Hindawi Publishing Corporation
ISRN Biodiversity
Volume 2013, Article ID 593087, 6 pages
http://dx.doi.org/10.1155/2013/593087

Research Article

Seasonal Colonization of Arbuscular Mycorrhiza Fungi in the Roots of *Camellia sinensis* (Tea) in Different Tea Gardens of India

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Received 18 October 2013; Accepted 3 December 2013

Academic Editors: I. Bisht and A. Chistoserdov

Study describes Arbuscular Mycorrhiza (AM) fungi colonization within the roots of cultivated tea plants (*Camellia sinensis*) at four sites, that is, Goodrich, Archadia, IIP, and Vasant Vihar of Doon Valley, Dehradun, India, from April, 2008, to March, 2009. Microscopic study of sterilized and stained root segments showed presence of four species namely *Glomus fasciculatum*, *G. mosseae*, *Gigaspora margarita*, and *Acaulospora scrobiculata* belonging to three genera of mycorrhizal fungi. Maximum AM colonization was observed during April–September and minimum was observed for December-January months of the year. Comparative study of AM fungi colonization at four sites during rainy season showed maximum colonization (100%) at Archadia site having soil with high organic matter, less acidity, and low phosphorus (P) whereas minimum (64.59%) at IIP with low organic matter, more acidity, and high P content. However, no variation in nitrogen content was observed at all four sites. Study suggested a positive relation of percentage root colonization with soil organic matter and negative relation with acidity and P content of soil. Study concludes that the percentage AM colonization is the function of seasonal variation in physicochemical properties of soil and presence of AM inoculums in the soil at a particular time.

1. Introduction

Increasing concern over industry based development and related risk of environment, energy, and food security has stimulated scientists to develop and design biosystems as alternate or supplementary sources of biofertilizer for sustainable practices, particularly for agriculture and remediation of degraded lands. Mycorrhizas—a symbiotic association between the fungi and the roots of higher plants [1]—are receiving more attention worldwide for their ability to enhance the uptake and absorption of relatively immobile nutrients and minerals of plants due to comparatively large surface area of mycelium : root ratio [2]. Mycorrhizal associations occur naturally with more than 95% of terrestrial plants, of which 65% belong to Arbuscular Mycorrhiza (AM) fungi [3, 4]. Inoculation of AM fungi with different plants showed increased uptake of nitrogen (N) by plants [5], promotion of plant growth [6], exchange of water and mineral with soil [7], resistance to stress and drought and in some cases to soil pathogens [8, 9], and metals toxicity resistance to plants [10]. Establishment of mycorrhizal associations supports up to 80% of N and 90% P requirements of plants [11].

Camellia sinensis is widely used for the cheapest aromatic beverages in the world [12]. Consumption of *C. sinensis* has profound effects on health as it contains more than 700 chemicals such as flavanoids, amino acids, vitamins (C, E, and K), caffeine, and polysaccharides. Beneficial role of *C. sinensis* in normalizing blood pressure, lipid depression activity, prevention of coronary heart diseases, and diabetes by reducing the blood glucose activity as well as the presence of catechins (an antioxidant) has elevated this plant at industry level and has profound effect on economic growth of a country. However, application of chemical fertilizers for quantitative production is adversely affecting the quality of tea. Therefore,

