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Efficiency of Bioagents in Controlling Root-Knot Nematode on *Acacia* Plants in Egypt

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Abstract: A greenhouse experiment was conducted to assess the influence of *Azospirillum brasilense*, *Pseudomonas fluorescens*, *Azotobacter chroorcoccum*, mixed genera of *Arbascular mycorrhizae* (AM) fungi and oxamyl for controlling *Meloidogyne incognita* on *Acacia farnesiana* (L.) Willd and *A. saligna* (Labill), in a complete randomize design, in two seasons (2009 and 2010). Final nematode population (juveniles in soil, developmental stages, females, egg masses and eggs number in root) and number of galls and rate of buildup of root-knot nematode were determined. Growth parameters (plant height, number of branches/plant, dry weights of shoots (stems + leaves) as well as roots/plant and root length); nodulation parameters (number and dry weight of nodules/plant, as well as nitrogenase activity) and chemical analysis (N , P, K, protein, total carbohydrates percentages and total chlorophylls (a+b) and carotenoids contents in leaves) were also determined. Results indicated that both oxamyl and *Arbascular mycorrhizae* were the most effective treatments in decreasing the final nematode population in both soil and roots, number of galls and rate of buildup of root knot nematode. Also, they recorded the maximum plant growth, nodulation parameters and chemical components in the leaves of the two species. While, the least effective one was *Azospirillum brasilense*. *Pseudomonas fluorescens* and *Azotobacter chroorcoccum* occupied an intermediate position. In conclusion, mixed genera of *Arbascular mycorrhizae* fungi can be used for controlling *Meloidogyne incognita* on *Acacia farnesiana* and *Acacia saligna* instead of nematicide that cause severe pollution of ecosystems.

Key words: *Acacia farnesiana* · *Acacia saligna* · *Meloidogyne incognita* · Bioagents · *Azospirillum brasilense* · *Pseudomonas fluorescens* · *Azotobacter chroorcoccum* · *Arbascular mycorrhizae* fungi and oxamyl

INTRODUCTION

Leguminous tree species such as *Acacia* could be good candidates to grow in soils very deficient in nitrogen because of their associated rhizobial symbioses constitute a source of N input to the ecosystem. This nitrogen is returned to the soil by the natural loss of leaves which improves the soil fertility and its physical properties through maintenance of soil organic matter, or soil aggregation [1, 2]. In addition, they provide high-quality animal fodder, timber, fuel wood, charcoal, gums and other products [3].

Root-knot nematodes, *Meloidogyne* spp., are parasitic to *Acacia* species and can cause extensive damage to the infected plants [4, 5]. Due to severe environmental problems, achieving a sustainable agriculture will require avoidance of chemical controlling plant parasitic nematodes. Recently, numerous researches have focused on biological control agents with the objective of controlling the parasitic nematodes and to overcome the nematode damage by using mycorrhizal fungus [6, 7] or bacteria such as *Pseudomonas fluorescens* [8, 9], *Azospirillum brasilense* [10] and *Azotobacter chroorcoccum* [11].

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It is, therefore, the objective of the present research was controlling root-knot nematode; *Meloidogyne incognita*; on *Acacia farnesiana* (L.) Willd and *A. saligna* (Labill) by using different biological antagonists in comparing with nematicide in order to improve the growth and the chemical components of the pathogen plants.

MATERIALS AND METHODS

The experiment was conducted at the experimental greenhouse of Nematodes Research Department, Plant Pathology Research Institute, Agricultural Research Center (ARC), Giza, Egypt. and at the Experimental Laboratory of the Natural Resources Department, Institute of African Research and Studies, Cairo University, Giza, Egypt.

Pods of *Acacia farnesiana* (L.) Willd were collected from Al-Wahaat El-baharya, sixth of October Governorate, Egypt and seeds of *Acacia saligna* (Labill) were obtained from Sadat Research Station, Desert Development Center, American University in Cairo, Menofia Governorate, Egypt, on May, during the period of the experiment, 2009 and 2010.

