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Research Article

Biomass Allocation Plasticity of An Invasive Alien Weed *Cannabis sativa* (L.): Its Implication for Its Invasiveness in an Indian Dry Tropical Urban Region

Anjali Anjali, Arvind Kumar, Narendra Kumar and Rup Narayan

Ecology Research Lab, Department of Botany, Chaudhary Charan Singh University, Meerut-250004

Abstract

India is a dry tropical region with a wide variety of native flora in the past few decades; many foreign plant species started invading into its ecosystems due to its rich soil quality. Besides this, anthropogenic interruptions ongoing in the urban and peri-urban areas, provides the environment for these species to get established and thrived with ease. Any invasive weed adopts various invasive strategies to colonize and naturalize itself in the newer environments. Out of these strategies, efficient biomass allocation pattern is directly associated with their invasiveness. The present study is aimed at understanding the invasiveness of a tropical Central Asian weed, *Cannabis sativa* L. in an Indian dry tropical region. The weed was studied for its above ground biomass (AGB) allocation patterns, at maturing stage in different parts i.e. stem, leaves, branches and reproductive parts with their mass fractions; along with studying for other plant traits i.e. shoot length, basal diameter, number of leaves, leaf area, branches, nodes and gender of plant. Besides this, soil characteristics were also investigated for a better understanding of the dynamics. The highest aboveground biomass was allocated to stem (12.25 g/plant) followed by leaves (4.89 g/plant), branches (3.16 g/plant) and reproductive parts (2.24g/plant). Mean phenotypic plasticity index of biomass fractions plant traits was higher than for morphological plant- traits which is indicative of biomass allocation plasticity as an undisputed invasive trait in the studied weed plant. Further, relatively higher allocation into stems and leaves and less allocation into the seeds but the production of large number of seeds per plants relative to number of leaves could be directly linked to its rapidly expanding invasive trait.

Keywords: *Cannabis sativa, Biomass-allocation, Invasiveness, phenotypic plasticity.*

Introduction

Cannabis sativa (L). is a herbacious plant from *Cannabaceae* family which is known by various vernacular names with most popular one as "marijuana" or 'hemp". Due to its magnificent adaptability to different climates, it is widespread across the world, in spite of its nativeness to Central Asia (Mc- partlandet, *et al.*, 2019). Gradually, its cultivation got spread rapidly across Asia and Europe leading it to a status where its cultivation nowadays, is practiced both legally and illegally across various parts of the world (Van Bakel, *et al.*, 2011).

Cannabis has a long cultivation history (Russo, 2007) which started when naturally occurring *Cannabis* stands were destroyed due to the anthropogenic activities, which led the earlier farmers to grow *Cannabis* plants nearby their residences (Clarke and Merlin, 2016). Most recent records, document the occurrence of *Cannabis* plants in more than 135 countries and territories. Out of which, it has been declared invasive in 50 of these countries (Canavan, *et al.*, 2022). In wild form, *Cannabis* is seen easily growing across the whole country of India and its cultivation is

***Corresponding Author:**
Anjali Anjali;
DOI: http://dx.doi.org/10.21746/aps.2024.13.11.3

widespread from North to South, which never get officially accounted due to lesser surveys and data in the country (Chouvy, 2019; Govindswamy, 2022).

The potential for invasiveness will be decided by the level of domestication of plant (Molina-Montenegro, *et al*., 2014; Iqbal, *et al*., 2020). *Cannabis* can thrive both in domesticated as well as wild environment. Life history, phenotypic plasticity, competitive ability, hybridization, genetic variation, dispersal capability, tolerance to a wide range of environmental conditions, resistance and allelopathic effects are some species oriented traits which chiefly contributes to the invasiveness of given plant species (Ullah, *et al*., 2022; Cranberg and Keller, 2023). *Cannabis* possesses high competitive ability, rapid growth, annual life form, and photosynthetic efficiency to sustain its invasive ability (Guo, *et al*., 2018). In wild habitats, cannabis gets colonized so densely that it reduces the light availability, which hampers the growth of surrounding native plants (Noreen, *et al*., 2019; Haq, *et al*., 2020). Besides this, seeds of *Cannabis* possess the efficiency to escape and travel to a significant distances from their cultivation point. Its seeds were more buoyant than other 93 invasive species recorded. (Moravcova, *et al*., 2010), which allowed them to float and be carried by rivers. *Cannabis sativa* has been identified as invader which is aggressively trying to overshadow the native species through its dominance in Indian dry tropical regions. Such invasive weeds have been observed to expand their area of influence in the anthropic regions through phenotypic plasticity and allelopathic influence (Gupta and Narayan, 2010, 2011, 2012; Aggrawal and Narayan, 2017). Besides this, there is little ecological investigation on invasive impact on native flora of this weed, in the mushrooming urban area of National Capital Region (NCR) Of Delhi. Meerut region coming under this zone, in the past few decades, went through many industrialization and other anthropogenic activities like Rapid rail project, setting and evolution of many industries which tampered the basic structure of the native ecosystem, thus allowing the scope of invasion by more invasive exotic species with better strategies of survival in novel environment.

