# Anatomical and Phenotypic Study of *Dianthus amurensis* L. and *Zinnia elegans* by the effect of aqueous extracts of leaves Myrtus and Mentha

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**Abstract**
The study includes an experiment conducted in a greenhouse of the College of Science/Mosul University to study allelopathic effects of aqueous extract for myrtle and mint that preparation at 5%w:v in growth and anatomical characters.
Results show decrease in germination percentage of *Dianthus amurensis* L. and *Zinnia elegans* when treated with aqueous extract of mint amounted to (12.5,36.14%), when treated with mint extract caused increase in (shoot height, root system length, and dry weight of seedlings as well as an increase in leaf area of Dianthus amurensis L. that (27.11, 28.57, 6.42 and 48.74%) while the aqueous extract of mint caused a reduction in germination percentage, shoot height and root length of Zinnia elegans that( 5.88,23.91%) .As will the result anatomically shows that a reduction in stomata number in all treatments, high percentage reducing (56%) in *Dianthus amurensis* by effect Myrtle increases in stomata index, stomata density, and length of stomata in Dianthus amurensis L. leaves by the effect of mint aqueous extract that (39.13, 49.98 and 20%) . found significant differences in the studied characteristics of the cross section, as the aqueous extracts of Myrtle and mint caused an increase in the diameter of the section for *Dianthus amurensis* and was accompanied by an increase in the length of the shoots of plants treated with mint extract
**Key Words:** Allelopathy, *Zinnia elegans*, Myrtle, Mint, Allelochemicals

## Introduction

The use of plant extracts for medicinal plants is one of the important means in agricultural systems, and given the importance of the active substances in these extracts (1) , researchers have turned towards them to learn more about these materials and their effects, as the use of medicinal plants has become increasingly common in the recent period and several areas and different countries for what they contain important active ingredients, including Myrtle, mint, which is characterized by its high content of active substances are known as Natural product, which belong to a large family of different chemical substances that have a wide range of biological effects that have led to a variety of uses, mainly in agricultural and veterinary medicine (2). Natural products have a wide range of applications because of their wide range of biological effects. Natural products derived from plants, animals, and minerals.
The plant "Myrtle" belongs to the Myrtaceae family, which is evergreen shrubs and the important part of the myrtle is the leaves, flowers, and seeds (3). Myrtle contains volatile oils, the most important of which are cyanol and myrtol, as well as resin and tannin substances.
As for Mint, it is a semi-perennial herbaceous plant, the part used for the whole plant, mint is cultivated in homes and nurseries and in most countries, Egypt, Iraq, and European cities, and grows in the wild (4). Mint contains volatile oils, alkaloids, glycosides, tannins, gums, and resins, as well as Menthol, Turpentine, and Tannic acid.
Allelopathy is an environmental phenomenon associated with the secondary metabolic products produced by plants, this phenomenon is considered an important environmental mechanism and has a prominent role in natural ecosystems through its effect on plants in ecological communities.
Numerous studies have shown that allelopathy results from the release of secondary metabolites into the environment in various ways to affect the plants neighboring them or


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that follow them in agriculture. It was found that many types of trees and shrubs had allelopathic effects on their neighboring plants and sometimes on themselves (5).

*Dianthus amurensis L. and Zinnia elegans.* It reproduction with seeds and is considered one of the ornamental annals in Iraq and it is one of the plants that are frequently cultivated in parks, public gardens, and homes. Its cultivation is often associated with myrtle and mint Which is known to have an allelopathic effect on the plant species associated with or after planting.

Since the *Myrtus Communis* L. and *Mentha spicata* L. are grown in parks, public gardens and home, and through our personal observations we found that the aforementioned ornamental plants are cultivated under or near these plants. **Aim of Study** : Study allelopathic effects of water extracts of leaves Myrtle and mint, under greenhouse conditions, in seed germination, some growth characteristics, and anatomical characteristics of two types of flowering plants.

**Materials and Methods**

**Study of allelopathic effects of plant residues**

The study included an experiment in the greenhouse of the Biology Department / College of Science / University of Mosul to find out the effect of aqueous extracts of plant residues of Myrtle( *Myrtus Communis* L.) and Mint( *Mentha spicata* L) on germination and growth of two types of flowers: *Dianthus amurensis* L. and *Zinnia elegans*.