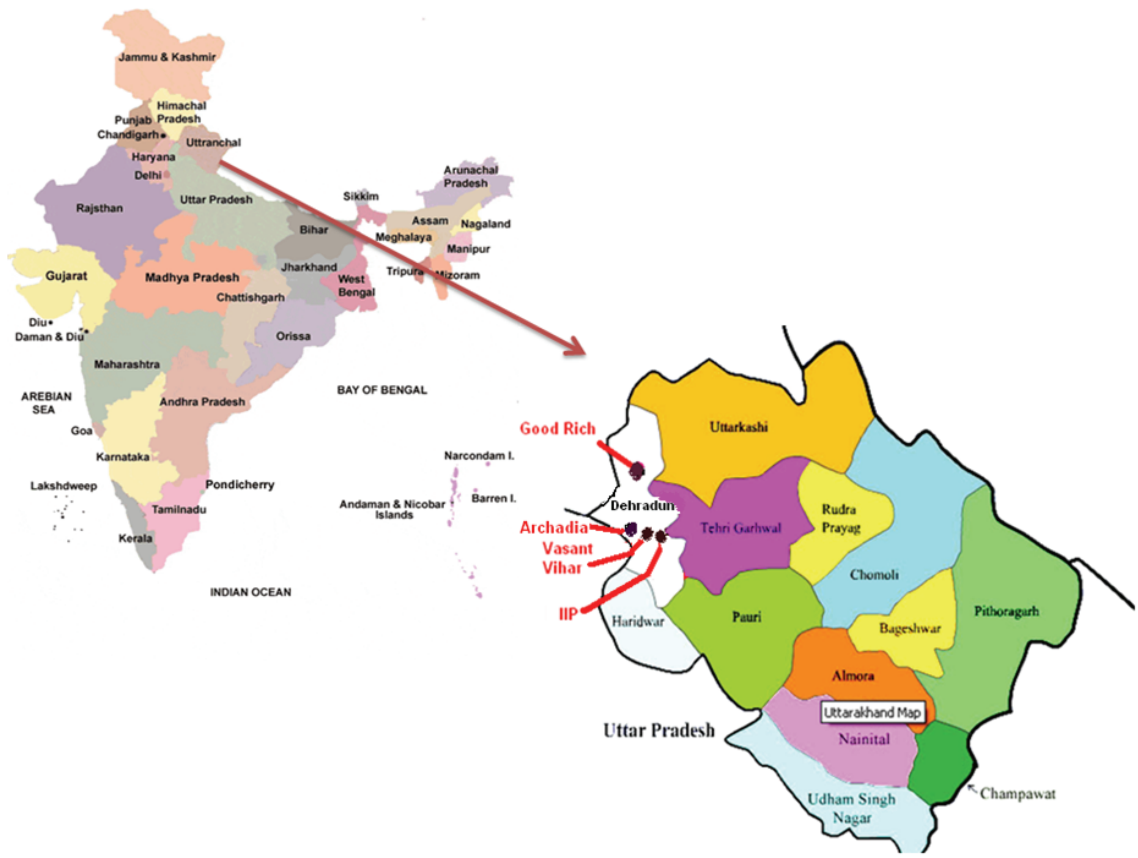


Figure 1: Location of four study sites on geographical map.

AM fungi colonizing the tea plants are getting more attention for their ability to support the growth. Although, percentage root colonization of AM fungi with *C. sinensis* roots [13, 14] and with other plants like Pine-Oak forests [15] in the Himalayan region has been variously studied, detailed study on percentage root colonization with respect to seasonal change that might support the annual nutrient management is scarce. Considering the significant nutrient contribution of AM fungi and ultimate requirement of sustainable nutrient management, present study is proposed to enumerate the colonization of AM fungi in the roots of *C. sinensis* during different months for a year.

2. Materials and Methods

2.1. Study Area. The study was conducted on 4th year of tea plantation at four selected sites, namely, Goodrich, Archadia, IIP and Vasant Vihar located at the foot hills of the Himalayas at Doon Valley, Dehradun, India, from April, 2008, to March, 2009. Geographical location of four different sites has been presented in Figure 1. Dehradun is located at the latitude of $30^{\circ}19'$N and longitude of $78^{\circ}04'$E and is situated between the Himalayas in the north and Shivalik range in the south. Climate of Dehradun is generally temperate and varies from hot in summers to severely cold in winter. Average annual temperature ranges from 5 to 35°C and average annual rainfall is 2073.3 mm. Study was conducted in the area of 100 m × 70 m for each selected site. The distance between Goodrich (north end of the study site) and IIP (south end of the study site) sites is 73 Km. Archadia and Vasant Vihar are located at a distance of 48 and 66 Km from Goodrich site, respectively.

2.2. Root Sampling. Percentage root colonization of AM fungi was documented with root samples collected from the "root zones" of the *Camellia sinensis* plants at the depth of 15 to 20 cm below the soil surface. Root sampling was done randomly between 8 and 10 am in middle of every month for one year. Eighty root samples were collected in conical flasks for each selected site separately and brought to the laboratory. Study of the root samples for AM colonization was performed on the very next day of the collection. Roots were kept in running tap water for half an hour and washed thrice with sterile double distilled water to avoid the presence of other microbes or AM fungi adsorbed to the root surface.

