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# VERTICAL DISTRIBUTION OF AM COLONIZATION AND RELATIONSHIP WITH SOIL PROPERTIES IN MEDICINAL PLANTS OF BCSIR RESERVE FOREST CHITTAGONG, BANGLADESH

**MILTON HALDER, SAMINA AKHTER, NUSRAT JAHAN MOURI AND MD SAIDUR RAHMAN***

*BCSIR Laboratories Chittagong, Chittagong-4220, Bangladesh*

*Key words*: Mycorrhizal colonization, Edaphic factors, Vertical soil depth

**Abstract**

Investigation on arbuscular mycorrhizal (AM) association with medicinal plants has been conducted with emphasis on mycorhizal colonization distribution along the vertical soil depth as well as their relationship with edaphic factors. Soil (0 - 10 and 10 - 20 cm depth) and root samples were collected between January and February in 2015. The mean per cent colonization of AMF was noted highest (100%) for *Asparagus racemosus, Calotropis gigantea, Terminalia bellirica, Terminalia chebula* followed by *Azadirachta indica, Adhatoda vasica* (about 88.89 ± 3.57%) and *Asparagus racemosus*, and *Calotropis gigantea* (about 76.92 ± 7.73 and 76 ± 4.72% respectively) *Adhatoda vasica* (50 ± 4.37%) for surface and sub soil, respectively. Soil pH, EC, K, Na, OM, OC, P, S, and fungal colonization intensity decreased with the increase of soil depth. Pearson's correlation revealed that arbuscular mycorrhiza is well established with the selected medicinal plants and exhibites variations depending on edaphic factors in the study area.

## Introduction

Mycorrhizal colonization in plants revealed a surprising relationship between the mutualistic mycorrhizal fungi and nutritional requirements of plants. Arbuscular mycorrhizal fungi (AMF) is ubiquitous soil borne fungi under Glomeromycota that forms mutualistic association with the roots of common plants of terrestrial ecosystems (Smith and Read 2008). The host plants provide the fungal partner with soluble carbon sources, and the fungal symbionts enhance the uptake of certain nutrients by root (Li *et al*. 2006), increase plants' resistance against pathogens (Graham 2001) and drought (Augé 2001). AM fungal symbionts are usually distributed at the top layer of the soil profile where labile nutrients are released; however in some cases it may be exceptional. AM colonized roots can extend deep into the soil profile (Allen 1991) and the vertical distribution depends on the soil resources and other edapho-climatic factors. Differences in topographical position, organic matter content, drainage condition, moisture contents etc. play key role on the distribution of AM colonization. In general, increase in soil pH, nutrient status and salinity are related to a decrease in VAM root colonization and spore density (Abbott and Robson 1991). However, although, some works have been performed on the AM colonization of non-medicinal plants so far, no study on the vertical distribution of AM colonization in medicinal plants, spore density and their relationship with edaphic factors in the field conditions have been performed in the context of Bangladesh (Dhar *et al.* 2005). The present research therefore, has been conducted to investigate the vertical distribution of mycorrhizal colonization and relationship with edaphic factors in the association with medicinal plants growing in the BCSIR research field of Chittagong, Bangladesh.

*Author for correspondence: <saidurgene@yahoo.com>.

## Materials and Methods

The BCSIR reserve forest is specialized for research and conservation of medicinal, aromatic and locally available fruit plants. It is situated at 22°24’35.4" N 91°49’00.6" E in the south-eastern part of Bangladesh. The area of the forest is aproximately 100 acres that support about especially for about 1600 species.

Rhizosphere soil and root samples of selected highly valued 10 medicinal plants (Table 1) were collected during January to February, 2015. Prevailing precipitation was 0.02 - 1.31mm (per day) during the period of sample collection. Three replicated root and rhizosphere soil samples were collected vertically from top soil layer of 0 - 10 cm and 10 - 20 cm from the rhizosphere zones of the selected medicinal plants.