**Bioassay for plant extracts**

To study the effect of aqueous extracts of leaves of *Myrtus Communis* L. and *Mentha spicata* L. on the germination and growth of two types of flowers *Dianthus amurensis* L. and *Zinnia elegans*, the extracts were prepared at concentrations of 5% w:v (5 g / 100 mL DW) mixed with Mator stirrer. For 30 minutes and then filtered using a Buechner funnel fitted with Whatman No. 1 and collect clear solution.

10 seeds were planted for each of the tested plant species, 0.5 cm deep from the soil surface in pots, then watered with aqueous extract (previously prepared).

For plants with myrtle and mint at 5%, and placed in greenhouse, after 15 days of planting, (6),the germination percentages were calculated. After 60 days of germination, the length of the shoots and roots were measured, then they were dried in oven 70 C° for 72 hours, and their dry weights were recorded.

**Estimate total chlorophyll content**

Estimate total chlorophyll according to the (7) method, 0.5 g of plant leaves were taken and 10 ml of 95% ethyl alcohol was added to it after cutting it and placing it in the dark for 24 hours. The volume reached 30 ml, then it was filtered and the absorbance was measured at 649 and 665 nm with a spectrophotometer. The amount of chlorophyll a, b, and total chlorophyll a was calculated. According to the following equations:

Ch a =13.7(A665) - 5.76(A649)

Ch b = 25.8(A649) – 7.6(A665)

Total chlorophyll = Ch a + Ch b

**Anatomical traits**

As for the anatomical study, fresh samples were taken two months after germination for the purpose of studying the anatomical features, which included the following:

**Leaf preparation:**

The Leaf epidermis was prepared according to (8). The samples were examined using an Optima optical microscope and measurements were taken using the Ocular micrometer (7x). The dimensions of the stomata, the shape of the stomatal complexes, and the calculation of the stomatal index and stomatal frequency were studied. In the microscopic field, according to (9), as follows:

Stomatal index(%)= number of stomata / number of stomata + number of epidermal cells x 100

Stomatal density (mm2) = number of stomata / number of epidermal cells

**Stem cross sections**

Free-hand sectioning method was used to prepare cross-sections of stems, diameter number of vascular bundles, and bundle dimensions were studied (10).

**Statistical analysis**



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Treatments were replicated four times and using a randomized complete block design. Data were initially analyzed using analysis Duncan's Multiple Range Test at p 0.05 (11).

**Result and Dissection**

The results in Table (1) indicate a variation in effect of aqueous extracts of leaves (Myrtle, Mint), as we find a decrease in germination percentage of *Dianthus amurensis* L. and *Zinnia elegans* when treated with aqueous extract of mint amounted to (12.5,36.14%). The effect is due to fact that the seeds are more sensitive to the allelopathic compounds produced by the plant, as effect mechanism of these compounds on germination may be due to their effect on embryo cells division or drinking water or as a result of their effect on other metabolic activities (12).

The biological test for aqueous extracts indicates an increase in (shoot height, root system length, and dry weight of seedlings as well as an increase in leaf area) of *Dianthus amurensis* L. when treated with mint extract that (27.11, 28.57, 6.42 and 48.74%), while the aqueous extract of mint caused a reduction in germination percentage, shoot height and root length of *Zinnia elegans* that( 5.88,23.91%) may be due to the inhibitory effect of mint extract due to the nature of compounds it released to environment, as studies have found that it contains saponins, glycosides, alkaloids and flavonoids (13)

Among the results, we find a difference in the effect between Myrtle and Mint aqueous extracts due to this genotypic variation ,as that these plants belong to different families and contain the quantity and quality of chemical compounds different , such as Alkaloids, tannins, glycosides and other compounds (14). in study ( 13) that indicted to mint contains most of the secondary metabolites, while the results showed that Myrtle does not contain terpenes.