2.3. Research Methods. AM fungi colonization with the root samples was assessed following the slide method of Giovannetti and Mosse [16]. Root samples were cut into pieces of 1 cm length and stained with 0.05% cotton blue in lactophenol. Excess stain was removed with clear lactophenol. Root segments (1 cm long) were selected randomly from stained samples and mounted on microscopic slides in groups of 10 pieces. Presence and absence of infection were recorded microscopically in each of the 80 root segments for each site

Table 1: Percentage root colonization of AM fungi in the roots of tea plants at Archadia, Goodrich, IIP, and Vasant Vihar sites during different months of the year.

	Months	Sites				Level of significance	CD
		Archadia	Goodrich	IIP	Vasant Vihar		
AM colonization	Jan	33.20	31.16	16.63	36.34	$***$	7.053
	Feb	40.49	31.97	19.46	37.35		
	Mar	59.67	38.45	28.80	40.67		
	Apr	70.97	51.05	35.39	63.75		
	May	70.96	68.75	42.06	85.90		
	Jun	96.86	83.64	64.59	92.69		
	Jul	100.00	83.62	47.49	92.59		
	Aug	78.34	73.49	39.48	86.56		
	Sep	76.31	67.40	29.35	82.42		
	Oct	49.12	52.82	30.85	58.57		
	Nov	39.07	43.96	21.99	52.24		
	Dec	32.50	41.82	21.10	31.20		

$***$: significant at 0.001.

separately for different months of the year and percentage root colonization was calculated. Mycorrhizal fungi were studied for their cellular organization, color, shape and size, wall structure, and position of the vesicles and arbuscules and identified with the help of the literature [17–19]. Micrograph of AM fungi was assessed under a stereomicroscope (Olympus SZ H10 research microscope).

2.4. Analysis. pH of the soil sample was determined in 1 : 5 suspension of soil : deionised water electrometrically using glass electrode pH meter 335 (Systronics India Limited). Organic carbon was determined by using Walkley and Black's rapid titration method [20] and total nitrogen (N) and phosphorus (P) were determined using the method of Jackson [21].

2.5. Statistical Analysis. Data presented in the tables are the mean values of the analysis. Data produced for different parameters were subjected to multifactor analysis using SPSS software version 10.0.

3. Results

The study describes the differences in monthly colonization of AM fungi in the *C. sinensis* roots round the year at four different sites. Different stages of AM fungi inside root of *C. sinensis* have also been studied and presented in Figure 2.

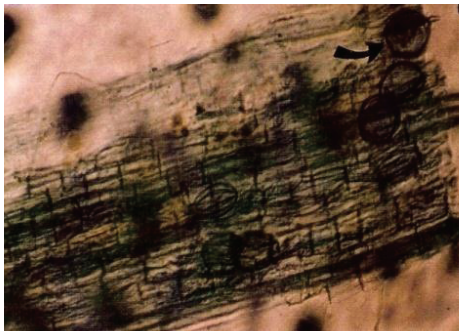
Extensive study carried out to enumerate the AM fungi colonization in the roots of *C. sinensis* showed differences in percentage root colonization (PRC) during different months of the year at four selected sites (Table 1). Study showed maximum AM fungi colonization at Archadia (100%) followed by Vasant Vihar (92.69%), Goodrich (83.64%), and IIP (64.59%) sites (Table 1). Detailed analysis of percentage root colonization during different months of the year showed that the PRC for all four sites was above/around the annual mean from April to September (hot and humid summer) and below the annual mean from October to March (cold winter). Annual mean was 62.29, 55.68, 33.10, and 63.36 for Archadia, Goodrich, IIP, and Vasant Vihar, respectively (Table 2). AM fungi colonization started to decrease with the onset of cold winter, that is, from the month of October, and reached to lowest during the coldest months of December-January and started to increase with onset of rainy summer. Differential colonization of tea plants with AM fungi during different months of the year indicated the possible role of climatic and edaphic factors.

Table 2: Statistical analysis of percentage root colonization of AM fungi in the roots of *C. sinensis* at four sites.

