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Research Article

FUNCTIONAL, REPRODUCTIVE AND BIOCHEMICAL TRAITS OF INVASIVE *Chromolaena odorata* IN DRY AND MOIST SITES OF RAMECHHAP DISTRICT, NEPAL

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ABSTRACT

Chromolaena odorata (Siam weed) is the worst invasive weed, spreading in tropical and subtropical regions of the Old World and invading different habitats even in stressful environments. Specific mechanisms regarding how the weed has developed adaptive traits for growing in adverse ecological circumstances such as dry environment are less explored. This study has evaluated morphological, reproductive and biochemical traits of *C. odorata* in invaded dry and moist sites. Specific leaf area (SLA), reproductive traits (floral branches, capitula, and seeds) and biochemical traits (chlorophyll, carotenoid, proline, and leaf nitrogen) were measured by sampling plants from dry and moist sites from Ramechhap district, Nepal. Analyzing the results, the SLA was high but photosynthetic pigments and leaf nitrogen were reduced in the dry site. The weed had an increased number of mature capitula and seed mass against reduction in the number of floral branches in dry sites. Elevated concentration of the osmolyte 'proline' was found in the dry sites. Reducing the number of floral branches might help energy allocation to earlier maturation of capitula and increased seed mass at the dry site. The results will be useful to understand the adaptive biology of *C. odorata* and will also have significance in understanding their trait plasticity, which ultimately can be applied for predicting future invasions.

Keywords: Drought-prone areas, invasions, plasticity, trait variation

INTRODUCTION

Chromolaena odorata (L.) R. M. King & H. Rob. (Asteraceae), native to tropical America, is known as Siam weed which represents one among 100 of the world's worst invasive alien species (Lowe *et al.*, 2000). Being native to tropical America, this perennial shrub has been spreading rapidly and encroaching different ecosystems in tropical and subtropical regions of the Old-World continents, Africa and Asia (Yu *et al.*, 2016; Debnath *et al.*, 2018). It has been a serious weed along the roadsides and degraded areas, abandoned lands and pastures out of its native range (Zachariades *et al.*, 2009). Apart from these areas, its invasion has extended to forest ecosystems, posing a significant threat to native species in the tropical to subtropical regions (Thapa *et al.*, 2016; Debnath *et al.*, 2018). Its negative impacts on native species due to creating monodominant stand and shading (Gbètoho *et al.*, 2018), allelopathy (Hu *et al.*, 2013), and altering soil quality (Yuan *et al.*, 2024) are major concerns for biodiversity and ecosystem health.

Chromolaena odorata produces a large number of light and minute seeds. The seeds germinate faster with high germination percent, and greater number of seedling emergence occurs at surface soil (Ismail *et al.*, 1996; Zachariades *et al.*, 2009). These traits benefit the plant for its rapid colonization and establishment. Besides these, Liao *et al.* (2019) found longer shoot, and higher biomass and leaf area of *C. odorata* in the introduced areas than the native populations, highlighting stronger plastic response of the weed. Similarly, Li *et al.* (2024a) found its greater phenotypic plasticity in root traits such as root to shoot ratio, specific root length, and branching intensity than the native populations. These are the evidences showing ability of invasion success of *C. odorata,* adapting phenotypically in new environment. Other studies also revealed that *C. odorata* under different environmental conditions relies on its functional response based on its ecotypic differentiation and phenotypic plasticity as strategy of invasion success (Joshi *et al.*, 2011; te Beest *et al.*, 2013). Phenotypic plasticity and adaptive ecotypic differentiation in plants play a significant role to persist them even in adverse environmental conditions (Palacio-López & Gianoli, 2011; Caruso *et al.*, 2020).

Regarding plasticity and functional adaptations, specific leaf area, photosynthetic pigments, leaf nitrogen and osmolytes like proline are the key traits that show variations, serving as the vital indicators reflecting adaptive features under stressful environment in many plant species (Heuer, 2010; Liu *et al.*, 2023; Mathur *et al.*, 2014; Nacry *et al.*, 2013). Additionally, the reproductive traits also signify adaptational responses to optimize reproduction, ensuring the plants' survival, growth, and propagation (Araújo *et al.*, 2015; Hampton *et al.*, 2016). Li *et al.* (2024b) found that the introduced populations of *C. odorata* in Asia had higher seed numbers per capitula with lighter seeds and high dispersal ability than the population in its native range. However, insight on the biochemical and reproductive traits under contrasting environmental conditions is still limited on the weed.

