



















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  $\mu\text{g}/\text{mL}$  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  $\beta$ -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,  $\beta$ -carotene content was significant and affected by parasitism. The investigation showed a notable reduction in  $\beta$ -carotene levels, with a 28-34% decrease during *O. lutea* infection and a significant 50-57% reduction during *C. alba* infection (Table 6). Since  $\beta$ -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  $\beta$ -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  $\beta$ -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  $\beta$ -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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