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Diversity of Rhizosphere Fungi and VAM Fungal Spores Associated with *Murraya koenigii* (L.) Spreng. From Hamirpur District of Himachal Pradesh

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Abstract **– The study was conducted to isolate and identify the rhizosphere fungi and AM spores from the soil samples of *Murraya koenigii* (Curry patta) growing in three different sites of Hamirpur District of Himachal Pradesh. For the isolation of Rhizosphere fungi Dilute Plate Method was followed. Isolation and identification of AM spores was done by using Schenck and Perez (1990), manual. Investigations on rhizosphere fungi and VAM spores isolated from the root adhering soil samples of curry patta revealed the presence of thirty species of fungi and seven species of VAM fungi belonging to three genera were present. A comparison of seasonal distribution of these fungal associates revealed that twenty species belonging to 16 fungal genera (*Alternaria*, *Aspergillus, Chaetomium, Coniothyrium, Curvularia, Emericella, Fusarium, Gliocladium, Mortierella, Mucor, Penicillium, Phoma, Pythium, Rhizoctonia, Rhizopus* and *Trichoderma*) were reported in rainy season and eleven species of fungi belonging to seven genera (*Aspergillus, Cunninghmella, Fusarium, Mortierella, Penicillium, Rhizoctonia* and *Trichoderma*) were recorded in spring season. Eight species belonging to six genera (*Alternaria, Aspergillus, Baratalinia, Coniothyrium, Chaetomium*, and *Trichoderma*) were recorded in summer season and seven species of fungi belonging to four genera (*Aspergillus, Gliocladium, Mortierella* and *Penicillium*) were recorded in winter. The VAM fungi include three genera *Acaulospora*, *Gigaspora* and *Glomus. Acaulospora* was the dominant genus represented by four species (i.e. *A. delicata*, *A. foveata*, A. *mellae* and A. *spinosa*). The genus *Glomus* was represented by two species (i.e. *Glomus geosporum* and *Glomus macrocarpum*). The genus Gigaspora was represented by a single species (i.e. *Gigaspora calospora*). The results showed the association of rhizosphere as well as VAM (Vesicular *Arbuscular Mycorrhiza*) fungi in *Murraya koenigii*.**

Keywords **– Rhizosphere fungi, VAM, *Acaulospora*, *Gigaspora*, Curry Patta.**

I. INTRODUCTION

Murraya koenigii (L.) Spreng. is an important member of family Rutaceae. The genus *Murraya* include five species of which, the leaves of *Murraya koenigii* are used as an ingredient in curry. The common name of this plant is Curry Patta. It is distributed in India, China and Philippine islands. In India it grows in Himachal, Punjab, Parts of Assam (Lakhimpur), Bengal, Deccan, Konkan, Western Ghats of Bombay to Travan core and Ceylon.

The 'rhizosphere' generally refers to the zone of soil surrounding the roots with higher microbial activity than in soil remote from roots [1]. The rhizosphere microorganisms increase the ability of plants to acquire nutrients from soil by either increasing the extent of root system (through the fungal hyphae) or solubilising macronutrients such as phosphorous or sulphur [2].

VAM fungi are ubiquitous in distribution. They occur over a wide ecological zone. It has been estimated that VAM can be found in 70% of all plant families [3]. Members of order Glomales form mycorrhizal relationship with most agronomically important angiosperms, some gymnosperms, as well as certain bryophytes and pteridophytes and even a few algae [4].

The susceptibility of *Acacia sp.* seedlings to colonization by AM fungi appeared to be seasonal. It has been concluded that colonisation increased with increasing daytime temperatures and day length [5]. Studies have shown that VAM fungi associated with *Taxus baccata* contain 14 VAM spores. The VAM spores were of *Glomus aggregatus, Glomus albidum, Glomus citrocolun, Glomus fasciculatum, Glomus macrocarpum, Glomus gigantea, Glomus formosum* and *Acaulospora nicolsonii* [6].