So, the present investigation aimed at assessing the plant traits and biomass allocation pattern of *Cannabis sativa* w.r.t to its different plant parts to study its survival strategy in novel environments.

Materials and Methods

Study Plant

Cannabis sativa is an annual plant with dioecious nature i.e. male and female flowers are found on separate plants. It is a cross-pollinated plant with strong deep tap roots and erect stems. The stems are mostly branched with furrowed and angular exterior surface. It mostly has a woody interior but sometimes hollow in the internode area. The height varies from 1 to 6 meters. The branches are arranged in either opposite or alternate fashion. The roots are branched taproot, which goes deep to the lengths of 30-60 cm, but it may go even deeper up to 2.5 meters in loose soils. The leaves are usually of palmate type with stark green color and possess a maximum of seven lobes. The size and shape of leaves varies as per the genetic origin. Leaves are arranged opposite, or alternate or spiral with leaflets with length range of 6-11 cm and 2-15 mm width. Leaves are coarsely serrated at the margins, with scattered, resinous trichomes on abaxial and adaxial surfaces. Numerous flower heads which are located on long leafy stems and at the axil of each emerging leaf comprise the inflorescence. The female flowers (pistilate) are sessile and occurs in pairs at each internode of every branch and sub-branches. Male plants are usually taller and less robust than the female plants. Male plants have mixed inflorescence. Cymose clusters form off a central racemose axis. Plants have one male inflorescence per each leaf axil. The seed is ellipsoid, slightly compressed and smooth with an average size of 2-5 mm long. It is generally brownish in color and blotched in appearance. (Raman, *et al*., 2017; Hesami, *et al*., 2023)

Study Area
This study was conducted in Chaudhary Charan Singh University, Meerut campus (28°96'98. 21'' N lat., 77°73'58. 62'' E long. and 224 m ASL), which is located in the National Capital Region of Delhi at Meerut. The study area was naturally protected in the less commuted area of the university, where the natural occurrence of annual and perineal exotic and native flora could be easily spotted at the time of study. Of these plant species, *Cannabis sativa* came into the noticeable presence as its colonies started to grow at rapid rates and is able to spread at rates which not only impacts the native flora, but also the constituents of soils and diversity structure around. The climatic conditions of the selected site area are semi-arid, which typically covers three major seasons – Summers (March – June), Rainy (July to September) and winters (November – February). Average temperature for studied period was 42.5 °C in summers.

Plant Samples for Biomass Allocation Study:
Two hundred healthy and mature plants of *Cannabis sativa* (100 male and 100 females) were collected in full blooming reproductive phase (ripening up of flowering and seeding happening along), from different patches of *Cannabis* colonies existing in the selected site area during the month of mid-May to mid-June 2022. The plants were harvested from the site, and plant parts were separated into leaves, stem, reproductive part and branches, with the help of a sharp knife. Then, plant level traits i.e. Shoot length (cm), basal diameter (cm), gender of plant, number of leaves, leaf area, number of branches, branchlets, stem and nodes were recorded. Two hundred fresh leaves of varying sizes (100 for male and 100 for female), of matured plant individuals were randomly plucked from study site and leaf area of all leaves was then measured by digital leaf area meter (Systronics). Further, the plants were separated into different parts for estimating biomass fractions of leaves with petioles, branches with branchlets, stems, and reproductive parts (inflorescence/flowers/seeds). All these segregated plant parts were then put into the oven set at 80 °C for 48 hours straight, for releasing all the moisture content and then their dry weights were measured. The mass fractions of different plant parts i.e. stem mass fractions (SMF), leaf mass fraction (LMF), branch mass fraction (BMF) and reproductive mass fraction (RMF) were estimated as the biomass of each part relative to the total aboveground biomass (AGB) and calculated separately for male and female plants. Total number of seeds produced per plant was calculated by manual counting through forceps and needle for 20 plants. The biomass of each seed was estimated based on dry weight of randomly selected 1000 seeds.