In Nepal, *C. odorata* has been spread in the forests, fallow lands, shrublands and grasslands at tropical to subtropical regions (Shrestha *et al.*, 2018). In these regions, Nepal exhibits a wide range of climatic and geographical variations, vegetation types, and soil characteristics, even within relatively small areas. For instance, Ramechhap district of Nepal exhibits the significant climatic and topographical variations. The district has rugged rocky dry landscapes which often experience lower precipitation where vegetation is sparse (Joshi & Dangol, 2018) and other regions which perceive more rainfall represent moister sites support denser vegetation. These landscapes, whether moist or dry, are experiencing severe colonization of the invasive weed *C. odorata*. It seems that native plants in the dry sites are stressed by aridity, where *C. odorata* is takings the opportunity for spread and impose the negative impacts on them (LaForgia *et al.*, 2020; Gioria *et al.*, 2023). In such scenarios, this weed might have developed the ability to adapt in different environmental conditions within the introduced areas.

Previous studies have compared the adaptation traits of *C. odorata* between its native and introduced population and explained its higher plasticity in the introduced range as mentioned above. Such assessments under varied environmental conditions within the invaded regions seem to be very limited. Some of the studies investigated the structural and physiological traits of *C. odorata* in greenhouse conditions by exposing them with different light and water conditions (Zhang & Wen, 2009; Naidoo & Naidoo, 2018). They observed that the plant is able to grow successfully even at high light or low soil water regimes, showing high water use efficiency. Alongside the controlled experiments, trait assessment in real field scenarios can give valuable insight into how *C. odorata* adapts to diverse environmental conditions as a successive invader. Hence, this study was carried out with objective to understand changes in the vital morphological, reproductive and biochemical traits of *C. odorata* in drier and moist environments in the Ramechhap district of Nepal.

MATERIALS AND METHODS

Study sites

This study was carried out in Ramechhap district of Bagmati Province, Nepal. The district is characterized by moderate vulnerability to drought specially the southern part (MoFE, 2021). The climate data of the district shows a tendency towards higher temperatures, more dry months, and lower annual rainfall (Joshi & Dongol, 2018). This study was carried out in two sites near the headquarter (Manthali) of Ramechhap district (Fig. 1). Site first was the Bhote Paakho Forest in Ramechhap Municipality (elevation 605-690 masl, 27.3655°N/ 86.073°E), which lies towards the south-east side of Manthali facing northward. The forest was a mixed type characterized by the presence of *Lagerstroemia parviflora*, *Shorea robusta, Woodfordia fruticosa*, *Mimosa rubicaulis, Colebrookea oppositifolia* etc. Due to dense vegetation and north facing slope the forest was found to be moist and hence it is named the 'moist site' hereafter.

The second site was Ramite Community Forest in Manthali Municipality (elevation 470-630 masl., 27.3814°N/ 86.0417°E) that lies towards the northwest side of Manthali facing southward. The forest has sparse vegetation characterized by presence of *Aegle marmelos, Senegalia catechu* and *Annona squamosa.* Due to southward facing rocky slope and sparse vegetation, the site was found to be drier than Bhote Paakho Forest. Therefore, this site is referred as the 'dry site' hereafter.

These moist and dry sites are situated approximately 5 km distance from each other, with the Tamakoshi River and the Manthali separating them. The area of moist and dry forests (moist and dry sites) was ca. 31 hectare and 34 hectares, respectively. Both the sites were invaded by *C. odorata;* comparatively high invasion was observed in the dry site (80% of the total area) than the moist site (ca. 50% of the total area) (field observation, 2022). According to the local people, *C. odorata* was introduced in the sites about 10-15 years ago. Hence, both the study sites were found to be different in terms of vegetation canopy, species composition, aspect, temperature, and level of *C. odorata* infestation.

Sampling design and study parameters

Plot sampling: Altogether 14 plots (quadrats) of size 5×5 m^2 were established in each site for sampling *C. odorata* to analyze trait variation. Three horizontal transects were made in each site and each of the transects had 4-5 plots. The distance between two transects and two plots was 100 to 200 m. The field work was conducted during November 2022.

Soil moisture index (SMI): The SMI of two seasons (pre-monsoon: April 18, 2022, and post-monsoon: November 20, 2022) was calculated using Landsat 8 image downloaded from the United States Geological Survey (USGS) Earth Explorer website (USGS, 2022). The SMI function was based on the utilization of the Normalized Difference Vegetation Index (NDVI) and Land Surface Temperature (LST) which were calculated using multispectral satellite imagery for each pixel following Zeng *et al.* (2004). The SMI was calculated for each plot sampled in both sites and the mean value was computed. The SMI map was generated using QGIS 3.28.3 (QGIS Development Team, 2022). For field verification, soil samples were collected from each plot (mentioned above) at 0-15 cm depth. The samples were collected from five cores (4 corners and the center of each plot) and they were mixed to make a composite sample for each plot. Soil moisture content of each plot was determined by weighing the freshly collected soil in the field, drying it in an oven, and then weighing the dry soil. Fresh weight of soil was taken in the field, and dry weight was taken at Central Department of Botany, Tribhuvan University, Kathmandu, Nepal.