II. Materials and Methods

Collection, Isolation and Identification of Rhizosphere Fungi and am Spores:

For taking soil samples, three regions (Gasota, Bijhari and Bhota) of Hamirpur district were selected. Collections were made during December, 2008, April, 2009, June, 2009 and July 2009. Materials used in the present study were roots, leaves, bark and soil samples from the rhizosphere of the medicinally important plant *Murraya koenigii* (L.) Spreng. For the isolation of rhizosphere fungi, Dilution plate method was followed [7], [8]. The media used for culturing rhizosphere fungi were Czapek's Dox [9] and Potato Dextrose Agar [10]. For identification, temporary mounts of fungi were made in 0.1% cotton blue and Lactophenol. An adequate high power microscope was used for observing the slides and then the cultures were identified [11], [12].

Isolation of VAM spores was done by "Wet Sieving and Decanting Technique" [13]. AMF spore identification and their morphological characteristics like shape, size, texture, wall layers, attached hyphae, sporocarps were determined by using Manual [14].

III. Observations

Thirty species of fungi belonging to 19 genera (i.e*., Alternaria, Aspergillus, Baratalinia, Chaetomium, Cladosporium, Coniothyrium, Cunninghamella, Curvularia, Emericella, Fusarium, Gliocladium, Mortierella, Mucor, Penicillium, Phoma, Pythium, Rhizoctonia, Rhizopus* and *Trichoderma* were isolated from the root adhering soil samples of *Murraya koenigii* (Table - 1) the genus *Alternaria* was represented by a single species (*Alternaria alternata*). The genus *Aspergillus* was represented by six species (i.e. *A. niger, A. glaucus, A. fumigatus, A. wentii, A. ustus* and *A. flavus*). The genus *Penicillium* was found to be represented by two species (i.e. *Penicillium citrium* and *Penicillium funiculosum*).

The other 13 genera (i.e. *Baratalinia, Chaetomium, Cladosporium, Coniothyrium, Curvularia, Emericella, Gliocladium, Mortierella, Mucor, Phoma, Pythium, Rhizoctonia* and *Rhizopus*) were represented by single species each (i.e., *Bartalinia* sp., *Chaetomium* sp., *Cladosporium* sp. *Coniothyrium* sp., *Curvularia parasadi*, *Emricella nidulans, Gliocladium roseum, Mortierella* sp., *Mucor* sp., *Phoma* sp., *Pythium* sp., *Rhizoctonia solani* and *Rhizopus oryzae*). The genus *Cunninghamella* and *Trichoderma* were represented by two and three species each (i.e. *Cunninghamella elegans* and *Cunninghamella echinulata, Trichoderma harzianum, Trichoderma viride* and *Trichoderma* sp.) respectively.

A comparison of seasonal distribution of these fungal isolates revealed that twenty species belonging to 16 fungal genera (i.e. *Alternaria, Aspergillus*, *Chaetomium, Coniothyrium, Curvularia, Emericella, Fusarium, Gliocladium, Mortierella, Mucor, Penicillium, Phoma, Pythium, Rhizoctonia, Rhizopus* and *Trichoderma*) were recorded in rainy season and 11 species of fungi belonging to seven genera (*Aspergillus, Cunninghmella, Fusarium, Mortierella, Penicillium, Rhizoctonia* and *Trichoderma*) were recorded in spring season. 8 species

belonging to six genera (*Alternaria, Aspergillus*, *Baratalinia, Coniothyrium*, *Chaetomium, and Trichoderma*) were recorded in summer season and seven species of fungi belonging to four genera (*Aspergillus, Gliocladium*, *Mortierella* and *Penicillium*) were recorded in winter (Table 2, Fig. 2).

Further grouping of these fungal isolates from the root adhering soil samples of *Murraya koenigii* into different fungal subdivisions revealed that Ten belong to Deuteromycotonia, four belong to Zygomycotina, four fall in Ascomycotina and one belong to Mastigomycotina (Table 3, Fig. 1).

IV. Isolation and Identification of Different VAM Fungal Spores of Murraya Koenigii (L.) Spreng.

The soil samples from rhizosphere of *Murraya koenigii* were analyzed for the presence of various VAM fungi. The fungal spores were isolated by Wet Sieving and Decanting Technique [13].

Seven species of VAM fungal spores belonging to 3 genera (*Acaulospora, Gigaspora* and *Glomus*) were isolated from the rhizosphere soil samples of *Murraya koenigii*. Maximum numbers of four species were reported from *Acaulospora.* These were Acaulospora *delicata, Acaulospora foveata*, *Acaulospora mellae* and *Acaulospora spinosa*. The genus *Glomus* was represented by two species (i.e. *Glomus geosporum* and *Glomus macrocarpum*). The genus *Gigaspora* was represented by a single species of *Gigaspora calospora* (Table 4).