Soil Analysis
Five surface soil samples (0-5 cm) were collected randomly from the studied site area in the month of May- June, 2022. Then this soil was sieved with a 2 mm (about 0.08 in) sieve and physico – chemical characteristics i.e. soil moisture content, pH, conductivity and total organic Carbon were studied (Walkley and Black, 1934).

Plasticity Indices and Statistical Analysis:
The phenomenon of phenotypic plasticity is usually found in invasive plants, which typically refers to the ability of the plant to alter its physiology and morphology to adapt to the changing environments well (Schlichting, 1986, Nictora, *et al.*, 2010). The plasticity indices were calculated by using the equation (Funk, *et al.*, 2008, Lamarque, *et al.*, 2013).

$$P_I = \frac{\text{Maximum value- Minimum value}}{\text{Maximum value}}$$

The plasticity range of mature individuals in reproductive phase was compared by calculating the individual plasticity indices (PIv) of each plant traits (Valladares, *et al.*, 2006). The maximum value of index ranges from zero (no plasticity) to one (maximum plasticity), which is evaluated by finding the difference between the maximum and minimum values of the trait and dividing it by the highest value at a site (Gupta and Narayan, 2012). Further, the mean of all plasticity indices evaluated for individual plant traits was found by finding the average of all variables. Pearson Correlation coefficients and t-test were done using SPSS software.

Results

Plant Traits

It was observed that, the mature plants of *Cannabis* in studied area have a mean stem length of 116 cm and mean base diameter 1.24 cm (Table 1). There was a direct strong positive correlation between the INF with the NB ($p<0.01$) in male plant whereas, there is a direct positive associativity between the total NB and BRN with the INF in female plants ($p<0.01$). But, if the proportions and allocation in terms of biomass is talked about for both the genders, much greater investments were seen in stem (11.53 g/individual, including branches and stems), followed by leaves (4.89g/individual). Surprisingly, biomass allocated to the reproductive parts was very less i.e. 2.24 g/ individual, but the number of seed produced by a tallest healthy female found (10-12 ft.) plant reached to 2500-3000 per plant. The biomass of each seed averaged 2.01 mg, which makes it extremely light weight and apt for dispersal, definitely contributing to its invasive ability. Lesser biomass of the reproductive parts may be attributed to the type of floral arrangements.

Besides this, the total above ground biomass (AGB) of an adult Cannabis plant was recorded to 21.82 g, of this the major contribution was of stem and branches (14.69g) which primarily is the support system of the plant followed by leaves (4.89g). This order can be easily seen by the arrangement of the mass fractions in order SMF>LMF>BMF>RMF (table 1).

This plant possessed an average of 15 branches and 13 branchlets (for the collective pool). The average number of leaves per plant recorded was 137, with total leaf biomass average of 4.87g/individual, giving an average biomass of single leaf to be 35.54 mg.

On further investigating the strategy of biomass allocation in an adult plant for collective pool w.r.t. different aboveground plant parts, biomass was found maximum in stem (52.84%), followed by leaves (22.41%), branches (14.48 %) and least to reproductive parts (10.26 %) [Pi Charts (a)]

Soil samples from study sites were found to be slightly acidic to neutral (mean pH 7.52) with mean organic carbon of 1.97% and 9.83% moisture content (Table 2)

Plasticity Indices

The plasticity indices (PIv) for morphological plant traits had a range of 0.664 to 0.978 and for plant-level original biomass fraction traits 0.621-0.983 (Ref Table 3). The estimated mean phenotypic plasticity of biomass fraction plant traits (PIv 0.866) was relatively higher than plant-level morphological traits (PIv 0.829) evaluated for the study site for collective pool. The difference between the mean phenotypic plasticity for morphological traits and biomass fractions were found to be almost similar in both male and female population (0.123) [Table 3]

Besides this, Plasticity indices for SL, BD, IN, SMF and NN were found to be lower than LN, LA, BN, LMF, RMF and BMF for collective population.

Table 1: Plant level traits (Mean±S.E) of adult individuals of *Cannabis sativa* (n=200), 100 males and 100 females.

