which distinctly indicated a trade-off between growth-associated characteristics and oxidative stress-defense metabolism. Faisalabad and Khanewal infested plants aligned with high MDA, H_2O_2, antioxidant enzymes, and secondary metabolites, along this axis, which indicated a costly reorganization of the metabolism in response to combined climatic and parasitic challenges (Kaur *et al.*, 2025). The existence of high temperature and low humidity in this domain of stress emphasized the contribution of both factors to increasing the severity of the infestation and the physiological load on the host (Bennett & Groten, 2022). Conversely, the healthy ecotypes of Wah Cant positively loaded chlorophyll, leaf area, amino acids and biomass, which is in line with the colder and wetter conditions that maintained the photosynthetic integrity (Akula & Ravishankar, 2011). Toba Tek Singh was in a middle position where it did not grow fully but activated defense pathways in semi-humid, irrigated environments (Sharma *et al.*, 2012). The PC2 also separated the severity of infestation, as canopy loss and mortality were associated with high protease and β-amylase activity, which are signs of increased catabolic turnover and nutrient remobilisation in the plants with heavy infestation (Ayvaci *et al.*, 2025). The ionic characteristics switching to stress-related loadings double the significance of ion imbalance in parasitic disruption (Irving & Cameron, 2016).

The multiple linear regression analysis (Fig. 5) numerically explained the way climatic gradients control *Cuscuta* incidence and effects across sites of the study. High- and low-risk zones were very clearly differentiated by the regression surface, which suggests that success of parasites is limited by clear environmental thresholds. Humidity was also ranked as the strongest of all predictors with positive correlations with infestation intensity, canopy loss, and host mortality. This highlights the significance of atmospheric moisture in supporting haustorial attachment, parasite turgor, and solute withdrawal especially in warm but with moderately humid climates like Faisalabad and Khanewal (Jeschke & Hilpert, 1997). The secondary reinforcing role was on temperature which increased parasitic activity, especially when combined with adequate humidity conditions. On the contrary, continuous cooling down or excessive moisture as in the case of Wah Cant inhibited the growth of parasites even though there is a lot of moisture (Akula & Ravishankar, 2011). Ionic imbalance, canopy decay and host mortality showed the same humidity-dependent patterns that remind us that warm humid air masses increase the stress of the host and resources loss (Irving & Cameron, 2016). The regression results, combined with the heatmap and PCA dynamics, supported the core contribution of climate, in particular, humidity, to the organization of *Cuscuta-Conocarpus* interactions and the establishment of high-risk settings.

Conclusion

The results indicate that the *Cuscuta* infestation of *Conocarpus erectus* is under strict influence of a combination of environmental factors and physiological reactions of the host. Hot climatic conditions with moderate levels of humidity were conducive to the establishment of parasites, their hypertrophy, and a reduction in the canopy of the host. Comparatively, climatic extremes i.e. hot-arid or cool-humid limited survival and infestation ability of *Cuscuta*. Among all the environmental factors, it was found that humidity was the most prevalent predictive factor of infestation severity, host mortality, and canopy loss. This served to demonstrate that the parasite requires the presence of atmospheric moisture to be able to effectively attach itself and extract resources. The temperature served as an augmenting but secondary factor. The best infestation potential was observed in semi-arid and humid climates and the less favorable or cooler climates reduced the performance of the parasites. Physiological adaptations were environment dependent in host plants. There was a trade-off between growth and defense in severe infestations when temperature conducive to growth was in the presence of harsh-humid conditions and when osmolyte content, oxidative stress, and antioxidant and phenolic responses were high. Intermediate conditions favoured moderate attack with partial physiological compensation, and plants in drier or cooler habitats either exhibited excessive oxidative injury or inhibited recovery of metabolic activity. On the whole, the findings prove climate to be a key controller of the *Cuscuta* success and host stress response that depend on the physiological resilience and metabolic plasticity as the main determinants of the infestation capacity and host tolerance. Regions that were highly humid, moderately rainy with a high temperature were seen to be most susceptible in terms of outbreak. This highlighted the importance of specific surveillance and early control in urban plantations. Early diagnosis can be facilitated by the evaluation of physiological parameters, like an increased oxidative markers and phenolics and decreased osmolytes. It could increase host resistance to parasitic pressure due to enhanced osmolytes stability and antioxidant capacity.

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