**Table 1. List of medicinal plant species selected for the collection of roots and rhizosphere soil sample in the BCSIR reserve forest, Chittagong.**

| Sl. No. | Scientific name | Family | Habit |
|---|---|---|---|
| 1 | *Achyranthes aspera* L. | Amaranthaceae | Undershrub |
| 2 | *Adhatoda vasica* Nees | Acanthaceae | Shrub |
| 3 | *Andrographis paniculata* (Burm.f.) Wall. Nees | ,, | Herb |
| 4 | *Asparagus racemosus* Willd. | Asparagaceae | ,, |
| 5 | *Averrhoa carambola* L | Oxalidaceae | Tree |
| 6 | *Azadirachta indica* A. Juss. | Meliaceae | ,, |
| 7 | *Calotropis gigantea* (L.) W.T. Aiton | Apocynaceae | Shrub |
| 8 | *Rauvolfia serpentina* (L.) Benth. *ex* Kurz | ,, | Herb |
| 9 | *Terminalia bellirica* (Gaertn.) Roxb. | Combretaceae | Tree |
| 10 | *T. chebula* Retz. | ,, | ,, |

Soils were sieved to separate unwanted gravels particles with a 2 mm sieve. Soil samples were preserved at room temperature by closing the polybags to avoid the loss of moisture content. From each soil sample, three subsamples were studied for analyzing the soil properties. Soil pH (soil : water = 1 : 2.5) and EC (soil : water = 1 : 2.5) were determined. Available nutrients (Na, K and P) as well as OC and SOM (Jackson 1973), daily ambient and soil temperature and moisture for both top and sub soil were measured.

Adhered soil particles were separated from the root and roots were washed and chopped into 1 cm pieces, stained by following the procedures of Philips and Hayman (1970). A total of 25 segments of roots were mounted on the microscopic slides with 50% glycerol and smashed softly after placing a cover glass on the root pieces. Root segments were observed by a compound microscope at (20 × 10) magnification. Per cent root colonization was recorded and calculated. Presence of mycelium was regarded as the AM positive and total mycelial colonization was treated as root colonization. Per cent root colonization was calculated by using the following formula.

$$\text{Per cent colonization} = \frac{\text{Number of AM positive segments}}{\text{Total number of segments observed}} \times 100$$

Statistical analyses were performed by using SPSS 22.0 version and MS Excel 2007 software. ANOVA was done for DMRT at 0.05 significance level. Pearson correlation was also performed to determine the relationship between the edaphic factors and AM colonization of the host plant species.

**Results and Discussion**

Highest arbuscular mycorrhizal fungi (AMF) colonization rate (100%) among the root samples collected from top soil, was recorded in the root cortical of *Asparagus racemosus, Calotropis gigantea, Terminalia bellirica* and *T. chebula* followed by *Azadirachta indica* (88.89 ± 3.57%) and *Adhatoda vasica* (78.57 ± 8.34%). The lowest colonization was found in the roots of *Andrographis paniculata* (44.44 ± 7.86%) but no colonization was observed for *Achyranthes aspera* (Fig. 1). In case of sub soil, the degree of colonization was totally different in comparison to top soil. In sub soil, highest AMF colonization was measured in *Asparagus racemosus* (76.92 ± 7.73%) and *Calotropis gigantea* (76 ± 4.72%) followed by *Adhatoda vasica* (50 ± 4.37%) and least colonization was observed in *Rauvolfia serpentina* (33.33 ± 6.25%) and *Terminalia bellirica* (30 ± 5.63%) but no colonization was found in *Achyranthes aspera, Andrographis paniculata* and *Averrhoa carambola.* AMF colonization was higher in roots collected from top soil (0 - 10 cm) in comparison to roots of sub soil (10 - 20 cm) of all studied medicinal plants in the BCSIR reserve forest. AMF colonization decreased with depth which was supported by the result of other researchers (Alejandra *et al.* 2014). Microbial activity is highest in top soil in comparison to sub soil. Abott and Robson (1991) suggest that there is an exponential decrease of both mycorrhizal colonization and spore number with soil depth. Vyas and Gupta (2014) also found both colonization per cent and intensity that decreased with increasing depth in Tall grass or True prairie species. The decreasing colonization rates towards the vertical depth in the roots of medicinal plants of BCSIR reserve forest might be due to the low oxyzen diffusion rate, high moisture content and low nutrient availability.