Traits	Male Plant N=100	Female plant N=100	Overall Pool , n=200	p-value t-test(for A&B)
Number of leaves (LN)	91.39±10.24	**183.22**±17.23	137.76±13.24	<0.01
Number of branches (BN)	15.92±1.35	**16.82**±3.34	15.46±2.34	<0.05
Number of Branchlets (BRN)	12.89±1.21	25.82±1.54	13.45±2.54	<0.01
Number of Inflorescences (INF)	78.54±5.34	**45.02**±2.34	56.78±3.27	<0.01
Number of Nodes (NN)	16.68±0.50	**20.99**±0.98	17.17±0.50	<0.04
Shoot length (SL) (cm)	104.84±7.53	**128.02**±4.57	116±3.58	<0.01
Basal diameter (BD) (cm)	0.95±0.03	**1.74**±0.02	**1.24±0.04**	<0.01
Leaf Area (LA)	31.48±3.54	**39.18**±3.87	35.09±3.46	<0.04
Leaf biomass (LB) (g)	3.26±0.49	**4.63**±0.56	4.89±0.58	<0.05
Branch biomass (BB) (with branchlets) (g)	2.97±0.59	**3.64**±0.24	3.16±0.37	<0.05
Reproductive biomass (RB) (g)	1.97±0.21	**2.51**±0.54	2.24±0.49	<0.03
Stem biomass (SB) (g)	8.94±1.23	**12.54**±0.93	11.53±1.29	<0.01
Aboveground biomass (AGB) (g)	17.14±1.25	**23.32**±0.98	21.82±2.34	<0.01
Leaf mass fraction (LMF)	19.01±0.007	**19.85**±0.002	22.41±0.005	ns
Branch mass fraction (BMF)	17.32±0.006	**15.61**±0.003	14.48±0.005	<0.05
Reproductive mass fraction (RMF)	11.49±0.003	**10.76**±0.002	10.26±0.092	<0.05
Stem mass fraction (SMF)	52.11±0.023	**53.77**±0.09	52.84±0.029	<0.05

Table 2: Physico-chemical characteristics of soils at the study site

Soil Characteristics	Mean±S.E.
Moisture content (%) (winter soil)	9.83±0.36
pH	7.52±0.15
Conductivity (mS)	0.29±0.10
Total Organic Carbon (%)	1.97±0.12

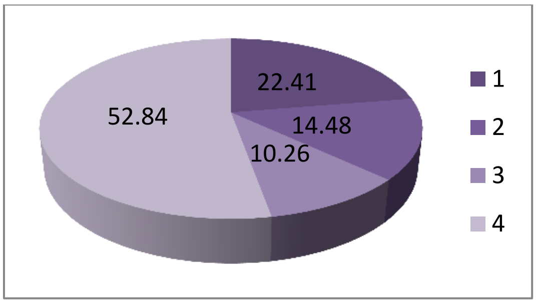
Table 3: Plasticity indices of different plant-traits (morphological and plant part biomass fraction) in adult individuals of an exotic weed *Cannabis sativa* in a dry tropical urban eco system (See Table 1 for codes)

For Plant (n=200)	
Morphological(overall)	
LN	0.975
BN	0.978
IN	0.777
NN	0.751
SL	0.664
BD	0.786
LA	0.975
Mean value	**0.829**
Biomass fraction	
LMF	0.942

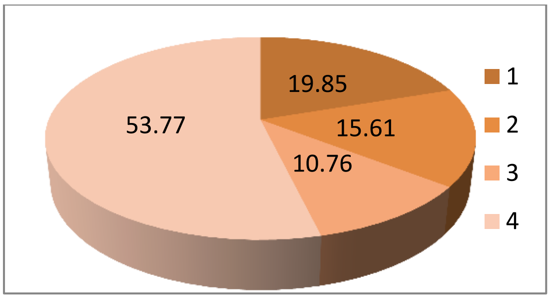
SMF	0.621
BMF	0.983
RMF	0.918
Mean Value	**0.866**
For Male Plant (n=100)	
Morphological	
LN	0.651
BN	0.959
IN	0.918
NN	0.714
SL	0.703
BD	0.969
LA	0.915
Mean value	**0.789**
Biomass fraction	
LMF	0.841
SMF	0.916
BMF	0.973
RMF	0.921
Mean Value	**0.912**
For female plant AGB (n=100) morphologcal	
LN	0.878
BN	0.878
IN	0.666
NN	0.842
SL	0.753
BD	0.817
LA	0.923
Mean value	**0.812**
Biomass fraction	
LMF	0.941
SMF	0.948
BMF	0.911
RMF	0.939
Mean Value	**0.934**

Table 4: Comparative data of various invasive species in Indian dry tropical region, on the basis of Above Ground Biomass allocation fraction in different parts of the plants

