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A comprehensive analysis of morphological and biochemical parameters of *Medicago sativa* during biotic stress

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ABSTRACT

Background: Parasitism is an abiotic stress that significantly threatens plant growth and severely impacts crop productivity. Plants have developed complicated regulatory mechanisms to cope with stressful conditions, undergoing morphological and biochemical changes.

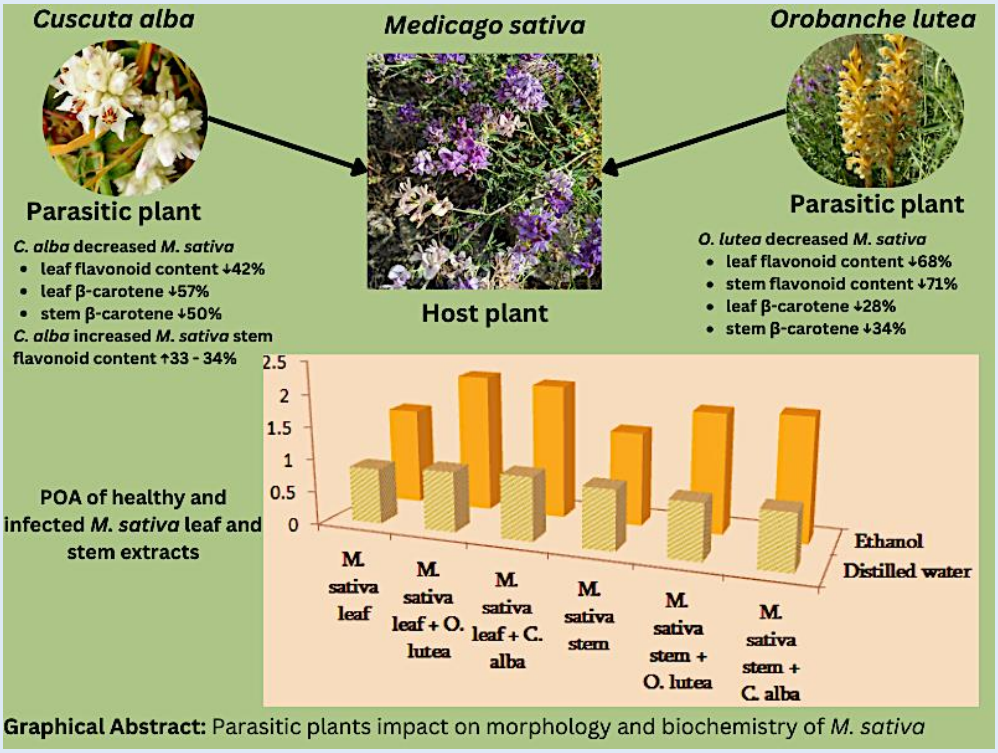
Objective: The article presents the first comprehensive analysis of the *Medicago sativa* response to the penetration of flowering parasites *Orobanche lutea* and *Cuscuta alba*. The outcome of this study aim to enhance crop quality comprehend their bioactive properties and pinpoint optimal strategies for their utilization in preventing and treating human and animal health issues.

Methods: *Medicago sativa, Orobanche lutea* and *Cuscuta alba* were collected in the Ararat province, located in southeastern Armenia. The plants' morphological structures and leaf parameters were observed. The prooxidant activity was determined by the potentiometric method. The content of total phenols, flavonoids and catechins were determined by a spectrophotometric method. Pigments were separated and identified using thin layer chromatography and a spectrophotometric method.

Results: The *in-situ* observations of *M. sativa* plant and *ex-situ* measurements of its leaves infected by *O. lutea* and *C. alba* reveal a significant negative impact on both the reproductive success and the vegetative aspects. Infected plants exhibited minimal inflorescence development, also, substantial reductions in leaf dimensions were observed. The analysis of prooxidant activity revealed that *M. sativa* exhibits inherent prooxidant properties, which are amplified in the presence of parasites. The influence of dodder on prooxidant activity is much more significant (14-38%) than that of broomrape (8-42%). The percentage changes in total phenolic content were more pronounced in the presence of *C. alba*, but flavonoid decline was more pronounced in the presence of *O. lutea*, suggesting a differential impact of the two parasites. Although catechin content was not affected, photosynthetic pigments of the host plant were significantly (28-57%) reduced by the parasites.