Table 1. List of Rhizosphere Fungi Isolated from the Soil Samples of *Murraya Koenigii* (L.) Spreng.

Sr. No.	Name of Fungus Isolated
1	*Alternaria alternata*
2	*Aspergillus flavus*
3	*Aspergillus fumigatus*
4	*Aspergillus glaucus*
5	*Aspergillus niger*
6	*Aspergillus ustus*
7	*Aspergillus wentii*
8	*Baratalinia sp.*
9	*Chaetomium sp.*
10	*Cladosporium sp.*
11	*Coniothyrium sp.*
12	*Cunninghamella echinulata*
13	*Cunninghamella elegans*
14	*Curvularia parasadi*
15	*Emericella nidulans*
16	*Fusarium moniliforme*
17	*Fusarium oxysporum*

18	*Fusarium solani*
19	*Gliocladium roseum*
20	*Mortierella sp.*
21	*Mucor sp.*
22	*Penicillium citrinum*
23	*Penicillium funiculosum*
24	*Phoma sp.*
25	*Pythium sp.*
26	*Rhizoctonia solani*
27	*Rhizopus oryzae*
28	*Trichoderma harzianum*
29	*Trichoderma viride*
30	*Trichoderma sp.*

Table 2. Comparison of Occurrence of Rhizosphere Fungal Species of *Murraya Koenigii* in different Seasons.

Sr. No.	Name of Fungus Isolated	Winter	Spring	Summer	Rainy
1	*Alternaria alternata*	-	-	+	-
2	*Aspergillus flavus*	+	-	-	-
3	*Aspergillus fumigatus*	-	+	+	+
4	*Aspergillus glaucus*	+	-	-	+
5	*Aspergillus niger*	-	+	+	+
6	*Aspergillus ustus*	+	-	-	-
7	*Aspergillus wentii*	-	+	+	+
8	*Bartalinia sp.*	-	-	+	-
9	*Chaetomium sp.*	-	-	+	+
10	*Cladosporium sp.*	-	-	-	+
11	*Coniothyrium sp.*	-	-	+	+
12	*Cunninghamella echinulata*	-	+	-	-
13	*Cunninghamella elegans*	-	+	-	-
14	*Curvularia parasadi*	-	-	-	+
15	*Emericella nidulans*	-	-	-	+
16	*Fusarium moniliforme*	-	-	-	+
17	*Fusarium oxysporum*	-	+	-	-

Sr. No.	Name of Fungus Isolated	Winter	Spring	Summer	Rainy
18	*Fusarium solani*	-	+	-	+
19	*Gliocladium roseum*	+	-	-	+
20	*Mortierella sp.*	+	+	-	+
21	*Mucor sp.*	-	-	-	+
22	*Penicillium citrinum*	+	+	-	+
23	*Penicillium funiculosum*	+	-	-	+
24	*Phoma sp.*	-	-	-	+
25	*Pythium sp.*	-	-	-	+
26	*Rhizoctonia solani*	-	+	-	-
27	*Rhizopus oryzae*	-	-	-	+
28	*Trichoderma harzianum*	-	-	-	+
29	*Trichoderma viride*	-	-	+	-
30	*Trichoderma sp.*	-	+	-	-

(+) = (Present), (-) = (Absent).

Table 3. Categorization of Rhizosphere Fungi of *Murraya Koenigii* in Different Fungal Subdivisions.

Sr. No.	Subdivision	Genus
1	Mastigomycotina	*Pythium*
2	Zygomycotina	*Cunninghamella, Mortierella, Mucor, Rhizopus*
3	Ascomycotina	*Aspergillus, Chaetomium, Emericella, Penicillium*
4	Deuteromycotina	*Cladosporium, Alternaria, Baratalinia, Coniothyrium, Curvularia, Fusarium, Phoma, Rhizoctonia, Trichoderma, Gliocladium.*

Table 4. List of VAM Fungal Spores Isolated from the Root Adhering Soil Samples of *Murraya Koenigii*.