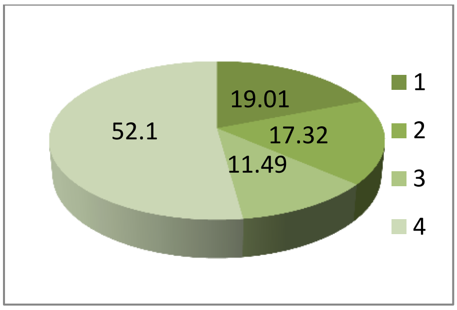
Plant species	Reproductive part (%)	Leaf (%)	Stem (%)	References
Parthenium hysterophorus	7.0	46.5	46.5	Gupta, 2008
Achyranthes aspera	8.1	45.4	46.5	Gupta, 2008
Cassia obtusifolia	12.2	41.1	46.7	Gupta, 2008
Sida cordifolia	24.0	37.0	39.0	Singhal and Narayan, 2014
Sida acuta	13.0	49.0	38.0	Singhal and Narayan, 2014
Ageratum conyzoides	26	12.6	61.1	Chaudhary, *et al*., 2015
Chenopodium murale	27.4	26.4	46.2	Gupta and Narayan, 2012
Hyptis suaveolens	29.5	19.6	50.9	Lomas and Narayan 2023
Cannabis sativa	10.26	22.41	52.84	Present study



(a) Biomass allocation strategy of adult plant individuals of exotic invasive weed *Cannabis sativa* adult plants in an Indian dry tropical region. (n=200)



(b) Biomass allocation strategy of adult plant individuals of exotic invasive weed *Cannabis sativa* female plants in an Indian dry tropical region.



(c) Biomass allocation strategy of adult plant individuals of exotic invasive weed *Cannabis sativa* male plants in an Indian dry tropical region.
Note: 1=LB, 2=BB, 3=RB, 4=SB in Percentage

Discussion

To survive in a novel environment, invasive plants need to adapt various strategies, out of which phenotypic plasticity can be considered as the most potent for colonization success (Lehmann and Rebele, 2005). On further noticing deeply the biomass distribution in mature individuals at sites of its dominance, it can be inferred that the invasiveness of the plant can be attributed to light biomass of seeds and high buoyancy (Moravcova, *et al*., 2010) and also the high seed count per plant in spite of less biomass (lesser than previously recorded invasive species; table 4.) attributed to reproductive part (2.24g/plant) see table1. This high count of seed formation and dispersal makes it the most potent trait of an invader for rapid colonization (Parker, *et al*., 2003). Also in the mature plant studied, it allocated less than one fifth part of its aboveground biomass to plant part involved in photosynthesis, can probably take trends to different shifts (Lomas, 2023).

At the seeding stage , a mature adult, showed 52.84% allocation to the stem recorded in the present study , greater than commonly existing weeds and only little lesser than the highest recorded of *Ageratum conyzoides* (Table 4) in the area. This high stem allocation is due to its taller heights, which are the result of the competition with the surrounding invasive species, like *Chenopodium murale* etc. (Gupta and Narayan, 2012).

Besides this, around 65 % percent of its AGB is allocated to its support system i.e stem and branches. This huge portion of investment to support structure *viz.* stem and branch not only contributes to its perennial nature but also allows it to outcompete the neighbor invaders and native plants, by hampering the reach of availability of light for photosynthesis (Noreen, *et al*., 2019, Haq, *et al*., 2020) along with providing it the enhanced ability to exploit the aboveground resources like. CO_2, solar energy etc., through the leaf areas of exposed leaves on stem and branches. In addition to this, the relative organically rich soils, which was evident in current dry tropical study site with soil organic carbon of 1.97%, the competition for above ground resources comes into the sight. (Poorter and Nagel, 2000). Dry matter allocation strategy of a plant species is often impacted by the ontogenies and soil resource states (Maron, *et al*., 2004). High plasticity indices for LN, BN, LA, LMF, RMF and BMF in the studied plant species shows high phenotypic plasticity of this invasive plant, which has been considered an important characteristic trait that facilitates invasiveness of the alien plants (Gupta and Narayan, 2012). Also, invasive plants possess a high net productivity which shows their ability to contribute significantly to the total soil organic carbon through their biomass, which eventually creates a loop of positive feedback which indirectly supports their own growth (Joan G. Ehrenfeld 2003; Ming Nie, *et al*., 2017).

Conclusion

The monstrous expansion rates in *Cannabis sativa,* in Indian dry tropical region could be attributed to the high seed count per plant and light seed weight along with high plasticity indices of LN, LA, LMF, RMF and BMF. Its perennial nature can be attributed to its maximum biomass allocation in stems followed by leaves.

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