Conclusion: Findings indicate a clear morphological alteration induced by parasitic infestation. The parasites, particularly *O. lutea* can cause temporal isolation and allochronic speciation in *M. sativa* populations. Biochemical analysis highlighted a complex interplay between the host and the parasite. The biochemical impact of the parasite is reflected on the host's primary and secondary metabolism. The observed morphological and biochemical changes highlight the need for further research to explore potential mitigation strategies, selective herbicide development, and biocontrol measures against parasitic plant infestations in agricultural ecosystems.

Keywords: alfalfa; *Orobanche lutea; Cuscuta alba;* parasites plants; morphophysiological observations; bioactivity.



Graphical Abstract: Parasitic plants impact on morphology and biochemistry of *M. sativa*

INTRODUCTION

Medicago sativa L., commonly referred to as lucerne or alfalfa, is a perennial flowering plant in the Fabaceae family. The plant has been widely cultivated since ancient times as a forage crop for livestock because of its high proteins and considerable biomass. It has various other

agricultural and medicinal uses [1-6]. It grows at different altitudes and in a wide variety of environmental conditions in Armenia. Adapting to all these different conditions, *M. sativa* has varying forms in its characteristics [7-8].

Alfalfa is not only valuable for animal nutrition, providing a high-protein forage that supports livestock health and productivity, but it is also widely appreciated for human consumption. In several countries, alfalfa sprouts are prized as a nutritious addition to salads, baked goods, sauces, and soups, due to their rich profile of vitamins, minerals, and other bioactive compounds. [9-11]. Edible plants serve as primary origins of bioactive substances known as nutraceuticals, operating through various mechanisms to promote human health [12-13]. Alfalfa also is increasingly recognized for its health benefits. In traditional herbal medicine, *M. sativa* is employed for addressing various health conditions. In Indian and Ayurvedic traditions, it is renowned for its attributes as an agent to treat the female reproductive system, to lower cholesterol levels, its abundance in crucial enzymes, minerals, and vitamins, and its preventive qualities against conditions such as hypertension, diabetes, peptic ulcers arthritis pain, and fluid retention [13-14]. Alfalfa's richness in minerals and high chlorophyll content plays a key role in promoting the well-being of bones and teeth and supports the growth of connective tissue, making it particularly beneficial for treating wounds, ulcers, as a remedy for kidney diseases, and antibacterial and antifungal agent [15-19].

Alfalfa tea is used to enhance the digestive and nervous system as well as for the management of arthritis and scurvy [20-21].

In traditional Persian medicine, *M. sativa* seeds have been recommended as a treatment for male infertility [22]. The plant was used in Europe, as an anti-hemorrhagic, galactagogue and to improve blood circulation and digestion in traditional medicine [23-24].

The plant has also been widely cultivated as a fodder and medicinal plant in Armenia for centuries. As noted by Amirdovlat Amasiatsi, in Armenia *M. sativa* has a history of application in addressing blood clotting disorders, soothing nervous hand tremors, alleviating coughs and its seeds augment lactation in breastfeeding mothers [25].

This triple role of alfalfa as a vital agricultural resource, a functional food and medicinal plants highlights its importance in dietary, health and farming systems worldwide. The limitations of current drug treatments have sparked interest in studying bioactive compounds of the functional foods to help slow the progression of chronic diseases such as hypertension, type 2 diabetes, and etc. [26]. The phytochemical analysis of alfalfa extracts showed the presence of a wide sector of biologically active substances in *M. sativa*. It has been documented to be a source of alkaloids (stachydrine, homostachydrine), aminoacids (arginine, asparginine, cystine, histidine, isoleucine, leucine, methionine, tryptophan, valine), coumarins (medicagol, sativol, trifoliol, lucernol, 4-o-methyl coumesterol, 3-methoxycoumesterol, 11,12–dimethoxy-7-hydroxylcoumesterol), flavonoids (quercetin, myricetin, luteolin, apigenin, chrysoeriol, tricin, coumestrol, biochanin A, genistein, etc.), benzofuran neolignans, saponins (pentacyclic triterpenoid saponins, medicagenic and zanhic acids, hederagenin, soyasapogenol A, B, and C, bayogenin glycoside), steroids (stigmasterol, campesterol, cycloartenol, β-sitosterol), acids (lauric, maleic, malic, malonic, myristic, oxalic, palmitic, quinic), purines (adenine, guanine, xanthine, hypoxanthine), canavanine, amino acids (medicanine, lysine, arginine, histidine, tyrosine, phenylalanine, methionine, aspartic acid, glutamic acid, asparagine, serine, alanine, threonine), a range of vitamins (A, B_1, B_6, B_{12}, C, D, E, K), ketones (myristone, alfalfone), polysaccharides (fucose, arabinose, galactose, glucose, xylose, mannose, etc.) and other constituents such as fructose, pectin, chlorophyll,