Sr. No.	VAM Fungus Isolated
1	*Acaulospora delicata*
2	*Acaulospora foveata*
3	*Acaulospora mellae*
4	*Acaulospora spinosa*
5	*Gigaspora calospora*
6	*Glomus geosporum*
7	*Glomus macrocarpum*

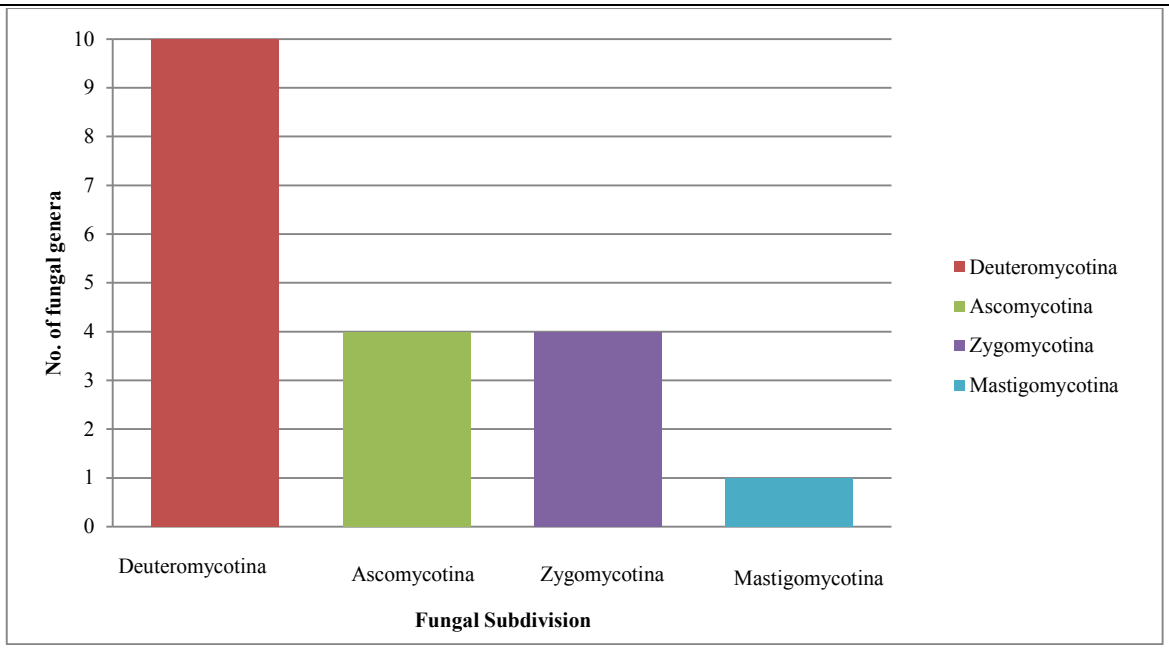

Fig. 1. Histogram showing the distribution of fungal genera (in different fungal subdivisions) isolated from the rhizosphere soil samples of *Murraya koenigii*.