Specific leaf area (SLA): From each plot, three mature individuals (ramets) of *C. odorata* were randomly selected and 5-8 matured leaves were plucked from each individual. Photographs of the leaves were taken on the spot and leaf area was calculated using the software ImageJ (ImageJ 1.53k). The leaves were transferred to the laboratory and dried in a hot air oven at 80˚C for 72 hours (Wilson, 1999). The SLA was calculated using the formula: SLA (cm^2/g) = Leaf area/Leaf dry mass (Vile *et al.*, 2005).

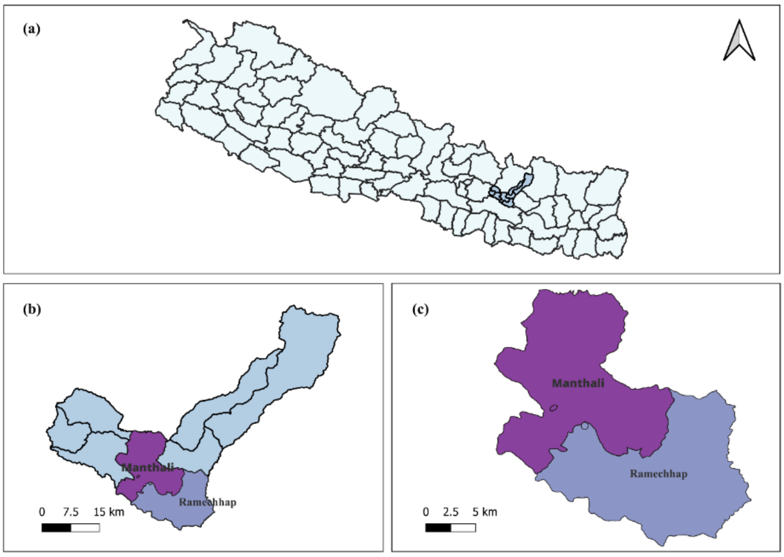

Figure 1. Map of study sites (a) Nepal showing Ramechhap district (b) Manthali and Ramechhap Municipalities (c) Municipalities showing the community forests (RCF and BCF)

Biochemical traits: The biochemical traits, chlorophyll, carotenoids, proline, and leaf nitrogen contents were measured in the leaves of *C. odorata*. Leaves were sampled from each plot as mentioned in the SLA. The leaves were wrapped by aluminum foil immediately after collection to protect from light and kept in icebox for transporting them to laboratory from the field. Photosynthetic pigments were determined by spectrophotometry after grinding 500 mg of fresh leaf/sampling plot in 20 mL of acetone (80%) with final volume of the filtrate as described by Bajracharya (1999). Proline (osmolyte, aiding plant stress tolerance) was measured following Bates *et al.* (1973). To extract the proline, 250 mg of fresh leaf/sampling plot was crushed in 1.5 mL of 3% sulfosalicylic acid, then the content was estimated spectrometrically by measuring absorbance at 520 nm, with the results compared to the standard curve created with l-proline standard. Leaf nitrogen content (LNC) was measured by taking 500 mg oven dried leaf sample with conc. H_2SO_4 in a digestion flask and processed further following Kjeldahl digestion distillation method (Horneck & Miller, 1998).

Reproductive traits: Altogether, 30 matured plants were sampled randomly from each site for reproductive trait analysis. The number of floral branches and number of capitula per plant was counted for each individual. Mature individual of *C. odorata* gives floral branches, which ends with heads bearing minute flowers. Fully developed capitula with fresh and blooming flowers were classified as immature, while those having flowers with maturation signs such as dried and browning involucre and florets, were considered mature. Total number of capitula per plant, including both mature and immature capitula were counted. The mature capitula of each plant was collected and seeds from each capitulum were extracted. Number of seeds per capitulum was counted and mass of seeds (300 oven-dried seeds at 70°C for 48 hours) was measured.

Statistical analysis

The independent sample t-test was used to compare SLA, chlorophyll, leaf nitrogen, and seed mass of *C. odorata* between two sites. Mann-Whitney U test was used to compare contents of proline, carotenoids, and SMI between two sites as the data were not homogeneously distributed. Poisson regression was used for comparing the number of seeds per capitulum in dry and moist site. As the count data exhibited over-dispersion, negative binomial regression was used to compare number of capitula, mature capitula and floral branches between dry and moist sites. The software R (Version 4.3.1) (R Core Team, 2022) was used for all analyses.