Table (1) The effect of the aqueous extracts of Myrtle and Mint on germination and seedling growth of two types of flowers

| Types of plant | Extract aqueous | Gr% | SL Cm | RL Cm | DW mg | RB | LF $Cm^2$ |
|---|---|---|---|---|---|---|---|
| ***Dianthu amurensis*** | control | 80b | 5.9b | 6.3b | 1.237b | 6c | 1.99c |
| | Myrtle | 83a | 5c | 5.2c | 0.777c | 8b | 2.44b |
| | Mint | 70c | 7.5a | 8.1a | 1.309a | 9a | 2.96a |
| Effect of Type | | 77a | 6.13a | 6.53a | 1.107a | 7.6a | 2.46a |
| ***Zinnia elegans*** | control | 83b | 1.8b | 4.6b | 0.446b | 5a | 0.39c |
| | Myrtle | 86a | 2.0a | 5.9a | 0.446b | 4b | 0.49b |
| | Mint | 53c | 1,7c | 3.5c | 0,475a | 3c | 0.99a |
| Effect of Type | | 74b | 1.8b | 4.6b | 0.455b | 4b | 0.623b |

*Gr%= Germination percentage , SL= Shoot length, RL=Root length, DW= Dry weight, RB=Root Branches LF=leaf area

Results in Table (2) show effect of aqueous extracts, myrtle, mint, on leaves anatomical traits of two types of flowers (*Zinnia elegans* and *Dianthus amurensis*), that a reduction in stomata number in all treatments, high percentage reducing (56%) in *Dianthus amurensis* by effect Myrtle figure(1), as well as a decrease in both Stomatal index (%) and Stomatal density by effect of Myrtle aqueous extract that (39.13, 49.98 and 20%) The reason may be due to the presence of active allelopathic compounds that can affect the nutritional status and abundance of mineral substances in the soil (15).

we found an increase in stomata length of leaves of *Dianthus amurensis* plant and reason



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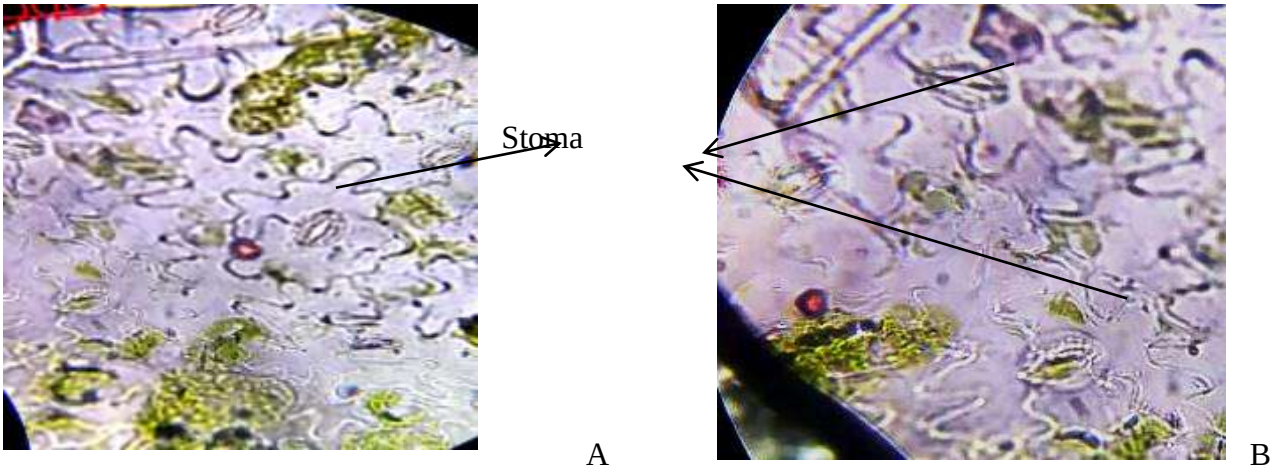
for this increase is due to reduction in stomata number, as there is an inverse relationship between the number of stomata and its dimensions (16). result show reduction in stomatal width of Mint , highest percentage of reduction (55.55%) in *Zinnia elegans* , reason for this inhibitory effect may be due to its high content of resins, essential oils and tannins.