and minerals [16, 27-31]. The outcomes of numerous experiments indicate that *M. sativa* exhibits various pharmacological properties, including analgesic, anticancer, antidiabetic, anti-inflammatory, antimicrobial, antioxidant, antiulcer, anxiolytic, cardioprotective, dermatological, estrogenic, hepatoprotective, hypocholesterolemic, hypolipidemic, immunomodulatory, neuroprotective, photodegradation, and reproductive effects [15-17, 21, 30, 32-40].

In recent years, growing risk factors have driven a global shift toward the use of medicinal plants and natural foods in the treatment of diseases affecting humans, animals, and plants. New diseases require identifying new bioactive compounds and mechanisms of action, which can be discovered, for example, during comprehensive studies of the relationship between parasite plants and host plants. New natural compounds are synthesized as a response to the penetration of the parasite into the host plant. Typical parasites for alfalfa in Armenia are *Orobanche lutea* (Desf.) Nyman and *Cuscuta alba* C. Presl. Hence, in this work, we aimed to evaluate the bioactivity of alfalfa ethanolic and water extracts in parasite-host relationship conditions.

The outcomes of this study aim to enhance functional food quality, comprehend their bioactive properties and pinpoint optimal strategies for their utilization in preventing and treating human and animal health issues.

METHODS

Equipment and Reagents: All reagents were purchased from Sigma-Aldrich GmbH (Sternheim, Germany) and were of analytical grade unless otherwise mentioned.

Plant Material: *M. sativa* was collected in June, from the field near Zangakatun village, Ararat province, located in southeastern Armenia, with latitude 39˚48´27˝N and 45°4´12˝E (Fig. 1). Most of the fields were partially infected either by *O. lutea* or by *C. alba*. We collected the samples from the healthy *M. sativa* plants, the infected *M. sativa* plants and both parasites. The samples collected were moved to the laboratory during that day. The photos of the plants were taken.

The voucher specimens have been stored in the Higher Plants Herbarium (ERCB) of Yerevan State University (*M. sativa* No. 13659; *O. lutea* No. 13660; *C. alba* No. 13698).

Figure 1. The location of the gathering area.

Morphophysiological Observations: To determine whether the presence of parasites affects the morphology, the plants were observed and documented both in the field and at the lab. The growing conditions and coordinates of the harvested plants were registered.

To determine the structural differences the width (W) and length (L) of leaves of the harvested plants were measured. 45 leaves from adult plants per group were selected. The measurement was conducted using graph paper (accuracy of 1 mm). The Standard Deviation (SD) and length-to-width ratio (L/W) were calculated.

Obtaining Extracts: The chosen fresh plant samples were air-dried in room conditions for 14 days. The dried samples (5.0 g) were placed on a magnetic stirrer with 50 mL distilled water or ethanol (70%) for extraction for 24 hours. The extracts were filtered using a 0.45 µm pore size filter. All the extracts were used within 3 hours after preparation [41].

Determination of Prooxidant Activity: The prooxidant activity (POA) was determined using potentiometric measurements of the oxidation-reduction potential (ORP) changes in the $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ mediator system caused by the sample extracts. A method described in Gevorgyan et al., 2017; was slightly modified to work with current samples and equipment [42]. 1.0 mL of sample was added to 3.0 mL of the $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ solution and incubated at 45°C for 30 minutes. The ORP changes were measured using a Hanna Edge pH meter with a HI-36180 combined platinum-reference electrode. The POA was assessed by comparing the ORP changes to a standard curve of the hydrogen peroxide (48-192 µg/mL).

Determination of Total Phenolic Content: The content of total phenols was determined by a modified spectrophotometric method using gallic acid as a standard. 1.0 mL of sample was added to tubes containing Folin-Ciocalteu's reagent (0.5 mL, 2.0 N) and water (7.0 mL). After 3 minutes, a sodium carbonate solution (0.5 mL, 7.5% w/v) was added. The mixture was incubated at 45°C for 60 minutes, and the absorbance was measured at 750 nm using a VWR V-1200 Visible Spectrophotometer. The polyphen ol concentration in the samples was determined using a standard curve of gallic acid (10-50 µg/mL) [43-44].