RESULTS

Soil moisture index (SMI)

SMI of the moist site was high comparing to the dry site during both seasons ($p < 0.001$, Fig. 2, Appendix 1). Based on the SMI values, moisture condition of both the sites is shown in Fig. 3. The SMI in the moist site did not vary between April and November ($p = 0.505$) while the dry site remained moister during November compared to April ($p = 0.026$, Fig. 2). Similarly, analyzing the soil moisture content, the Ramite Community Forest designated as the 'dry site' and Bhote Paakho Forest designated as the 'moist site' exhibited the moisture content 3.213±0.415% and 7.314±0.3%, respectively. The moisture was >50% lower in the dry site ($p < 0.001$) (bar graph not shown).

Specific leaf area (SLA)

Comparing the SLA of *C. odorata* between two sites, it varies significantly ($p = 0.017$). The SLA in dry site was high (61.50±1.41 cm^2/g) and low in the moist site (51.61±45 cm^2/g), respectively (Appendix 1).

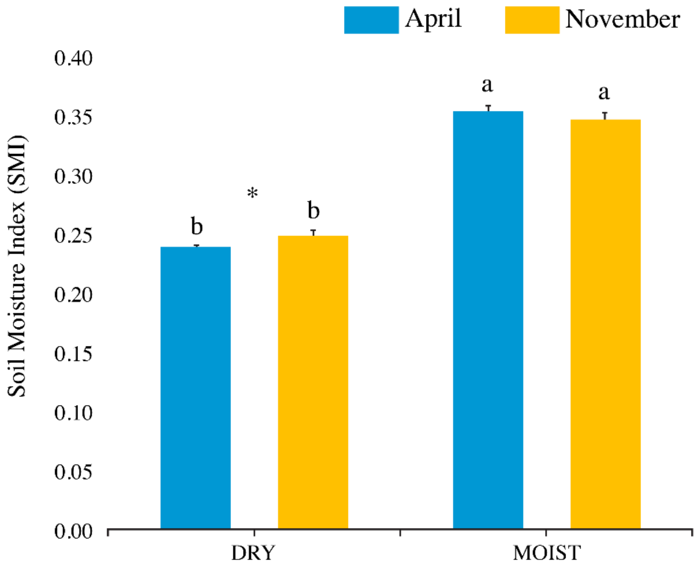

Figure 2. SMI of dry site and moist site. Different alphabets 'a, b' above error bars represent significant differences between dry and moist sites. The symbol * represents significant differences between April and November.

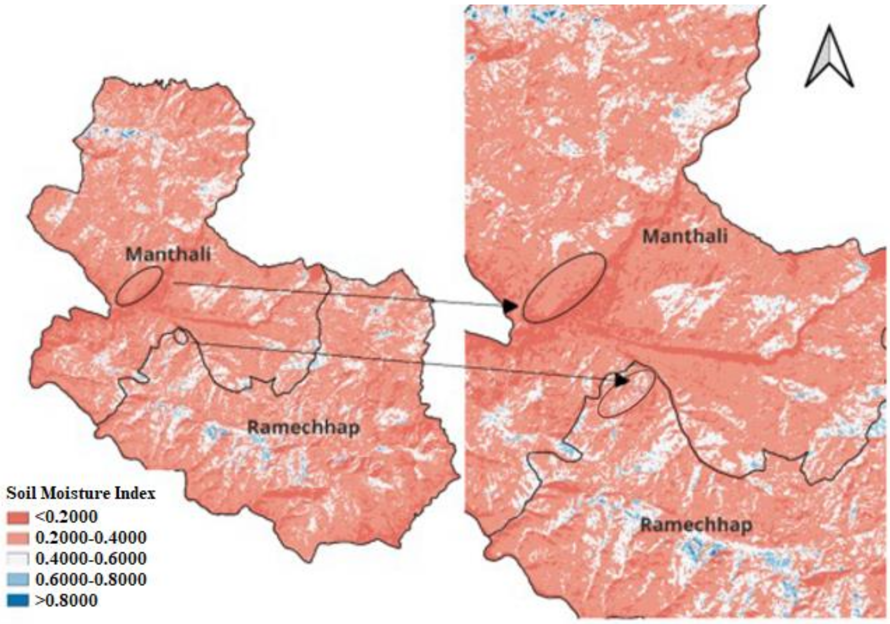

Figure 3. Soil Moisture Index (SMI) of dry site (Ramite Community Forest, Manthali) and moist site (Bhote Paakho Forest, Ramechhap) sites.