Table (2) the effect of aqueous extracts Myrtle and Mint in the anatomical features of the leaves of the two types of flowers

| Types of plant | Extract aqueous | Numbers of stomata | Stomatal index (%) | Stomatal density (mm2) | Stomatal length | Stomatal Width |
|---|---|---|---|---|---|---|
| ***Dianthus amurensis*** | Control | 64a | 23b | 31.37b | 30b | 24b |
| | Myrtle | 28c | 22bc | 29.16c | 36a | 30a |
| | Mint | 32b | 32a | 47.05a | 36a | 20c |
| ***Zinnia elegans*** | Control | 32a | 17b | 21.05b | 36a | 20db |
| | Myrtle | 20c | 16c | 20.83c | 36a | 26a |
| | Mint | 24b | 25a | 33.33a | 16b | 10c |




A B

Figure(1)effect of the aqueous extracts, myrtle on the anatomical features in *Dianthus amurensis* leaves

As for the anatomical characteristics of the stems, Table (3) found significant differences in the studied characteristics of the cross section, as the aqueous extracts of Myrtle and mint caused an increase in the diameter of the section for *Dianthus amurensis* and was accompanied by an increase in the length of the shoots of plants treated with mint extract, that the cause of the increase could be a result. Increasing the number of parenchymal cellsin essential tissue by the effect of allelopathic compounds on cell division and elongation; Because any increase in the size and weight of the plant requires the occurrence of cell division and its enlargement in (16), while the treatments caused a reduction in the number of vascular bundles, and the reason for this difference between increasing the diameter of the section and reducing the number of bundles may be due to incomplete



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differentiation of vascular bundles that due to the influence of allelopathic compounds (17).

Reduction of the segment diameter was also caused by most other treatments in *Zinnia elegans*.the reason for the reduction may be due to a deformation of the stem and vascular bundles (18) due to the influence of allelopathic compounds.

Table (3) The effect of the aqueous extracts of Myrtle and Mint on the anatomical features of the stems of the two types of flowers

| Types of plant | Extract aqueous | Shoot Sectional diameter | Vascular bundles | | |
|---|---|---|---|---|---|
| | | | Number | Length | Width |
| *Dianthus amurensis* | Control | 95.79c | 9a | 77c | 55c |
| | Myrtle | 109.48a | 8b | 120a | 72a |
| | Mint | 104.65b | 8b | 106b | 70b |
| *Zinnia elegans* | Control | 80.48a | 8a | 22c | 14c |
| | Myrtle | 51.52c | 5b | 34b | 20a |
| | Mint | 61.18b | 4c | 60a | 18b |

The results demonstrated a decrease in total chlorophyll content of *Zinnia elegans* When treated with mint aqueous extract (Figure 2), the reason for reduction in the chlorophyll content is may be due to role of allelopathic compounds in inhibiting the construction of chlorophyll and stimulating the process of its destruction (19). As for *Dianthus amurensis*, we find an increase in the content of leaves of chlorophyll when all treatments were accompanied by an increase In leaf area figure(3), Myrtle aqueous extract gave the highest increase rate of 43.16%.

the effect on chlorophyll content may be due to affecting nutrient absorption. (20) indicated that allelopathic compounds released from the *Alhagi maurorum* plant and *Cardaria draba* led to a decrease in the absorption of macro- and microelements in the wheat plant. Therefore, a decrease in absorption of the necessary elements or nutrients may lead to a decrease in seed germination, seedling growth, and weight.

The chlorophyll content is because these nutrients are essential and the plant needs them in appropriate quantities or concentrations in the process of germination and physiological processes in the plant. In another study (21) .

The aqueous extracts of plants (Oat Avena fatua, Polypogon hissaricus, Melilotus officiualis L.) had different effects on root length, plant height, chlorophyll concentration, and biomass of three wheat varieties.