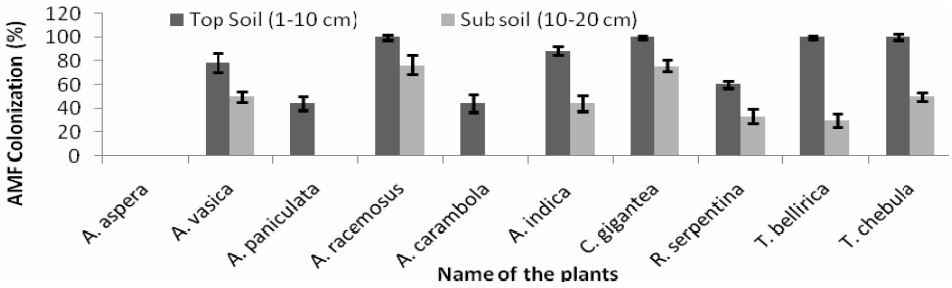


Fig. 1. AMF colonization in top and sub soil in rhizosphere zone of studied medicinal plants under BCSIR forest. Error bars indicate the standard deviation (Sd). n = 3.

Montilla *et al.* (1992) did not observe any significant differences in vertical distribution of AM colonization in tillage soil of Venezuela. Tillage may change soil properties and vertical AM colonization intensity (Abott and Robson 1991). In BCSIR reserve forest there is no disturbance from tillage or any other factors indicating the consistency of the results of the present study. Difference of edaphic factors between the top and sub soil existed. For all soil samples pH values ranged from extremely acidic to slightly acidic and decreased with increasing soil depth. The concentration of soil available P as well as K, EC, OM, OC attenuated with increasing soil depth vertically (Fig. 2 B, F, G, D, E, respectively). But soil moisture and soil temperature mostly increased across the vertical soil depth (Fig. 2 A, C, respectively). Significant differences in soil properties existed among the plants (Tables 2 and 5). Survival of AMF as well as subsequent spore germination and number depend on a species' adaptation and on the effects of edaphic factors of

**Table 2. Chemical properties of rhizosphere subsoil samples of different medicinal plant species growing in the BCSIR reserve forest, Chittagong.**

| Plant species | Colonization | | | Soil Properties | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Myce-lium | Vesicle | Arbus-cules | pH | EC | OM% | % OC | Na (mg/kg) | K (mg/kg) | P (mg/kg) | Day temp. | Soil temp. | Moisture (%) |
| 1. *Achyranthes aspera* | 00e | 00c | 00c | 3.81e | 238.00c | 2.97a | 1.72a | 74.73c | 44.18a | 6.95b | 20.5 | 20 | 0.76 |
| 2. *Adhatoda vasica* | 50b | 00c | 00c | 4.88c | 28.00i | 0.36f | 0.21f | 69.39d | 23.39f | 11.78a | 22 | 22 | 2.56 |
| 3. *Andrographis paniculata* | 00e | 00c | 00c | 5.13b | 274.00b | 0.12g | 0.07g | 74.73c | 21.66g | 1.68f | 22 | 19.5 | 1.73 |
| 4. *Asparagus racemosus* | 76.92a | 7.69b | 7.69bc | 5.45a | 52.00g | 0.85b | 0.49b | 85.40a | 20.79g | 2.80e | 22 | 21 | 3.36 |
| 5. *Averrhoa carambola* | 00e | 00c | 00c | 3.85e | 237.00c | 0.54d | 0.32e | 74.73c | 39.85b | 6.18c | 22 | 19.5 | 1.53 |
| 6. *Azadirachta indica* | 44.45bc | 00c | 11.11ab | 3.09f | 542.00a | 0.67d | 0.39cd | 74.73c | 28.5d | 5.71d | 22 | 18 | 1.15 |
| 7. *Calotropis gigantea* | 76.00a | 00c | 16a | 5.47a | 135.00d | 0.75c | 0.45c | 74.73c | 21.66g | 1.29f | 22 | 21.5 | 1.83 |
| 8. *Rauvolfia serpentina* | 33.33d | 22.22a | 00c | 4.15d | 30.00h | 0.79c | 0.46c | 74.73c | 20.79g | 2.06e | 25 | 22 | 2.58 |
| 9. *Terminalia bellirica* | 30cd | 00c | 10a | 4.10d | 64.00f | 0.30f | 0.18f | 80.06b | 27.72e | 1.51f | 21 | 20 | 1.46 |
| 10. *Terminalia chebula* | 50b | 00c | 10a | 4.11d | 68.00e | .64d | 0.37 de | 80.06b | 29.45c | 2.91e | 21 | 19.5 | 0.9 |