Biochemical traits

Total chlorophyll content in the leaves of *C. odorata* in the moist site was significantly higher than that of the dry site ($p < 0.001$, Fig. 4, Appendix 1). Similar was the case in the content of carotenoids. Significantly high concentration of carotenoid was found in the moist site comparing to the dry site ($p < 0.002$, Appendix 1). The differences in the content were >50% for both the pigments between dry and moist sites (Fig. 4).

Leaf nitrogen was also significantly higher in the moist site than the dry site ($p = 0.004$, Fig. 5A, Appendix 1). Contrasting to the values of photosynthetic pigments and nitrogen, the proline content in the dry site was significantly higher than that of the moist site ($p < 0.001$, Fig. 5B, Appendix 1).

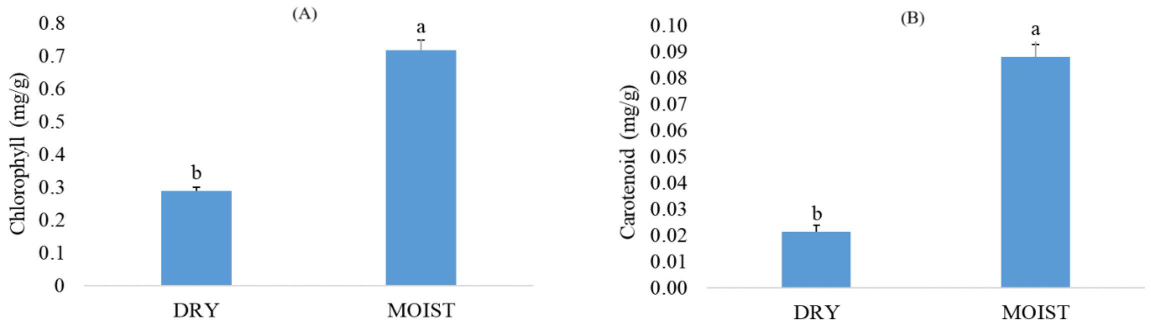

Figure 4. Concentration of photosynthetic pigments in *C. odorata* in dry and moist sites. Different alphabets 'a, b' above error bars represent significant differences in each chlorophyll and carotenoids between dry and moist sites.

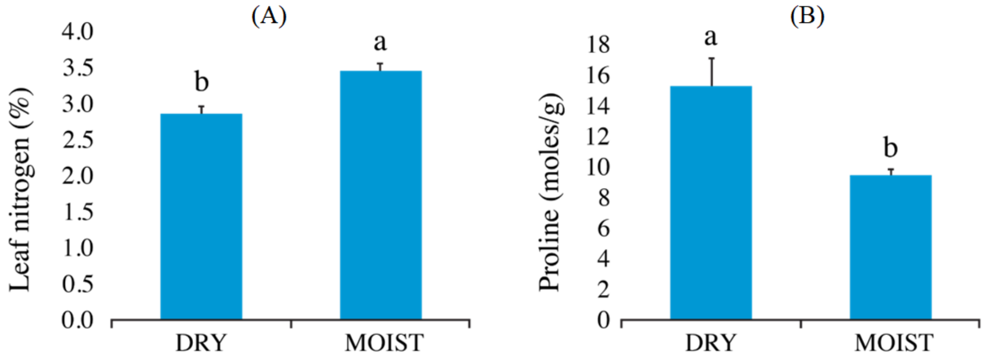

Figure 5. Concentration of leaf nitrogen (A) and proline (B) in *C. odorata* in dry and moist sites. Different alphabets 'a, b' above error bars represent significant differences between dry and moist sites in each leaf nitrogen and proline.

Reproductive parameters

The number of floral branches per plant in the moist site was greater than dry site ($p = 0.001$, Fig. 6A, Appendix 1). Though the number of seeds per capitula was not significantly different between two sites ($p = 0.391$; Fig. 6B), the seed mass in the dry site was significantly high than the moist site ($p < 0.001$, Fig. 6C, D; Appendix 1). Number of capitula was not significantly different between two sites ($p = 0.258$) but the number of matured capitula was greater in the dry site (190.93±22.98) than the moist site (only 22.1±5.68) ($p < 0.001$) (Fig. 7, Appendix 1).