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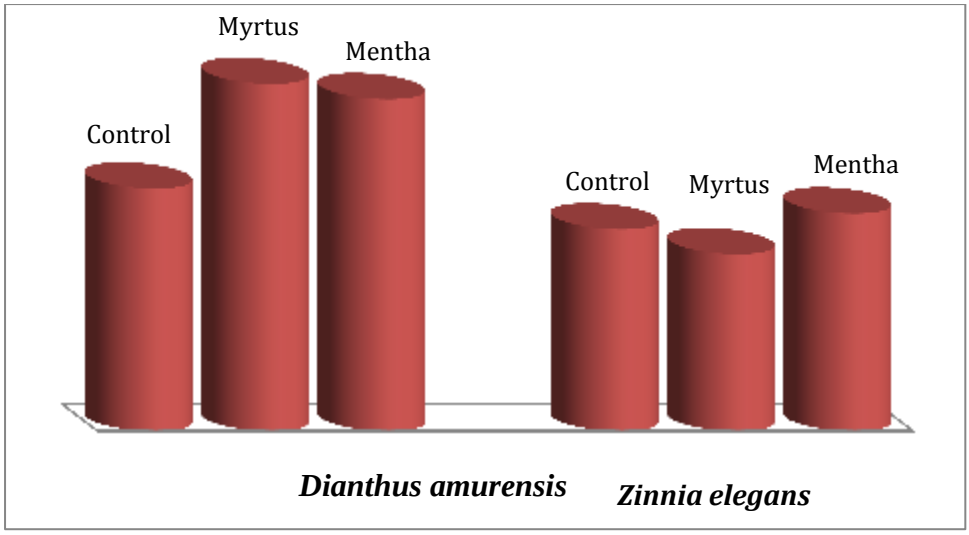
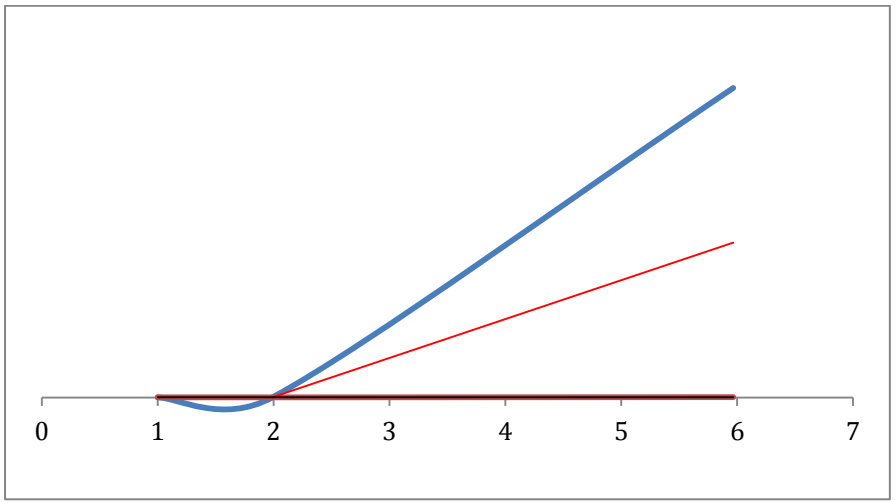



Figure(2) effect of Myrtle and Mint aqueous extract in chlorophyll content in *Dianthus amurensis* and *Zinnia elegans* leaves




Figure(3)Relationship between leaf area and chlorophyll in *Dianthus amurensis* That show increases in chlorophyll with increases leaves area

**Conclusion**

Study allelopathic effects of aqueous extract for myrtle and mint in growth and anatomical characters. show mint extract caused an increase in (shoot height, root system length, and dry weight of seedlings as well as an increase in leaf area of *Dianthus amurensis* L. . found significant differences in the studied characteristics of the cross-section, as the aqueous extracts of Myrtle and mint caused an increase in the diameter of the section for *Dianthus amurensis* and were accompanied by an increase in the length of the shoots of plants treated with mint extract.

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**Reference**

1. Abbas. M. M. and W. S. Hussain (2020). Allelopathic Potential of Aromatic plants on Germination and Growth of some Ornamental plants.