Statistical parameters	Sites			
	Archadia	Goodrich	IIP	Vasant Vihar
Mean	62.29	55.68	33.10	63.36
Range	67.50	52.48	47.96	61.49
Coefficient of range	0.51	0.46	0.59	0.50
SD	23.65	19.09	13.78	23.79

pH, organic matter, and nitrogen and phosphorus content of soil for four different sites are presented in Table 3. Relation between pH and organic matter of the soil and AM colonization was positive, that is, increase in pH and organic matter was directly related to increase in PRC. However, soil phosphorus has negative relation with percentage root colonization. IIP site with higher P content has low PRC whereas Archadia site with low soil P has high PRC. The P content of different sites might be presented as Archadia < Vasant Vihar < Goodrich < IIP whereas PRC as Archadia > Vasant Vihar > Goodrich > IIP. There were no significant differences in nitrogen status of the soil at different sites.

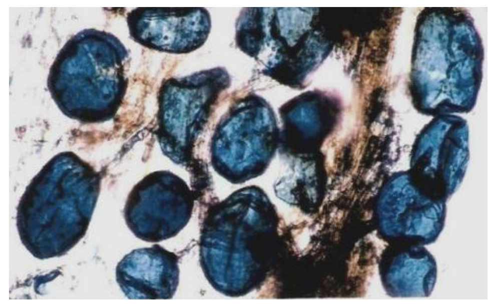
Statistical analysis of the AM colonization for selected sites around the year showed that AM colonization has great affinity with season and significantly different for all sites



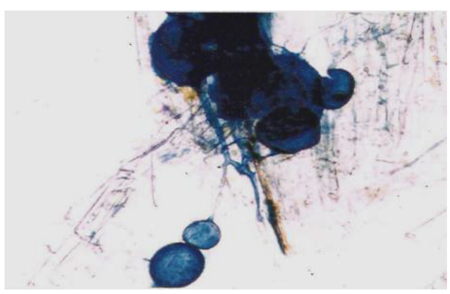
(a) Hyphae and vesicle in *C. sinensis* root cortex

(b) AM hyphae penetrating *C. sinensis* root

(c) Vesicle in the root of *C. sinensis*



(d) Hyphae swell apically to form vesicles



(e) Liberating spores and vesicles outside the root cortex

(f) Spore and vesicle in *C. sinensis* root

Figure 2: Different stages of AM colonization in the roots of *C. sinensis*.

(Table 1). Study gets support from the coefficient of range predicted in Table 2. In spite of significant differences in percentage colonization at four sites ranging from 100% (maximum) at Archadia site to 16.6% (minimum) at IIP site, annual coefficient of range was between 0.46 and 0.59. The study supports that the AM colonization with roots of *C. sinensis* is correlated to seasonal variation in climatic factors and also to AM inocula present at a given time. It was observed that minimum colonization at IIP site in the stress condition of light and temperature during December-January followed the similar trend of minimum colonization during favourable condition of May-June compared to that of other sites. However, the more is the colonization at Archadia site during December-January, the maximum was the colonization during May-June.

Microscopic study showed the presence of four species of AM fungi belonging to three genera of class Glomeromycota and were represented by *Glomus fasciculatum*, *G. mosseae* (family Glomaceae), *Gigaspora margarita*, and *Acaulospora scribiculata* (family Acaulosporeacae). Hyphae and vesicles were the most common structures observed for root segments under microscope (Figure 2(a)). However, hyphae were predominantly present for all selected sites (Figure 2(b)). The root samples of *C. sinensis* showed typical wider and aseptate hyphae growing inter- and intracellularly through the cortex and penetrating to the inner cortex (Figures 2(b) and 2(c)). Vesicles were mostly globose to subglobose or ellipsoidal in shape inside the roots of *C. sinensis* (Figure 2(d)). The arbuscules were observed in cells of the inner cortex. Vesicles with group of spores were also seen in the roots of *C. sinensis* (Figures 2(e) and 2(f)). Further investigation in terms of species abundance showed predominance of *Glomus* sp. followed by *Acaulospora scribiculata* and *Gigaspora margarita*.