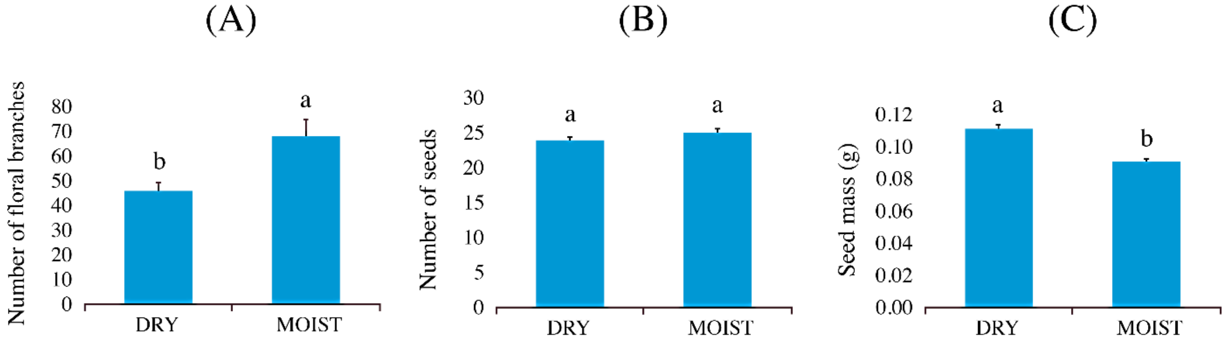

Figure 6. Reproductive parameters of *C. odorata* in dry and moist sites. (A) Number of floral branches per plant (B) Number of seeds per capitulum (C) Seed mass. Different alphabets 'a, b' above error bars represent significant differences between dry and moist sites.

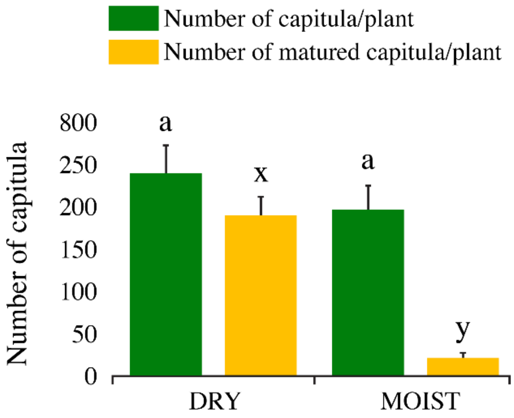

Figure 7: Number of total capitula and matured capitula per plant in dry and moist. Different alphabets 'a, b' above error bars represent significant differences between dry and moist sites.

DISCUSSION

SMI in dry and moist sites

This study compares vital functional traits of *C. odorata* between two community forests in Ramechhap district of Nepal, which were experiencing drier and moister conditions, analyzing soil moisture status. Given the high infestation levels of *C. odorata* at the drier site, we hypothesized that the functional traits of the weed vary between two sites having variation in soil moisture conditions.

The SMI of the dry site was found to be <0.3 during both pre-monsoon and post-monsoon season (Fig. 2). Range of SMI values (0 to 1) indicates the gradient of moisture level in soil (Saha *et al.*, 2019). The SMI <0.3 is considered as drought and >0.3 can be classified as no drought (Parida *et al.*, 2008). Map generated using SMI also indicated that the Ramite Community Forest is drier comparing to Bhote Paakho Forest as observed during field visit. Measurement of soil moisture content in laboratory also confirmed the result as expected. Such a significant variation in soil moisture level between these two sites and observation of *C. odorata* having disparities in level of invasion motivated to analyze trait variations of this weed from these sites.

Soil moisture index (SMI), a critical indicator of environmental health, considers NDVI and LST values, where NDVI represents the state of vegetation (density of vegetation) and LST gives the state of surface temperature (Karnieli *et al.,* 2010). The NDVI value ranges from -1 to +1 representing barren area to densely vegetated area, respectively (Wang & Feng, 2004). The average NDVI value of the dry site (0.239) indicates that the site is characterized by shrubs and grassland and the value of moist site (0.322) indicates sparse vegetation. As the value indicates, the dry site was observed with heavy invasion of *C. odorata* mixing in some patches with grass species *Heteropogon contortus* and *Themeda triandra.* The mother trees of species *Senegalia catechu* and *Aegle marmelos* were less frequent, allowing wide canopy gaps. These species are known to be drought tolerant species (Shukla *et al.*, 2014; Wang *et al.*, 2016). The moister site was observed green with large number of tree species like *Lagerstroemia parviflora*, *Shorea robusta* and *Woodfordia fruticosa,* with comparatively low infestation of *C. odorata.*

Greater specific leaf area (SLA) in dry sites

SLA among various leaf parameters is considered a key functional response of plants under varied stresses (Liu *et al.*, 2023). SLA is one of the key functional traits, which reflects adaptability of plants, growth rate, and competitive strategies across varied environmental conditions (Liu *et al.*, 2023). This trait is influenced by light and temperature variations, soil nutrient levels, and water stress (Liu & Stützel, 2004; Buajan *et al.*, 2017; Rosbakh *et al.*, 2015). At high light levels, many plants including invasive *Ageratina adenophora* decrease their SLA to reduce transpiration losses and optimize nutrient use (Wang & Feng, 2005). As per our observation in the dry site, there was larger gaps of tree canopy and light exposure to *C. odorata* leaves was high. We expect that the light condition may not be the cause of increased SLA in dry sites. Conversely, at the community level, the positive correlation between SLA and temperature, as explained by Rosbakh *et al.* (2015), can be justified by the greater SLA observed in the dry site.