*Different letters indicated significant differences at $p < 0.05$ level as shown by the DMRT.

the soil such as pH (Green *et al*. 1976). Vesicular arbuscular mycorrhiza occurs at greater maximum soil pH values (ca. 6.0) than do ecto or ericoid mycorrhizae (Peat and Fitter 1993). Vyas and Gupta (2014) conclude that soil pH has little direct effect on mycorrhizal population.

**Table 3. Pearson correlation coefficient among soil temperature, moisture, AMF colonization of subsoil of rhizosphere zone of medicinal plants under BCSIR reserve forest.**

| | Colonization | Soil temperature | Soil moisture |
|---|---|---|---|
| Colonization | 1 | 0.372* | 0.400* |
| Soil temp. | | 1 | 0.35 |
| Soil moisture | | | 1 |

Wang *et al.* (1993) had also reported field observations in Britain that percentage colonization was little affected by soil pH ranging from 4.5 to 7.5. In the study, pH ranges from 3.09 to 6.7 (Fig. 2 H) in both layers and differed significantly (Tables 4 and 6) at 0.05% level. Soil moisture plays key role for AMF colonization. Vyas and Gupta (2014) showed that the frequency of genera and species of VA mycorrhizal fungi varied with changes in soil moisture. In the present study AMF positively correlated (Table 3) with soil moisture content (0.05%) in sub soil. David *et al.* (2009) showed that pH and moisture positively varied with mycorrhizal colonization which is consistent with the present observation. Soil K has a stimulatory effect and minimum concen-tration is prerequisite for mycorrhization (Furlan and Bernier 1989).

**Table 4. Pearson correlation coefficient among the edaphic factors as well as AMF colonization of subsoil of rhizosphere zone of the studied medicinal plants in BCSIR reserve forest.**

| | pH | EC | OM | OC | Na | K | Colon. | Vesicl | Arbus. | P |
|---|---|---|---|---|---|---|---|---|---|---|
| Soil pH | 1 | | | | | | | | | |
| Soil EC | –0.55** | 1 | | | | | | | | |
| SOM | –0.29 | 0.109 | 1 | | | | | | | |
| SOC | –0.29 | 0.11 | 0.99** | 1 | | | | | | |
| Na | 0.21 | –0.24 | –0.01 | –0.001 | 1 | | | | | |
| K | –0.65** | 0.33 | 0.65** | 0.64** | –0.10 | 1 | | | | |
| Colonization | 0.42* | –0.33 | –0.28 | –0.26 | 0.31 | –0.62** | 1 | | | |
| Vesicle | –0.01 | –0.35 | –0.01 | –0.01 | 0.02 | –0.36* | 0.12 | 1 | | |
| Arbuscle | 0.005 | 0.09 | –0.18 | –0.16 | 0.35 | –0.15 | 0.60** | –0.26 | 1 | |
| P | –0.28 | 0.09 | 0.20 | 0.20 | –0.58** | 0.35 | –0.16 | –0.20 | –0.41* | 1 |

* and ** indicate correlation significant at 0.05 and 0.01 probability levels, respectively.

K concentration showed significant corelation ($p < 0.01$) with colonization for both top and sub soil. High concentration of Na or high salinity has adverse effects (Juniper and Abbott 1993) or decreases AMF colonization and spore germination and proliferation (Khaliel *et al.* 2011).