4. Discussion

AM fungi have been variously studied for their contribution in degraded land as well as their potential application in agriculturally cultivated plants for enhanced production [22].

Table 3: Annual averages of pH, organic matter, and nitrogen and phosphorus content at four sites.

Sites	pH	Organic matter (%)	Nitrogen (%)	Phosphorus (ppm)
Archadia	5.7	2.20	0.21 ± 0.03	12.60 ± 0.44
Goodrich	5.1	1.35	0.17 ± 0.02	25.64 ± 0.09
IIP	4.8	1.20	0.18 ± 0.01	28.17 ± 0.29
Vasant Vihar	5.4	1.55	0.18 ± 0.02	22.55 ± 0.02

The present study describes the colonization of *C. sinensis* roots with AM fungi round the year on monthly basis. Maximum root colonization during hot and humid summer (rainy season, i.e., during April–September) and decrease in colonization with the start of cold winter during the months of October–March were in accordance with the study reported by Chandra and Jamaluddin [23]. They also observed the similar result of maximum AM fungi colonization during rainy season in *C. sinensis* root. Gould et al. [24] also observed the maximum root colonization and spore population density during June and minimum during October-November on reclaimed sites of Archadia. Similar observation of negative relation between P content and AM colonization has been reported by Nagahashi et al. [25]. They reported that increase in P content significantly inhibited the number of branches and total hyphae length. Negative effect of P on AM colonization suggests that plant itself is capable of absorbing sufficient amount of P required for growth and development. Increase in P brings about anatomical and physiological changes in the roots that inhibit or limit the vigorous intraradical spread of AM fungi [25–27]. Positive effect of pH on AM colonization might be compared to the report by Medeiros et al. [28]. They reported the significant increase in root colonization of Sorghum plant with AM fungi with increasing pH. Positive relation of percentage root colonization of AM fungi with organic matter observed in this study was in accordance with Vaidya et al. [29] who reported increased AM population with increase in organic matter. Study reflects that the site Archadia having low phosphorus has high organic matter and high percentage of AM root colonization. In contrast, site IIP with high P content has low organic matter and low percentage of AM root colonization. It might be explained by the fact that high P content in the soil in the root limits the supply of organic carbon to the AM fungus of the colonized root thereby causing reduced extension of hyphae and development of spores [30].

Further investigation of differences in percentage colonization at different sites and differences in range might be attributed to the initial inocula of the AM fungi in the field under natural condition. It is apparent from the study that IIP site with less AM fungi inocula (lower PRC) has minimum percentage of root colonization during favorable condition of May-June; whereas Archadia site with more AM fungi inocula (higher PRC) has maximum percentage of root colonization during favorable condition of May-June (compared to all four sites). Moreover, dominance of *Glomus* sp. followed by *Acaaulospora* and *Gigaspora* sp. is comparable to Zhao et al. [22]. They reported similar observations of dominance of *Glomus* sp. and *Acaulospora* sp. in the tropical rainforests of Xishuangbanna in southwestern China. The differences in percentage root colonization and species abundance might be attributed to change in climatic conditions round the year. Varied diversity of AM fungi associated with different plants is already in the literature [31, 32]. In addition, seasonality, physiochemical properties of soil, host dependence, age of the host plants, and dormancy might play significant role in AM establishment and diversity [33]. The present study will certainly help in maintaining the nutrient status of the soil during lower rate of AM colonization by introducing comparatively high nutrient into the soil to maintain the growth of tea plants during stress period of cold.

5. Conclusion

The present study of percentage root colonization at different tea planted sites on monthly basis might contribute significantly in the future management of nutrient status of soil under field condition for better application of AM fungi for better yield. Establishment of AM fungi in field condition might support the proliferation of some important microbes that actively contribute in nitrogen and other nutrient cycling. The research output of present study concludes that maximum AM fungi colonization during rainy season is probably due to physico-chemical properties of soils and surroundings.

Acknowledgments

The authors wish to thank the owner of Goodrich Tea Estate (Vikasnagar) Mahant Shri Devendra Dass ji Maharaj (Darbar Shri Guru Ram Rai Ji Maharaj), Dehradun, for giving place to set up tea nursery, Mr. Digvijay Singh Rawat, the Manager of Tea Estate, for the tea plants given by them, and the Department of Botany, Govt. P.G. College, Rishikesh, for laboratory facilities. The authors are thankful to the University Grants Commission, New Delhi, and the Uttarakhand Council for Science and Technology, Dehradun, for financial assistance.