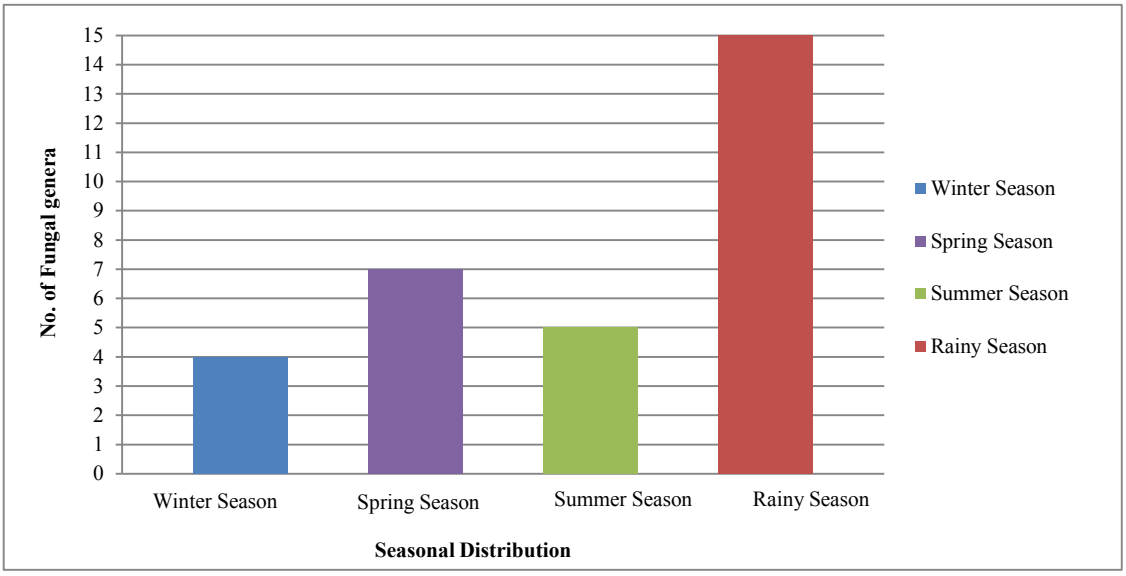

Fig. 2. Histogram showing distribution of different fungal genera isolated from the rhizosphere soil samples of *Murraya koenigii*.

V. DISCUSSION

Seasonal variation, disturbance of soil as well as texture of soil affects the AM diversity and number. The spore number was found high in Monsoon season followed by winter. The changing environmental conditions show greater effect on rhizosphere soil fungi and AM diversity.

Investigations on rhizosphere fungi from the root adhering soil samples of *Murraya koenigii* revealed the presence of thirty species of fungi. The different fungal genera isolated were *Alternaria, Aspergillus*, *Baratalinia, Chaetomium, Cladosporium, Coniothyrium, Cunninghamella, Curvularia, Emericella, Fusarium, Gliocladium, Mortierella, Mucor, Penicillium, Phoma, Pythium, Rhizoctonia, Rhizopus* and *Trichoderma*. These genera are represented by the following *species Alternaria alternata, Aspergillus flavus, A. fumigatus, A. glaucus, A. niger, A. ustus* and *A. wentii, Baratalinia* sp., *Chaetomium* sp., *Cladosporium* sp., *Coniothyrium* sp., *Cunninghamella*

echinulata, Cunninghamella elegans, Curvularia Parasadi, Emericella nidulans, Fusarium moniliforme, Fusarium oxysporum, Fusarium solani, Gliocladium roseum, Mortierella sp., *Mucor* sp., *Penicillium citrinum, Penicillium funiculosum*, *Phoma* sp., *Pythium* sp., *Rhizoctonia solani, Rhizopus oryzae, Trichoderma harzianum, Trichoderma viride and Trichoderma sp.* Studies conducted on the rhizosphere fungi from soil samples of *Aloe vera* and *Mentha viridis* have shown the presence of these main genera: *Absidia, Aspergillus, Baratalinia, Cladosporium, Coniothyrium, Curvularia, Fusarium, Myrothecium, Penicillium and Trematostroma* [15].

During present investigation, 7 species of VAM fungi belonging to 3 genera (*Acaulospora, Gigaspora* and *Glomus)* were isolated from the rhizosphere soil samples of *Murraya koenigii*. *Acaulospora* was the most dominant genus followed by four species (i.e. *Acaulospora delicata, Acaulospora foveata, Acaulospora mellae* and *Acaulospora spinosa.* The genus *Glomus* was represented by two species i.e. *Glomus geosporum* and *Glomus macrocarpum.* The genus *Gigaspora* was represented by a single species i.e. *Gigaspora calospora*. *Glomus fasciculatum, Glomus macrocarpum* and *Gigaspora sp.* isolated from the rhizosphere soil samples of *Sea buckthorn* [16]. The various VAM spores of *Acaulospora appendiculata, Entrophosphora sp., Gigaspora sp., Glomus fulvum* and *Glomus macrocarpum* isolated from the rhizosphere soil samples of *Terminalia chebula, Terminalia bellirica* and *Emblica officinalis* [17].