Seeds from the crushed pods of *Acacia farnesiana* were pre-treated with He–Ne laser at irradiance 1.70 W/cm^2 for 9 min. [12] and seeds of *Acacia saligna* were immersed in boiling water for 1 min. [13] to accelerate germination, then the seeds were sown on June1st, in both seasons, in a glasshouse, with average daily maximum temperature of 34°C and a minimum of 25°C, in a 25-cm diameter clay pots filled with 1: 2 (v/v) mixture of aerated steam sterilized loam and sand soil.

The seedlings of the two species, in both seasons, were inoculated after one month from the sown, with mixed spores of *Arbascular mycorrhizae* (AM) fungi from genera (*Glomus*, *Gigaspora* and *Acaulospora*) (500 spores/g) at a rate of 10g/hole. Once the mycorrhizal symbiosis was established, *Azospirillum brasilense* (NO40), *Pseudomonas fluorescens* (P.f.), *Azotobacter chroorcoccum* (A.ch.) were applied (10^6 CFU/ml) at a rate of 10 ml/ pot, which obtained from Soils, Water and Environ. Res. Inst., Agric. Research Center, Giza, Egypt.

After two weeks, nematode (N) inoculation was added with 3000 newly hatched second stage larvae of *Meloidogyne incognita* by making 3 holes at different depths (2-3 cm) around the roots and immediately after inoculation the roots were covered with soil. Then the previously microbial inoculants were re-added at the same rate, after 15 days of infestation. The treatments were as follows: nematode active larvae only (N), *Azospirillum brasilense* + N, *Pseudomonas fluorescens* + N, *Azotobacter chroorcoccum* + N, *Arbascular mycorrhizae* fungi + N and oxamyl (24 % EC) was applied at the rate of 0.03 ml./pot + N. Uninoculated healthy seedlings were also included as control. All pots were arranged in completely randomized design with five replicates.

Plants were harvested 60 days after nematode inoculation, in both seasons, all plants were carefully uprooted and nematode populations in soil (number of juveniles/ pot) were determined according to Franklin and Goodey [14]. Roots were stained by acid fuchsin in acetic acid according to Byrd *et al*. [15] and examined for number of developmental stages and females/ root. Egg masses, eggs /egg-mass of *Meloidogyne incognita* were extracted using the method described by Hussey and Barker [16]. In addition, the final nematode population (PF) and rate of buildup of *Meloidogyne incognita* (PF/PI) were calculated according to Oostenbrink, [17] as follows:

Final nematode population (PF) = (No. of egg-masses x No. of eggs/egg-masses) + No. of females + No. of developmental stages + No. of juveniles in soil/pot.

$$Rate\ of\ buildup\ of\ nematodes\ (PF/PI) = \frac{Final\ nematode\ population\ (PF)}{Initial\ nematode\ population\ (PI)}$$

Vegetative growth parameters, including plant height, number of branches/plant, dry weights of shoots (stems + leaves) and roots/plant, as well as root length were recorded. The nodulation parameters including number and dry weight of nodules/plant, as well as nitrogenase activity [18] were also determined. In addition, chemical analysis of fresh leaves samples was conducted to determine total chlorophylls (a+b) and carotenoids contents using the method described by Nornai [19]. The content of total carbohydrates in dried leaves samples was determined [20]. Dried leaves samples were digested and the extract was analyzed to determine N % using the modified micro-Kjeldahl method as described by Pregl [21], phosphorus according to Jackson [22] as well as potassium [23]. Also, protein % was determined as described by James [24].

All obtained data were subjected to statistical analysis of variance. The means were compared using the "Least Significant Difference (L.S.D.)" test at the 5% level, as described by Little and Hills [25].

RESULTS

All the treatments showed remarkable decrease of the number of root galls (Fig. 1) in *Acacia farnesiana* and *Acacia saligna*, in both seasons. Both oxamyl and mixed genera of *Arbascular mycorrhizae* fungi;