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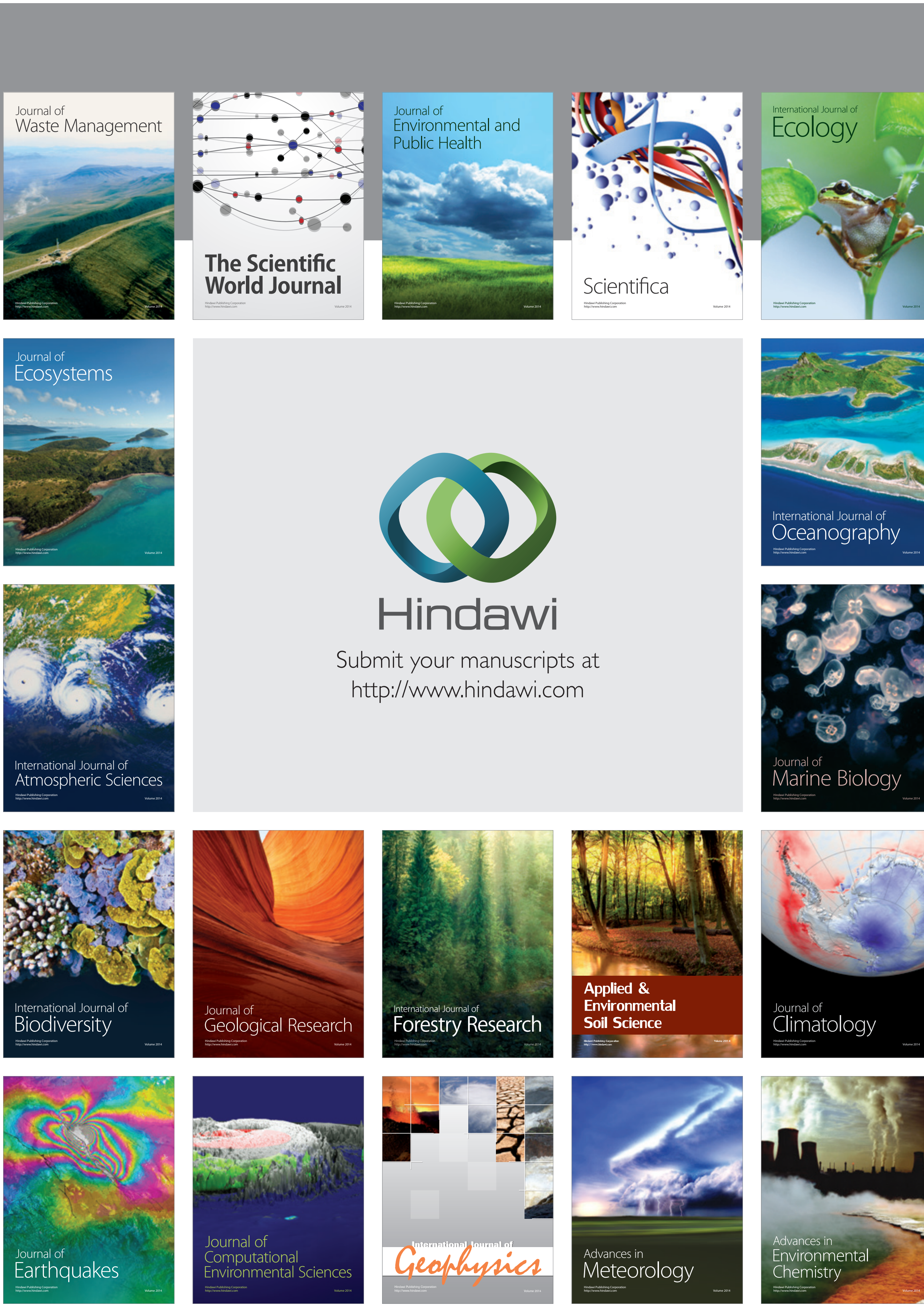
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