Extreme temperature and low rainfall may cause drought in plants and under such stress, plants develop thicker and smaller leaves (Wellstein *et al.*, 2017). Lower SLA is beneficial for plants, which enhance water-use efficiency as the plants' adaptive strategy (Pérez-Ramos *et al.*, 2013). The dry site in our study has been experiencing lower precipitation with high temperature comparing to the moist site. In contrast, Wellstein *et al.* (2017) found high SLA in the grasses species in sub-Mediterranean systems under drought conditions. They suggested that these species exhibit enhanced growth performance, possibly due to their allocation of more resources to their underground parts. This observation can be linked to *C. odorata* because it has underground corms that proliferate vegetative shoots (Joshi, 2006) under favorable conditions. To correlate effect of soil nutrients with

SLA, soil nutrients analysis of both the sites will give better understanding (Müller *et al.*, 2000).

Loss of photosynthetic pigments and nitrogen budget may be compensated by increasing SLA

This study also aimed to know some of the physiological traits of *C. odorata* that contribute to its adaptation in drier sites. Estimation of photosynthetic pigments offers valuable insight into the interactions between plants and their environment as the alteration in the pigments is vital functional response to reflect the plant's adaptive mechanism (Mathur *et al.*, 2014; Talebzadeh & Valeo, 2022).

Studies have shown that the chlorophyll content decreases under water stress due to various causes such as oxidative damage (Mibei *et al.*, 2017). This study reported that the chlorophylls and carotenoid pigments of *C. odorata* are diminished in the dry site (Fig. 4). As explained above, the dry site was characterized with larger tree canopy gaps and *C. odorata* leaves were fully exposed to sun light. The leaves exposed to very high light intensities decrease the chlorophyll content (Ma *et al.*, 2010). Reduced pigment concentration is directly linked to a reduced rate photosynthesis in the dry sites. Hence, the weed growing dry site might be stressed with lower productivity. Remarkably, SLA was found to be increased in *C. odorata* in dry site. Based on this fact, reduction in photosynthetic pigments might be compensated by increasing SLA in the dry site by *C. odorata*.

Alteration in leaf nitrogen is another functional response to indicate environmental stress in plants. Previous studies have highlighted that drought decreases nitrogen content in stressed plants (Saud *et al.*, 2017). Possible causes for nitrogen reduction include inhibition of root growth, poor soil-root contact, effects on cell turgidity and transpiration pull, and availability of soluble nitrogen in soil (He & Dijkstra, 2014). Many of the invasive plants can easily adapt to stressful environments, and it can be expected that nitrogen content may play a significant role in this adaptation. This study elucidated that *C. odorata* adaptation in dry conditions is not linked with the high nitrogen budget (Fig. 5A). Also, in this situation, increased SLA might have contributed to compensate for this loss in the plant body.

Proline accumulation is linked to *C. odorata* invasion in dry sites

Most plants respond to stresses by synthesizing osmolytes like proline (Ghosh *et al.*, 2021). Proline content is measured in crop plants as a drought indicator component but this chemical, in the case of wild and invasive plants, has been analyzed rarely. The proline helps plants tolerate stressful environments without experiencing oxidative bursts by maintaining cell turgidity or osmotic balance, and stabilizing membranes to prevent electrolyte leakage (Hayat *et al.*, 2012). This study tried to know whether *C. odorata* gets supported by this osmolyte to adapt in dry conditions and the result showed high proline in leaves collected from drier sites (Fig. 5B). As depicted by Filippou *et al.* (2014) this information adds insight on biochemical support against water stress for *C. odorata.*

Capitula mature earlier and production of seeds with high mass under dry conditions

The reproductive traits of plants are often -neglected aspects in trait-based ecology and in characterizing their response to environmental stress (E-Vojtkó *et al.*, 2020; Day Briggs & Anderson, 2024). Floral and seed traits may alter in plants to optimize reproduction and ensuring the plants' survival, growth, and propagation (Araújo *et al.*, 2015; Hampton *et al.*, 2016). In case of invasive plants, a large number of seeds, easily dispersible seeds, and other traits are the characteristic features (Mason *et al.*, 2008) but the connection of these traits to the mechanisms by which local adaptation contributes to the invasiveness of alien plants remains to be explored (Oduor *et al.*, 2016). This study measured some of the reproductive traits like the number of floral branches, capitula, and seeds of *C. odorata* (Fig. 6). Although the number of floral branches was less in dry sites, the seed mass and number of matured capitula per plant increased significantly (Fig. 7). From this, it is evident that the larger seeds are more resilient to drought as they have high amount of food storage, and sustain the seedling longer (Beaton & Dudley, 2010). Larger seeds are often correlated with nutrient-rich conditions of soil (Fenner, 1992) but the dry site in our study appeared nutrient-deficient as the site covers comparatively rocky slope than the moist site. Nevertheless, without specific soil nutrient data, we cannot conclusively link seed mass to soil nutrient levels. Moreover, lower amount nitrogen content in the leaves at dry site can increase seed mass as a strategy to allocate more resources to seed development ensuring seedling survival.