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LAP Lambert Academic Publishing. ISBN: 978-620-2-79593-7

2. Katz,L and RH Baltz (2016) . Natural product discovery: past, present, and future. J. *Ind. Microbiol. Biotechnol*. 43(2-3):155-76.
3. Abd al-Rahman, S. and I. H. Alwan (2009). Atlas of Iraqi Medicinal Plants (Part 1), Ministry of Agriculture, General Authority for Seed Inspection and Certification, Iraqi National Herbarium**.**
4. Arafa, A. A. (2008). Morphology of Spice Plants, Al-Awakening Press.
5. Rice, E. L.(1984). Allelopathy (Second ed.), Academic Press: 422 ISBN 978- 0-12-587058-0**.**
6. Hussein, W. S., J. A.Saeed, and A. M. Al-Maadi (2018). The effect of plant residues of some crops on the phenotypic and anatomical features of four types of bush (Kalgan, cornucopia, millet, or milk). *Al-Rafidain Agricultural Journal, M*. 46 (4): 393--405.
7. Knudson, L.L., T.w. Tibbitts and G.E. Edwards, (1977). Measurement of ozone injury by determination of chlorophyll concentration. *Plant Physiol*. 60:606-608.
8. Al-Ubaid, N. M. M. I. (2007). Morphological and Anatomical Study of Certain Species of *Brassicaceae* Races in Iraq. Mcs. Thesis / College of Education / University of Tikrit, / Iraq.
9. Al-Khazraji, T. A. and F. M. Aziz (1990). Practical in plant anatomy and microscopic preparations. Salah al-Din University / Ministry of Higher Education and Scientific Research / Iraq.
10. Hussain, W. S., N. A. H. A. Taher, and M. M. Abbas (2021). Allelopathic Effects of Chickpea on Anatomical Traits of Wheat Varieties *Triticum aestivum* L. *Al-Mukhtar Journal of Sciences*. 36(4): 363-372.
11. AL-Jehaishy ,W. S. H. (2017). The use of plant waste in the biological control of some bushes and their allelopathic effects on growth, and some physiological and anatomical traits. PhD thesis, College of Science / University of Mosul.
12. Hussain, W. S.(2010). The effect of artificial rain washings and aqueous washers of myrtle and orange leaves on the germination and growth of the *Godetia grandiflora* L and *Centurea plants*. *Centaurea Cyanus* L J. of the Faculty of Basic Education Relsearch, 100 (4): 490-501.
13. Hussain, W. S. and M. Abbas (2020). Evaluation of Allelopathic Potential for plant aqueous Extracts of Some Plants on Growth and Division of *Cheiranthus cheiri* and *corn Flower*. GSJ: 8 (7):1730-1740.
14. Al-Taie, A. M. I.(2012). The effect of aqueous extracts of lysates, ginger and castor beans on germination and growth of the barley plant *Hordeum vulgare* L.. Babylon University Journal of Pure and Applied Sciences, 4 (20): 1316-1327.
15. Scavo, A., C.Abbate and G. Mauromical (2019). Plant allelochemicals: agronomic, nutritional and ecological relevance in the soil system. Plant Soil, (442): 23–48.
16. Ormrod, D.J.(2011).Surface anatomy of weed leaves with particular reference to stomata. The university of British Columbia.
17. Cruz-Ortega, R., A.L. Anaya, B.E. Hernandez-Bautista and G. Laguna-Hernandez (1998) Effects of Allelochemical Stress Produced by *Sicyos deppei* on Seedling Root Ultrastructure of *Phaseolus vulgaris* and *Cucurbita ficifolia.* J. of Chem. Eco., 24: 2039-2057.
18. Hussein, W. S., J. A.Saeed and A. M. Al-Maadidi (2018). Detection of active compounds in the residues of some plant species, isolation and diagnosis of allelopathic compounds



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using HPLC technology. Al-Rafidain Science Journal, 27 (5) :41-32.
19. Al-Kanani, A. Y. H. (2013). Study of some industrial pollutants and their effect on the anatomical characteristics of some plant species in Samarra district. Master Thesis / College of Science / University of Tikrit / Iraq.
20. Mohammadkhani,N. and Servati, M. (2017). Nutrient concentration in wheat and soil under allelopaathy treatments. J. Plant Res. pp. 1-13.
21. Siyar, S., Majeed, A., Muhammad, Z., Ali, H., & Inayat, N. (2019). Allelopathic effect of aqueous extracts of three weed species on the growth and leaf chlorophyll content of bread wheat. Acta Ecologica Sinica, 39(1): 63-68.



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