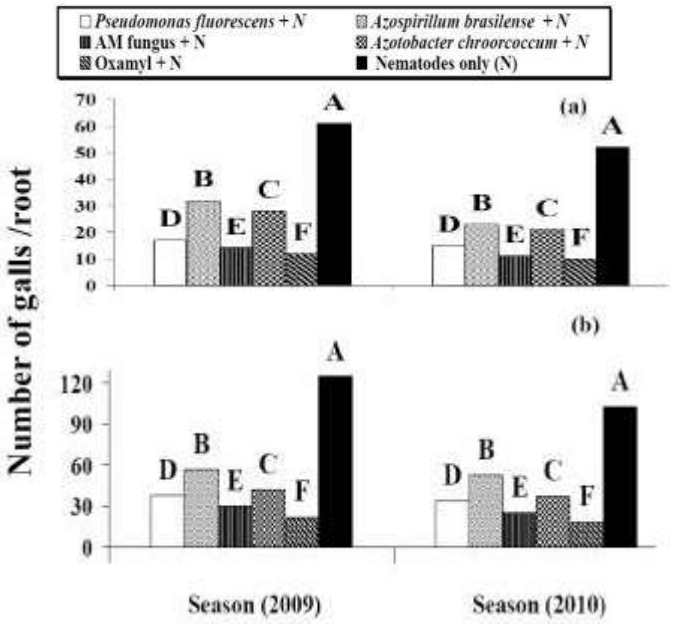

Fig. 1: Effect of some bioagents and oxamyl treatments on number of galls/root in infected a) *Acacia farnesiana* and b) *Acacia saligna* plants infected by *M. Incognita* under greenhouse conditions during the seasons of 2009 and 2010.

(*Glomus*, *Gigaspora* and *Acaulospora*); performed the lowest galls numbers in *Acacia farnesiana*; reached to 12 and 14, respectively, in season 2009 and reached to 10 and 11, respectively, in season 2010, compared with the other treatments. The same trend was found in *Acacia saligna* which reached to 22 and 29, respectively, in season 2009 and reached to 18 and 25, respectively, in season 2010. Whereas, *Azospirillum brasilense* resulted in the highest number of root galls in *Acacia farnesiana* and *Acacia saligna* reached to 32 and 57, respectively, in season 2009 and reached to 23 and 53, respectively, in season 2010. While *Pseudomonas flourescens* and *Azotobacter chroorcoccum* were occupied an intermediate position in decrease number of root galls. Data in Table 1 showed that all tested treatments, in both seasons, were effective in decreased the final nematode population and rate of buildup of root-knot nematode in both soil and roots, of the two species. Oxamyl and mixed genera of AM fungi were the most effective treatments, whereas the least effective one was *Azospirillum brasilense*. While, *Pseudomonas fluorescens* and *Azotobacter chroorcoccum* were occupied an intermediate position. The same trend obtained with the effect on juveniles in soil and developmental stages, females, egg-masses and eggs number in roots.

Analysis, in both seasons, indicated that the inoculation with *Meloidogyne incognita* significantly reduced all vegetative growth parameters (plant height, number of branches/plant, dry weights of shoots (stems + leaves) and roots, as well as root length) of *Acacia farnesiana* and *Acacia saligna* compared to the control, in both seasons (Table 2). While, the applications of oxamyl and mixed genera of AM fungi had an adverse effect on vegetative growth parameters, followed by the application of *Pseudomonas fluorescens*, *Azotobacter chroorcoccum* and *Azospirillum brasilense* to the pathogen plants of the two species, in both seasons.

Nodulation parameters (number of nodules, dry weight of nodules and nitogenase activity) in the two species significantly decreased as a result of inoculation with *M. incognita* compared to the control, in both seasons, (Tables 2, 3). Meanwhile, pathogen plants which received oxamyl and mixed genera of AM fungi resulted in the highest nodulation parameters, followed by pathogen plants inoculated with *Pseudomonas fluorescens*, *Azotobacter chroorcoccum* and *Azospirillum brasilense*, in both seasons.

Inoculation plants with *M. incognita*, in both seasons, had the same trend on chemical components in the leaves (nitrogen, phosphorus, potassium and total carbohydrates percentages as well as total chlorophylls (a+b) and carotenoids contents), of the two species (Table 3). The addition of oxamyl and mixed genera of AM fungi to the pathogen plants, caused significantly increased in chemical components in the leaves, followed by pathogens plants received the inoculum of *Pseudomonas fluorescens*, *Azotobacter chroorcoccum* and *Azospirillum brasilense*, in both seasons.