VI. Conclusion

The results have revealed the presence of 30 species of fungi. The species belong to 19 genera namely *Alternaria, Aspergillus, Baratalinia, Chaetomium, Cladosporium, Coniothyrium, Cunninghamella, Curvularia, Emericella, Fusarium, Gliocladium, Mortierella, Mucor, Penicillium, Phoma, Pythium, Rhizoctonia, Rhizopus* and *Trichoderma*. On further grouping of these fungal isolates into different subdivisions of fungi, it was found that ten of these genera belong to Deuteromycotina, four of them belong to Ascomycotina and Zygomycotina and one genus belong to Mastigomycotina. Seasonal distribution of these fungi revealed that 7 fungal species were recorded in winter season, 11 fungal species were recorded in spring season and 8 species were recorded in summer season. The maximum numbers of 20 species were recorded during rainy season from the rhizosphere soil of *Murraya koenigii*.

VAM spores were also isolated from the rhizosphere soil samples of *Murraya koenigii*. Total seven species of VAM fungi were isolated which belong to three genera i.e. (*Acaulospora, Gigaspora* and *Glomus*). The genus *Glomus* was represented by two species (i.e. *Glomus geosporum* and *Glomus macrocarpum*). The genus *Acaulospora* was represented by four species (i.e. *Acaulospora delicate, Acaulospora mellae, Acaulospora foveata* and *Acaulospora spinosa*). The genus *Gigaspora* was represented by a single species (i.e. *Gigaspora calospora).*

References

[1] Hiltner, Uber neue Erfahrungen and Probleme anf dem Gebiet der Bodenbakteriologie unter be sordrer Beruksichtigun of der Grundungung und Brache. *Arb dtsch Lanwit Ges,* 1904, **98:** 59-78 (Hiltner, on new experiences and problems in the field of soil bacteriology under special consideration of the foundation and fallow. Arb dtsch Lanwit Ges, 1904, 98: 59-78).
[2] S.E. Smith, and D.J. Read, "Mycorrhizal Symbiosis," Ed. 2. Academic Press, San Diego, 1927.
[3] E. Sieverding, "Vesicular arbuscular mycorrhiza management in Tropical Agrosystems," Technical Corp. of Germany Eshborn, Germany, 1991
[4] C.J. Alexopaulous, C.W. Mims, and M. Blackwell, "Introductory Mycology 4th edition", (Eds. Alexopaulous C.J., Mims, C.W. and Blackwell, M.) John Wiley and Sons, Inc. New York, 1996, pp. 153-154.
[5] J. Bell, S, Wells, D.A. Jasper and L.K. Abbott, "Field inoculation with arbuscular mycorrhizal fungi in rehabilitation of mine sites with native vegetation including *Acacia* sps", *Aus. System Bot, 2003,* **16:** 131-138.
[6] P. Sharma, "Studies on mycorrhiza and mycorrhizosphere of *Taxus baccata* Linn.", Ph.D. Thesis. H.P. University, Shimla, 2003.

[7] S.A. Wakesman, *Principles of Soil Microbiology*. London : Bailliere, Tindall and Cox, 1927.
[8] J.M. Warcup, "The soil plate method for the isolation of fungi from soil", *Nature*, 1950, 166: 117-118.
[9] K.B. Raper and C. Thom, "A manual of *Penicillia*", Williams and Wilkins Company, Baltimore, 1949, 875.
[10] T.E. Rawling, "Phytopathological and botanical research methods", John Wiley and Sons, London, 1933.
[11] H.L. Barnett and B.B. Hunter, "Illustrated genera of imperfect fungi", Burgess Publishing Company, 1972, 241.
[12] J.C. Gilman, "A manual of soil fungi", Oxford and IBH Publishing Corporation, 1967, 450.
[13] J.W. Gerdemann, and T.H. Nicolson, "Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting", *Trans. Br. Mycol. Soc., 1*963, **46:** 235-244.
[14] N.C. Schenck and Y. Perez, "Manual for identification of VAM fungi", University of Florida, Florida, USA, 1988, 1-248.
[15] A. Sagar and S. Thakur, "Studies on fungal associates of *Aloe vera* and *Mentha viridis*", *Plant Archives*, 2009, **9:** 369-374.
[16] S. Kumar and A. Sagar, "Microbial associates of Seabuckthorn (*Hippophae rhamnoides* Linn.)", M. Phil. Dissertation, Himachal Pradesh, University, 2005.
[17] M.Thakur and A. Sagar, "Fungal associates of *Terminalia Chebula, Terminalia bellirica and Emblica officinali", J. Pure and Appl. Microbio*, 2007, **2**: 301-306.

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