Determination of Total Flavonoid Content: The content of total flavonoids was determined by a modified spectrophotometric method using rutin as a standard. 1.0 mL of sample was added to tubes containing $AlCl_3$ (0.5 mL, 1% in methanol). The mixture was allowed to stand at room temperature for 10 minutes, and the absorbance was measured at 410 nm using a VWR V-1200 Visible Spectrophotometer. The flavonoid concentration in the samples was determined using a standard curve of rutin (0-0.5 µg/mL) [45].

Thin-layer Chromatography of Extracts: Thin-layer chromatography (TLC) was performed on a silica gel 60 F254 on aluminum plates (20 x 20 cm; Merck, Darmstadt, Germany) using toluene: acetone (6:4) as a mobile phase [46-47].

Determination of β-Carotene and Lycopene: For β-carotene and lycopene determination, the dried plant powder was vigorously shaken with an acetone:hexane mixture (4:6, 10 mL) for 30 min. The extract was centrifuged at 10 000 rpm for 3 minutes and filtered through filter with pore sizes of 0.45 um. The absorbance of the Filtrate was measured at 453, 505, and 663 nm. β-carotene and lycopene concentrations were calculated by the following formulae:

$$\text{lycopene (mg/100 mL)} = -0.0458 \times A663 + 0.372 \times A505 - 0.0806 \times A453$$

$$\beta\text{-carotene (mg/100 mL)} = 0.216 \times A663 - 0.304 \times A505 + 0.452 \times A453$$

The results are expressed as mg of carotenoid/g of extract [43].

Determination of Catechins: For catechin determination 50 g of powdered plant sample was taken. Then 40 mL of distilled water was added and placed on a magnetic stirrer at room temperature for 1 hour. The extract was washed several times by 40 mL of chloroform. A funnel separator was used. The washed water phases' absorbance was measured at 274 nm using Bioevopeak UV-Vis Spectrophotometer SP-LUV752 [48].

RESULTS

Morphological observations: The *in-situ* observations were conducted at the plant growing site. Observations showed that almost no inflorescence could be found on the plants affected by either parasite, even when the non-affected plants growing in the same field developed seeds. Besides, the measurements of the leaf length and width showed that both parasites significantly affect leaf size (Table 1).

Table 1. Width (W), length (L) and their relation (L/W) of healthy and infected *M. sativa* leaves (cm).

Sample	Healthy	Infected by *O. lutea*		Infected by *C. alba*	
***M. sativa* leaf L**	2.6±0.4	1.84±0.42	↓29%	1.76±0.39	↓32%
***M. sativa* leaf W**	1.17±0.3	0.62±0.09	↓47%	0.82±0.21	↓30%
L/W	2.22	2.98	↑34%	2.15	↓3%*

Data are expressed as the mean value ± SD (n = 45).
*insignificant decrease

Biochemical analysis: The measurements of POA showed that *M. sativa* intrinsic POA increases in the presence of a parasite (Table 2). *O. lutea* and *C. alba* increase POA only for alfalfa stems ethanolic extract and both water and ethanolic extracts of leaves.

Table 2. POA of healthy and infected M. sativa leaf and stem extracts.

Sample	Distilled water (x10^{-5} moles of electrons accepted)		Ethanol (x10^{-5} moles of electrons accepted)	
***M. sativa* leaf**	0.85±0.01	-	1.48±0.02	-
M. sativa* leaf infected by *O. lutea	0.92±0.04	↑8%	2.1±0.11	↑42%
M. sativa* leaf infected by *C. alba	0.97±0.06	↑14%	2.04±0.09	↑38%
***M. sativa* stem**	0.91±0.01	-	1.42±0.03	-
M. sativa* stem infected by *O. lutea	0.85±0.04	↓7%	1.82±0.07	↑28%
M. sativa* stem infected by *C. alba	0.85±0,03	↓7%	1.87±0.06	↑32%

Data are expressed as the mean value ± SD.

Analysis of total phenolic content showed that both parasites significantly decreased the polyphenolic compounds in the investigated organs of *M. sativa* (Table 3) – much more in the water extracts (up to 33%) than in the ethanolic extracts (up to 15%).