Table 1: Effect of some bioagents and oxamyl treatments on reproduction and buildup of infected *Acacia* plants by *Meloidogyne incognita* under greenhouse conditions during the seasons of 2009 and 2010

	Nematode populations in Acacia farnesiana													
		Root							Root					
Treatments	No. of juveniles in soil/pot	No. of developmental stages	No. of females	No. of egg-masses	No. of eggs/ egg-mass	PF*	Buildup (PF/PI)	No. of juveniles in soil/pot	No. of developmental stages	No. of females	No. of egg-masses	No. of eggs/ egg-mass	PF*	BuildUp (PF/PI)
	1st							2nd						
Pseudomonas fluorescens+ N	100	24	17	15	175	2766	0.92	80	18	15	13	161	2206	0.76
Azospirillumbrasilense + N	360	35	32	26	245	6797	2.27	180	31	23	20	222	4674	1.56
AM fungi + N	60	21	14	11	163	1888	0.63	40	15	11	9	148	1398	0.47
Azotobacter chroorcoccum + N	200	32	28	21	220	4880	1.63	120	23	21	18	195	3674	1.23
Oxamyl + N	40	14	12	9	160	1506	0.50	20	12	10	7	142	1036	0.35
Nematodes only (N)	1100	72	61	56	310	18593	6.20	880	55	52	45	284	13767	4.59
L.S.D. (5 %)	17.80	2.30	1.60	1.70	2. 90	274.70	0.09	18.90	2.80	1.9	1.80	5.20	292.80	0.11
	Nematode populations in Acacia saligna													
	1st							2nd						
Pseudomonas fluorescens+ N	140	49	38	32	344	11235	3.75	120	37	34	29	320	9471	3.16
Azospirillumbrasilense + N	300	63	57	50	382	19520	6.51	280	55	53	47	370	17778	5.93
AM fungi + N	100	39	29	22	310	6988	2.33	80	30	25	19	280	5455	1.82
Azotobacter chroorcoccum + N	280	59	45	38	366	14292	4.76	220	46	32	27	348	9694	3.23
Oxamyl + N	60	35	22	19	190	3727	1.42	40	32	18	15	175	2715	0.91
Nematodes only (N)	2600	122	125	120	458	57807	19.27	2460	110	103	92	447	43797	14.60
L.S.D. (5 %)	18.70	3.20	5.90	2.10	15.30	789.10	0.65	29.40	4.80	1.9	3.70	16.60	211.8	0.89

PF* = Final nematode population.

Table 2: Effect of some bioagents and oxamyl treatments on growth parameters and dry weight of nodules/plant of infected Acacia plants by M. incognita under greenhouse conditions during the seasons of 2009 and 2010

	Acacia farnesiana						Acacia saligna					
	Plant height (cm)		Root length (cm)		No. of branches/plant		Plant height (cm)		Root length (cm)		No. of branches/plant	
Treatments	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Pseudomonas fluorescens+ N	68.00	74.33	53.00	58.00	8.00	10.33	56.33	59.00	23.17	25.00	21.33	24.00
Azospirillumbrasilense + N	45.00	49.33	42.33	46.67	4.67	5.33	48.00	49.67	15.50	17.00	10.67	12.00
AM fungi + N	87.33	91.00	59.00	66.00	10.67	13.67	63.00	66.33	26.50	28.67	26.00	29.67
Azotobacter chroorcoccum + N	50.33	55.33	47.33	52.33	6.00	7.00	51.67	53.33	19.67	22.33	13.00	19.00
Oxamyl + N	87.67	91.67	59.67	66.33	11.33	14.33	63.67	67.00	27.83	29.17	26.33	30.33
Nematodes only (N)	35.67	38.00	28.00	34.33	1.67	2.67	23.00	27.33	9.33	12.00	7.00	9.67
Control	88.33	92.33	60.33	67.33	12.67	15.00	64.33	67.67	28.33	30.67	27.33	31.00
L.S.D. (5 %)	2.47	2.22	2.69	1.62	0.60	0.98	3.45	2.61	2.37	2.18	1.83	1.14
	Dry weight of shoots (g/ plant)		Dry weight of roots (g/ plant)		Dry weight of nodules (g/plant)		Dry weight of shoots (g/ plant)		Dry weight of roots (g/ plant)		Dry weight of nodules (g/plant)	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Pseudomonas fluorescens+ N	16.70	19.94	14.98	16.40	0.40	0.43	9.33	10.63	6.98	7.67	0.24	0.29
Azospirillum brasilense + N	9.79	11.64	10.77	12.00	0.28	0.32	5.23	6.17	3.67	4.03	0.16	0.19
AM fungi + N	17.90	21.93	17.12	18.00	0.47	0.50	11.00	11.50	8.00	9.50	0.26	0.32
Azotobacter chroorcoccum + N	13.38	14.81	13.35	15.00	0.33	0.39	7.07	8.68	5.03	5.82	0.19	0.22
Oxamyl + N	18.33	22.04	17.73	18.95	0.47	0.51	11.30	11.87	8.87	10.00	0.26	0.32
Nematodes only (N)	4.85	7.33	6.40	7.92	0.07	0.10	3.83	4.40	1.98	2.60	0.05	0.06
Control	19.03	22.65	18.13	19.33	0.50	0.54	11.73	12.40	9.03	10.87	0.32	0.36
L.S.D. (5 %)	0.96	1.00	0.92	1.45	0.05	0.04	0.94	0.47	0.66	0.71	0.01	0.02