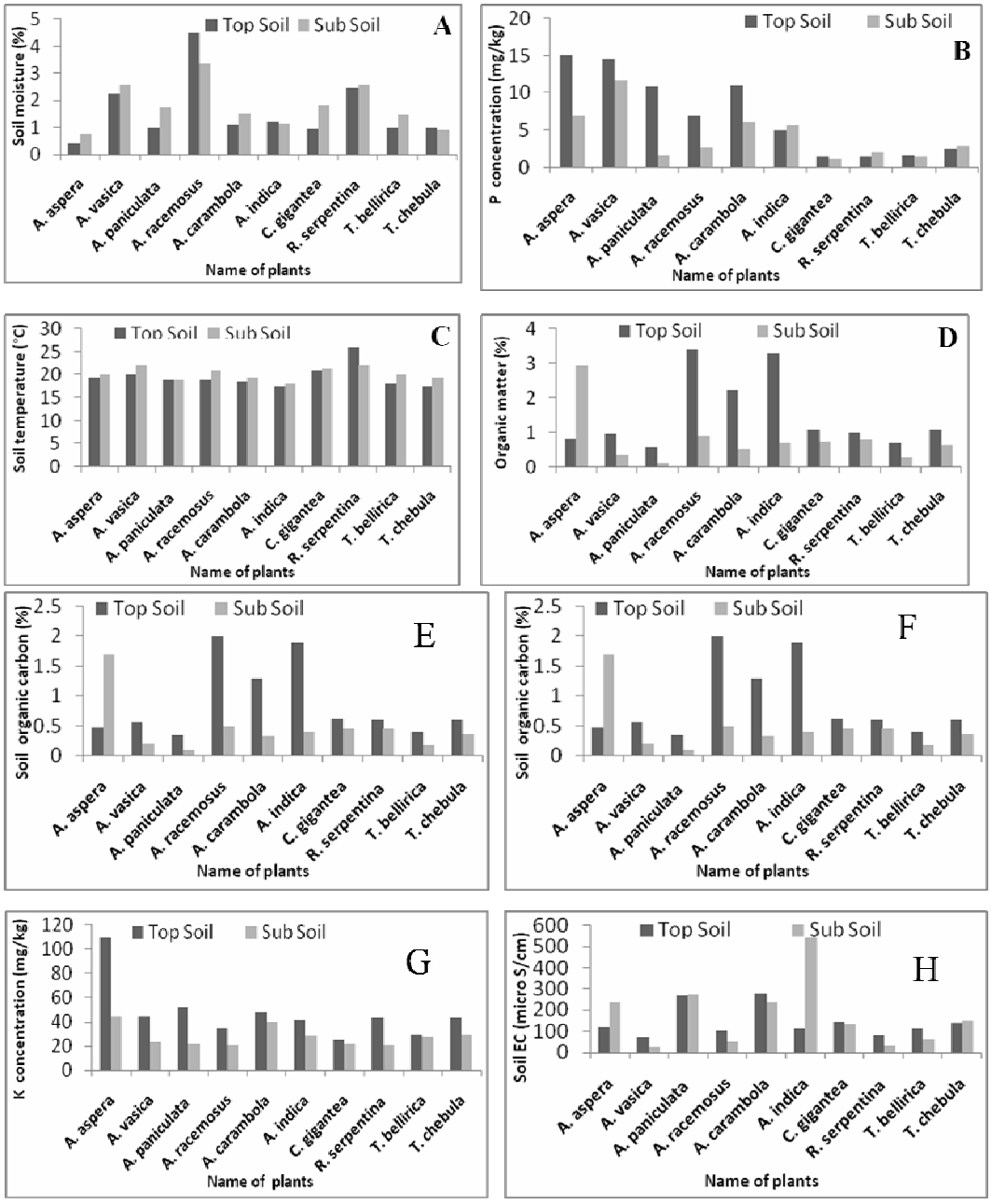


Fig. 2. Top and sub soil properties in rhizosphere zone of studied medicinal plants of BCSIR reserve forest of Chittagong.