Table 3. The total phenolic content of the healthy and the infected *M. sativa* leaves and the stem extracts.

Sample	Distilled water (g/L)		Ethanol (g/L)	
***M. sativa* leaf**	5.93±0.07	-	5.90±0.9	-
M. sativa* leaf infected by *O. lutea	4.20±0.18	↓29%	5.10±0.12	↓14%
M. sativa* leaf infected by *C. alba	3.95±0.13	↓33%	5.01±0.1	↓15%
***M. sativa* stem**	2.08±0.07	-	2.98±0.06	-
M. sativa* stem infected by *O. lutea	1.53±0.13	↓26%	2.63±0.12	↓12%
M. sativa* stem infected by *C. alba	1.55±0.17	↓25%	2.54±0.11	↓15%

Data are expressed as the mean value ± SD.

The flavonoid content in the leaf extracts decreased, much more by the influence *of O. lutea* (up to 68%) than *C. alba* (up to 42%). *O. lutea* decreased the flavonoid content of stems by 71% at most. However, *C. alba* surprisingly intensifies the flavonoid synthesis in the stem, by 33% on average (Table 4).

Table 4. Flavonoid content of healthy and infected *M. sativa* leaves and stems.

Sample	Distilled water (g/L)		Ethanol (g/L)	
***M. sativa* leaf**	2.38±0.12	-	4.45±0.26	-
M. sativa* leaf infected by *O. lutea	0.77±0.10	↓68%	1.7±0.21	↓62%
M. sativa* leaf infected by *C. alba	1.39±0.14	↓42%	2.6±0.31	↓42%
***M. sativa* stem**	1.44±0.08	-	1.34±0.09	-
M. sativa* stem infected by *O. lutea	0.42±0.04	↓71%	0.39±0.05	↓71%
M. sativa* stem infected by *C. alba	1.92±0.16	↑33%	1.8±0.13	↑34%
O. lutea	-	-	0.15±0.05	-
C. alba	-	-	47.53±5.53	-

Data are expressed as the mean value ± SD.

The analysis of the catechin content of plants' leaves and stems did not show a significant change during parasitism by either species (Table 5).

The measurements of lycopene and β-carotene showed that parasitism significantly affects the latter content (Table 6). Meanwhile, no lycopene was found in alfalfa leaves in detectable amounts. β-carotene content can be reduced by up to 34% during the infection with *O. lutea*, and by up to 57% during *C. alba.*

TLC of pigments showed that parasites drastically reduce the host plant's pigment amount, thus affecting photosynthesis (Fig. 2).

Table 5. Catechin content of healthy and infected *M. sativa* leaves and stems.

Sample	Catechins (ug/g of plant dry mass)	
***M. sativa* leaf**	304.95±28.14	-
M. sativa* leaf infected by *O. lutea	328.92±31.11	↑7.9%*
M. sativa* leaf infected by *C. alba	280.35±27.65	↓8.1%*
***M. sativa* stem**	87.02±7.04	-
M. sativa* stem infected by *O. lutea	85.58±7.54	↓1.7%*
M. sativa* stem infected by *C. alba	82.25±7.01	↓5.5%*

Data are expressed as the mean value ± SD.
*Insignificant difference

Table 6. Lycopene and β-carotene content of healthy and infected *M. sativa* leaves and stems.

Sample	Lycopene (mg/g of plant dry mass)		β-carotene (mg/g of plant dry mass)	
***M. sativa* leaf**	<0.01	-	5.48±0.51	-
M. sativa* leaf infected by *O. lutea	<0.01	-	3.95±0.29	↓28%
M. sativa* leaf infected by *C. alba	<0.01	-	2.34±0.24	↓57%
***M. sativa* stem**	<0.01	-	2.26±0.26	-
M. sativa* stem infected by *O. lutea	<0.01	-	1.50±0.12	↓34%
M. sativa* stem infected by *C. alba	<0.01	-	1.13±0.07	↓50%

Data are expressed as the mean value ± SD.