Table 3: Effect of some bioagents and oxamyl treatments on number of nodules/plant, nitrogenase activity, and chemical components in the leaves of infected *Acacia* plants by *M. incognita* under greenhouse conditions during the seasons of 2009 and 2010.

	Acacia farnesiana						*Acacia saligna*					
	Number of nodules/plant		Nitrogenase activity (mol. C_2H_4 / g dry weight nodules /hr)		Protien %		Number of nodules/plant		Nitrogenase activity (mol. C_2H_4 / g dry weight nodules /hr)		Protien %	
Treatments	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Pseudomonas fluorescens + N	25.33	29.00	67.89	73.36	20.85	21.69	18.67	19.33	52.23	55.20	17.04	18.77
Azospirillum brasilense + N	16.67	20.67	40.42	49.66	17.10	17.94	12.33	14.67	36.07	41.29	11.27	12.50
AM fungus + N	27.00	31.00	75.56	80.84	21.60	22.71	19.67	20.33	55.63	60.75	18.11	19.31
Azotobacter chroorcoccum + N	23.67	26.67	60.25	68.23	18.31	19.29	17.00	18.33	46.83	48.93	12.42	1 3 . 5 6
Oxamyl + N	27.33	31.33	76.13	81.13	22.60	23.67	20.00	20.67	56.14	61.04	22.21	22.52
Nematodes only (N)	7.00	10.33	10.01	12.09	14.52	15.44	4.00	6.33	6.79	7.42	8.86	9.77
Control	28.00	31.67	77.46	83.24	23.58	24.67	20.67	21.00	59.15	64.90	22.61	23.65
L.S.D. (5 %)	1.53	1.74	4.07	2.63	0.50	0.13	0.73	1.05	2.75	2.00	0.40	0.29
	N (% of dry weight)		P (% of dry weight)		K (% of dry weight)		N (% of dry weight)		P (% of dry weight)		K (% of dry weight)	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Pseudomonas fluorescens + N	3.34	3.47 D	0.25	0.28	1.49	1.72	2.73	3.00	0.20	0.25	1.32	1.41
Azospirillum brasilense + N	2.74	2.87 F	0.22	0.23	1.25	1.32	1.80	2.00	0.15	0.19	1.18	1.23
AM fungus + N	3.46	3.63 C	0.26	0.29	1.57	1.88	2.90	3.09	0.22	0.26	1.39	1.45
Azotobacter chroorcoccum +N	2.93	3.09 E	0.24	0.26	1.36	1.44	1.99	2.17	0.18	0.22	1.25	1.29
Oxamyl + N	3.62	3.79 B	0.30	0.33	1.73	1.93	3.55	3.60	0.27	0.31	1.42	1.49
Nematodes only (N)	2.32	2.47 G	0.16	0.18	0.99	1.04	1.42	1.56	0.10	0.15	0.89	0.93
Control	3.77	3.95 A	0.35	0.39	1.92	1.97	3.62	3.78	0.29	0.34	1.47	1.53
L.S.D. (5 %)	0.08	0.02	0.02	0.02	0.03	0.03	0.06	0.05	0.02	0.02	0.03	0.03
	Total chlorophylls (a+b) content (mg/g fresh weight)		Carotenoids content (mg/g fresh weight)		Total carbohydrates (% of dry weight)		Total chlorophylls (a+b) content (mg/g fresh weight)		Carotenoids content (mg/g fresh weight)		Total carbohydrates (% of dry weight)	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Pseudomonas fluorescens + N	2.67	2.75	1.31	1.35	17.03	19.23	1.36	1.47	0.67	0.72	12.67	15.07
Azospirillum brasilense + N	1.27	1.31	0.63	0.63	13.30	14.93	1.10	1.15	0.53	0.57	9.73	10.90
AM fungus + N	2.83	2.88	1.36	1.41	20.17	23.13	1.47	1.53	0.68	0.73	15.23	17.07
Azotobacter chroorcoccum + N	2.24	2.32	1.11	1.13	15.00	17.80	1.28	1.37	0.63	0.68	11.57	13.47
Oxamyl + N	2.88	2.92	1.39	1.43	20.83	23.73	1.49	1.54	0.71	0.75	15.87	17.80
Nematodes only (N)	0.54	0.67	0.23	0.31	6.87	7.80	0.41	0.45	0.18	0.23	4.97	6.07
Control	2.90	2.94	1.39	1.44	21.47	24.50	1.50	1.56	0.72	0.76	16.43	18.13
L.S.D. (5 %)	0.05	0.05	0.02	0.02	1.11	1.02	0.04	0.04	0.02	0.02	1.43	1.27