Li *et al.* (2024a) studied flower and seed traits of *C. odorata* in introduced populations in Asia comparing with native populations in America. They found that there was trade-off between seed numbers per capitula and seed mass in its native populations, but the relationship was not found in the introduced populations. In our study, number of high biomass-seeds at dry site was equal to the number of low biomass-seeds at the moist site. Here, loss in the number of floral branches might be traded-off with mature capitula earlier and seed mass in dry site (Fig. 6, 7). If the capitula mature earlier at dry site and later at moist site, the seeds shed and disperse earlier from early matured capitula in dry site than the moist site. It can be expected that the seeds matured earlier in November may germinate and seedlings may establish before those from the moist site. If so, the seedlings can secure resources and space early on, minimizing competition during the initial establishment phase and allowing them to establish and grow more effectively (Gioria *et al.*, 2018). But germination and seedling establishment before winter are generally less likely because of dry soil and low temperature. For many plants adapted to tropical and subtropical climates, germination before winter can be counter-productive as they often cannot tolerate low temperatures. Experiment on this weed carried out by Ismail *et al.* (1996) showed the alternating temperatures of 20/30 and 25/30°C is the optimum range for seed germination. Ping (2011) also concluded that the low temperature significantly reduces the germination, with no germination at 5°C and also, the germination is totally inhibited at osmotic stress higher than -0.8 MPa. Despite this, it is essential to assess seed germination and seedling development before the onset of winter in real field condition. Such evaluation can provide valuable insight into the relationship between seed germination and seedling establishment strategies of the weed and associated seasonal changes.

CONCLUSIONS

This study has evaluated valuable functional traits of *C. odorata* to adapt in dry environment. The weed is characterized for dry site with (i) high specific leaf area (SLA) and higher content of proline but less content of photosynthetic pigments and leaf nitrogen and (ii) a fewer number of floral branches but greater number of matured capitula and high seed mass. Based on these facts (a) *C. odorata* increases its SLA for the compensation of reduction in photosynthetic pigment and nitrogen contents. Further, it can be expected that *C. odorata* has opportunity to allocate more resources to its underground corms for proliferating vegetative shoots under favorable conditions by increasing SLA. Further comprehensive study needs for linking effects of light, temperature, and soil nutrients; (b) *C. odorata* matures the capitula earlier with the high number and production of seeds with high mass under drought conditions. Without specific data, seed mass cannot be conclusively linked to specific environmental parameter. However, the lower leaf nitrogen content in *C. odorata* at the dry site suggests that the plant allocates more resources to seed development for seedling survival. Fewer floral branches may be traded for earlier maturation of capitula and increased seed mass at the dry site. Early maturation and germination are crucial traits for securing resources and space before winter, which helps in establishing and minimizing competition during initial growth phases.

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AUTHOR CONTRIBUTIONS

SP conducted field works, laboratory works and wrote original draft. LBT, MKC, RRP conceptualized and supervised. All authors contributed to the interpretation of the results and text revisions.

CONFLICT OF INTEREST

The authors do not have any conflict of interest in the research work.

DATA AVAILABILITY STATEMENT

The data analyzed during the current study is available from the corresponding author on request.

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Appendix 1. Statistical details of functional traits of *C. odorata*

Mann-Whitney U test			
SN	**Parameters compared between dry and moist sites**	**W value**	**p value**
1	SMI (April)	00.00	<0.001
2	SMI (November)	00.00	<0.001
3	Proline	98.00	<0.001
4	Carotenoids	00.00	0.002
Independent sample t-test			
SN	**Parameters compared between dry and moist sites**	**t-value**	**p value**
5	SLA	02.651	0.017
6	Chlorophyll	-13.213	<0.001
7	Leaf nitrogen	-03.974	0.004
8	Seed mass	08.156	<0.001
Negative binomial regression			
SN	**Effect of sites**	**z-value**	**p value**
9	Number of capitula	-1.132	0.258
10	Number of matured capitula	-5.719	<0.001
11	Number of floral branches	3.283	0.001
Poisson Regression			
12	Number of seeds per capitulum	0.859	0.391