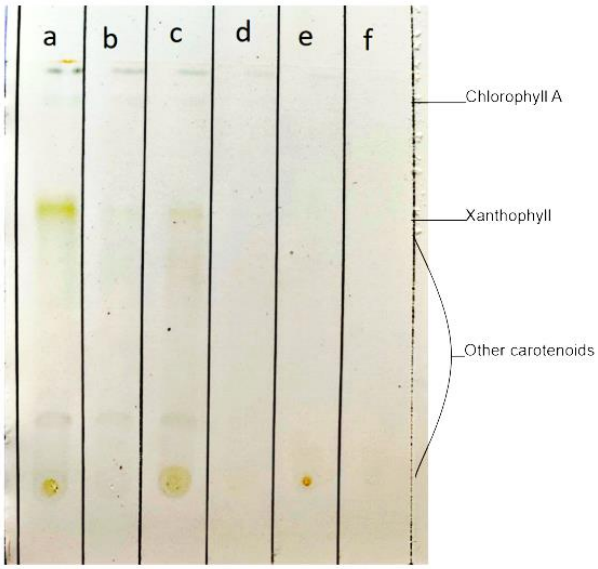


Figure 2. TLC of *M. sativa*: a – healthy leaves; b – healthy stem; c – leaves of the plant infected by *O. lutea*; d – stem of the plant infected by *O. lutea*; c – leaves of the plant infected by *C. alba*; d – stem of the plant infected by *C. alba*.

DISCUSSIONS

The morphological observations presented in the study provide valuable insights into the impact of parasitic plants (*O. lutea* and *C. alba*) on *M. sativa* plants. The fieldwork observations revealed a stark difference in the reproductive success of the infected plants. Almost no plant (less than 1%) affected by *C. alba* managed to develop flowers and when some did, inflorescences were incomplete and never got to the seed formation. The plants affected by *O. lutea* developed inflorescences way later than healthy plants. This can affect the fitness of the host plant as several of them will pollinate and develop seeds way later than the entire population. Temporal isolation can occur within the population as a result of *O. lutea* infection thus leading to allochronic speciation. Additionally, the detailed measurements of leaves highlighted the significant reduction of the leaf width and length when either parasite infected the crop (Table 1).

Changes of the leaf width and length showed that a parasite is a heavy burden. *O. lutea* caused a 29% and 47% reduction in the leaf length and width in the host, respectively. *C. alba* caused a 32% and 30% reduction on average. Meanwhile, under the *O. lutea* influence, the alfalfa leaves were more prolonged (L/W ratio increased by 34%); and *C. alba*, in contrast, caused a reduction in the leaf size (the width and length decreased by 30-32%) but left the length-to-width ratio approximately the same. These findings highlight the morphological

alterations induced by parasitic infestation, indicating a clear impact on the vegetative aspect of the host plant.

The studies report that the parasites can cause up to 80% weight loss in the host plants before their death [49-50].

Analysis of the POA showed that the *M. sativa* possesses an intrinsic activity, that in the presence of the parasites increases. Moreover, the influence of dodder on the POA is much more significant (from 14% to 38%) than that of broomrape (from 8% to 42%). Meanwhile, both parasites equally decreased the POA in the stem water extract (Table 2).

The analysis of the total phenolic and flavonoid content of the healthy and infected *M. sativa* leaves and stems detected the percentage changes in the total phenolic content were again more pronounced in the presence of *C. alba*, but the flavonoid decline was more pronounced in the presence of *O. lutea*, suggesting a differential impact of the two parasites (Tables 3 and 4).

The host plant may manage to convert the metabolism to synthesize prooxidant compounds to counter the parasite. This can help the host to counter parasite advancement and delay its development. Since the plant changes its metabolism, it lowers antioxidant compounds such as polyphenols to act as a hostile environment for parasites but fails. This can be an interesting biocontrol measure if one can promote similar changes prior to parasite introduction, the infection can be significantly delayed or even refused.

Besides, our findings highlight that parasitic plants have a unique secondary metabolism. Some significant amounts of flavonoids (up to 4.753 g per 100 g of dried plant material, or 47.53 µg/mL of ethanolic extracts) can be found in *C. alba*. This is the reason that several traditional medicines include different species of *Cuscuta* genus for liver treatments [51]. In contrast, *O. lutea* does not store a significant number of flavonoids and obviously, the coloring of the plant does not depend on them.

As analysis of the catechin content in leaves and stems of *M. sativa* didn't show significant variations during parasitism by abovementioned parasites (Table 5). We can conclude that catechins, which are known to act as antiparasitic compounds against different taxonomic groups of parasites [52], are not directly involved in the host plant's response to parasitic plant species. The stability of catechin levels in both infected and healthy individuals indicates that other biochemical pathways and defense mechanisms are more important in the plant's response to these parasites.