**Table 5. Chemical properties of rhizosphere topsoil samples of different medicinal plant species growing in the BCSIR reserve forest, Chittagong.**

| Plant species | Colonization (%) | | | Soil Properties | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mycell-ium | Vesi-cle | Arbu-scle | pH | Ec | OM% | OC% | Na (mg/kg) | K (mg/kg) | P (mg/kg) | Soil tem. | Soil tem. | Soil moisture |
| 1. *Achyranthes aspera* | 0f | 0e | 0 | 6.6b | 118e | 0.8g | 0.5g | 80.1c | 110.1a | 15.1a | 20.5 | 19.5 | 0.42 |
| 2. *Adhatoda vasica* | 78.57c | 0e | 14.3 | 4.9g | 71i | 0.97f | 0.56f | 806 | 44.18d | 14.6b | 22 | 20 | 2.24 |
| 3. *Andrographis paniculata* | 44.44e | 0e | 11.1 | 6.70a | 268b | 0.61i | 0.35i | 90.8a | 51.98b | 10.9c | 22 | 19 | 1.03 |
| 4. *Asparagus racemosus* | 100a | 10d | 10 | 5.43f | 101g | 3.42a | 1.98a | 85b | 34.65e | 6.95d | 22 | 19 | 4.52 |
| 5. *Averrhoa carambola* | 44.44e | 0e | 11.1 | 4.30i | 281a | 2.24c | 1.30c | 80.1c | 47.64c | 11.2c | 22 | 18.5 | 1.09 |
| 6. *Azadirachta indica* | 88.89b | 88.9a | 11.1 | 5.49e | 113f | 3.3b | 1.9b | 90.8a | 41.58d | 5.00e | 22 | 17.5 | 1.2 |
| 7. *Calotropis gigantea* | 100a | 0e | 40 | 5.6d | 147c | 1.1d | 0.6d | 80.1c | 25.12h | 1.60f | 22 | 21 | 0.93 |
| 8. *Rauvolfia serpentina* | 60d | 0e | 20 | 4.6h | 82h | 1.03e | 0.60e | 75d | 43.31d | 1.49f | 25 | 26 | 2.45 |
| 9. *Terminalia bellirica.* | 100a | 56b | 22.2 | 4.14j | 112f | 0.7h | 0.4h | 75d | 29.45f | 1.66f | 21 | 18 | 0.98 |
| 10. *Terminalia chebula* | 100a | 30 c | 40 | 5.85c | 141d | 1.1d | 0.6de | 80.1c | 43.31d | 2.50f | 21 | 17.5 | 0.98 |

*Different letters indicated significant differences at $p < 0.05$ level as shown by the DMRT.

Study sites of both top and sub soil showed salinity as well as Na concentration very low ranging from 542 to 28 µS and 69 to 90 mg/kg respectively (Table 2 and 5 ) due to that there was no effects of soil salinity on mycorrhiza colonization vertically. Cynthia *et al.* (2005) showed that under low-P conditions, encouragement of arbuscular mycorrhizal associations may enhance P uptake by crops early in the growing season. Liu *et al.* (2008) reported that under low P condition, the AM had a higher infection rate and contributed more to the P uptake; while under high P condition, it was in adverse situation which are consistent with the result of the present study.

**Table 6. Pearson correlation coefficients between edaphic factors and mycorrhiza colonization of top soil of studied medicinal plants of BCSIR reserve forest Chittagong.**

| | pH | Ec | OM | OC | Na | K | P | Colonization |
|---|---|---|---|---|---|---|---|---|
| pH | 1 | 0.189 | –0.13 | –0.13 | 0.63** | 0.53** | 0.32 | –0.38* |
| EC | | 1 | –0.05 | –0.05 | 0.40* | 0.04 | 0.29 | –0.31 |
| OM | | | 1 | 1.00** | 0.41* | –0.23 | –0.02 | 0.29 |
| OC | | | | 1 | 0.41* | –0.23 | –0.02 | 0.29 |
| Na | | | | | 1 | 0.08 | 0.35 | –0.08 |
| K | | | | | | 1 | 0.66** | –0.89** |
| P | | | | | | | 1 | –0.70** |
| Colonization | | | | | | | | 1 |

*, ** indicate correlation significant at 0.05 and 0.01 levels, respectively.

Results showed that mycorrhiza colonization was higher in surface soil in comparison to sub soil and AMF colonization significantly co-related to some edaphic factors such as soil pH, K, P, temperature and moisture. The present study thus brings out the fact that arbuscular mycorrhiza is a well established phenomenon in medicinal plants of BCSIR reserve forest Chittagong that exhibits variations depending on not only vertical soil depth but also edaphic factors.

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