DISCUSSION

Among the 4 biocontrol agents tested under greenhouse conditions, AM fungi in both *Acacia* roots had an effect on decreasing the final *M. incognita* population and the rate of nematode buildup. The physiological and biochemical changes caused by mycorrhizal fungi in the host plant generally reduce the severity of nematode population [26]. AMf inoculation increased also, phosphate-solubilizing microorganisms that cause an inhibitory effect on nematode development through releasing organic acids which are often accompanied with the release of other metabolites, mainly siderophores, phytohormones and lytic enzymes [27, 28]. In addition, the increased phenolic compounds, phytoalexins, lignin, phenols, sugars and amino acids (phenylalanine and serine) in mycorrhiza treated plants have been suggested to play an important role in the plant defense mechanism [29-31]. Also, PGPR strains (*Psudomonas fluorescens*, *Azotobacter chroococcum and Azospirillum brasilense*) inhibited egg hatching and killed juveniles by producing wide variety of antibiotics, siderophores, hydrolytic enzymes, organic compounds, HCN, phenol oxidation and protease [32, 33].

Our results indicated that the inoculation of AM fungi reduced plant stress caused by *Meloidogyne incognita* and increased plant growth, nodulation parameters as well as chemical components. Morphological root changes in mycorrhiza treated plants led to increasing the absorptive surface area of the whole host root system to water and mineral nutrients supply, particularly P, so that can improve biological N_2-fixation in

legumes and increase the number of nodulation sites and consequently the number of nodules per plant [34- 38]. *Psudomonas fluorescens*, *Azotobacter chroococcum and Azospirillum brasilense* may also improve plant growth, nodulation parameters as well as chemical components by the production of biologically active substances of growth hormones (IAA, gibberellins and auxins) [39], or by converting unavailable minerals and organic compounds into forms available to plants [40]. In addition, PGPR strains usually have been found to increase the root length and root biomass and this better developed root system may increases the mineral uptake in plants [41, 42].

CONCLUSION

Meloidogyne incognita on *Acacia farnesiana* and *Acacia saligna* can be controlled in order to improve growth characters and chemical components in pathogened plants by using mixed genera of *Arbascular mycorrhizae* fungi (*Glomus*, *Gigaspora* and *Acaulospora*) instead of nematicides that cause severe pollution of ecosystems.

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