The measurements of lycopene and β-carotene content proved to provide more significant results. Nevertheless, the amounts of lycopene present in *M. sativa* tissues were not detectable by the method implied (Table 6).

In contrast, β-carotene content was significant and affected by parasitism. The investigation showed a notable reduction in β-carotene levels, with a 28-34% decrease during *O. lutea* infection and a significant 50-57% reduction during *C. alba* infection (Table 6). Since β-carotene is an essential component of the photosynthetic apparatus as it is involved in photoprotection and light-harvesting processes, the observed decline suggests that parasitic infection disrupts the host plant's photosynthesis rate, leading to impaired growth and productivity. This reduction in β-carotene may reflect a broader degradation of carotenoids, which are crucial for maintaining the structural integrity of cells, chloroplasts, and other lipid structures protecting against oxidative stress [53]. Thus, the reduced amounts of β-carotene may also significantly affect the increase of the POA in infected plant samples. TLC of *M. sativa* pigments results further support the notion that parasitism severely influences the photosynthetic capabilities of the host plant. As the drastic reduction in pigment amounts observed through TLC suggests that parasitism not only diminishes carotenoid content but also affects other pigments

critical for photosynthesis such as chlorophylls. This reduction leads to a compromised photosynthetic apparatus, which would directly influence the energy balance and overall vitality of the plant. This further supports the morphological data as *O. lutea* infection reduces the leaf size less than *C. alba* infection.

Our findings underscore the significant morphological and biochemical shifts that occur *in M. sativa* during parasitism. The reduction in β-carotene content and the general decline in photosynthetic pigments as well as the decline of the secondary metabolism of the plant suggest that parasitic infection severely impacts the photosynthetic efficiency and overall fitness of the host plant. In contrast to biotic stress the drought stress leads to a notable reduction in yield while showing no significant impact on levels of antioxidants, anthocyanins, or phenols [54].

The comprehensive analysis of morphological and biochemical parameters provides a holistic understanding of the intricate relationship between the parasitic plants and their host. The observed changes in the leaf morphology, the POA, and the secondary metabolite content of both the host and parasite underscore the severity of the impact – offering valuable information for future research on potential mitigation strategies – selective herbicide development, or biocontrol measures against parasitic plant infestations in agricultural settings. Due to the differences in metabolism and accumulation of flavonoids in these two parasites, it can be a promising target to develop new herbicides to selectively eradicate them from the agroecosystems.

CONCLUSION

The *in-situ* observations of *M. sativa* plant and *ex-situ* measurements of its leaves infected by *O. lutea* and *C. alba* reveal a significant negative impact on both the reproductive success and the vegetative aspects. Infected plants exhibited minimal inflorescence development; also, substantial reductions in leaf dimensions were observed. Findings indicate a clear morphological alteration induced by parasitic infestation. Nevertheless, parasites, particularly *O. lutea* can cause temporal isolation and allochronic speciation in *M. sativa* populations.

Biochemical analysis highlighted an intrinsic the POA activity in *M. sativa*. That activity is increased in the presence of parasites, particularly *O. lutea*. Moreover, the study demonstrated a significant reduction in total phenolic and flavonoid content in the infected *M. sativa* leaves and stems. Although catechin content was not affected, photosynthetic pigments of the host plant were significantly (28-57%) reduced by the parasites. These findings suggest a complex interplay between the host and the parasite. The biochemical impact of the parasite is reflected on the host's primary and secondary metabolism.

The observed morphological and biochemical changes highlight the need for further research to explore potential mitigation strategies, selective herbicide development, and biocontrol measures against parasitic plant infestations in ecosystems. Understanding the relationship between parasitic plants and their hosts is crucial for developing targeted interventions. These interactions are meant to minimize the negative effects on the plant, its yield, nutritional value and health benefits.

Abbreviations: POA - prooxidant activity; TLC **-** thin-layer chromatography; ORP - oxidation-reduction potential.

Competing interests: Authors declare no conflict of interest.

Author contributions: NAZ and VSG conceived the concept. NGK and AVP collected of materials. LVM, IME, IVS and RGA performed the experiments. NAZ, VSG and

SGN revised and finalized the manuscript. All authors read and approved the final manuscript.

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