

Original Research Article

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Study of Micromycoflora and Fungal Diversity Analysis and Comparison among Three Different Ecosystems (Agricultural Land, Barren and Garden Land) of Khardah Municipality, 24 Parganas (N), West Bengal, India

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ABSTRACT

Soil microflora and their diversity study are now modern trends to know the proper soil health. Greater diversity of fungi in soil indicates better health of soil. In this experiment agricultural soil, barren land and garden soil were taken for their micromycoflora study and the diversity of microfungi were analysed by Simpson index and compared among the three types of soil. Total 563 colonies were isolated from the 38 soil samples collected from three different ecosystem types. Sixty eight (68) different species belonging to 31 genera were recorded. Maximum variation was observed in the agriculture soil, 350 colonies were isolated from 20 samples having 67 species and 31 genera. In all 54 species belonging to 28 genera were isolated from barren land samples. In the Garden soil samples, there were 123 colonies belonging to 30 genera and 66 species. Three genera (*Candida*, *Debaryomyces* and *Rhodotorula*) of yeasts were isolated and identified in three types of soil of this municipality and adjoining area. The study reveals that *T. viride* exists in maximum per cent of occurrence. It is a good indication of that as *T. viride* is natural biocontrol agent against many plant pathogens. *Aspergillus* was dominant in all the three types of soils followed by, *Trichoderma*, *Fusarium*, *Alternaria*, *Penicillium* and *Gloeosporium*. *Aspergillus* has 9 species, *Penicillium* has 6 species, *Curvularia* has 5 species. The diversity Simpson index (0.6139) is found in agriculture soil in comparison to barren (0.9763) and garden (0.950) soil. It means maximum diversity of fungi exists in the agriculture soil. Fungi get maximum and varied nutrient from agriculture soil. So agriculture soil supports maximum diversity of fungi.

Keywords

Soil,
Micromycoflora,
Simpson index,
Fungal diversity.

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Introduction

We are very much dependent on the soil for its needs in agriculture as the soil is the universal media for all kinds of crops and, of late, contributing to our energy supply with crops grown primarily for biofuels. Actually soil is a basic building block for life on earth. Soil is a dynamic and multifunctional system

that exists as thin layer on the Earth's crust. Sanchez and Swaminathan (2005) linked among the hunger, unhealthy people and unhealthy soils in Africa. The various combinations of soil forming factors have given rise to an exceptional diversity of soil types across the world. The kingdom Fungi is

one of the most diverse groups of organisms on Earth, and they are integral ecosystem agents that govern soil carbon cycling, plant nutrition, and pathology. Fungi are widely distributed in all terrestrial ecosystems, but the distribution of species, phyla, and functional groups has been poorly documented. On the basis of 365 global soil samples from natural ecosystems, Tedersoo *et al.*, (2014) determined the main drivers and biogeographic patterns of fungal diversity and community composition.

The properties of soils and associated environmental conditions govern the various ecosystem functions of soil such as decomposition and transformation of organic wastes, mediating nutrient cycles, and influencing populations of soil organisms such as bacteria and fungi. Recently scientists gave the attention on 'fertility' or 'quality' and more recently 'soil health'. The soil fungi occupied a very important position *i.e.*, decomposer of ecosystem. Their vital function with other microbes is the decomposition of organic matter and the release of the nutrients locked up in plants and animals, bringing about the recycling of nutrients.

There are near about 75,000 species of soil fungi are recorded in the world (Finlay 2007), and the soil formulation, soil fertility, and crop protection largely depend on microbial community (Schimel *et al.*, 2005). The diversity of soil fungi depends on the types of soils and organic matters and water holding capacity, pH, temperature and after all rhizosphere of soil.

Continuous urbanization on natural fields causes depletion of diversity of fungi and other microbes of soil and rhizosphere are changing. Biocontrol agents for control of crops from many plant diseases, antibiotic (Penicillin, Griseofulvin, etc) producing fungi

and other industrial important fungi are habited in soil; moreover soil borne plant pathogens exist in soil. With the advent of chemical fertilizers and pesticides /fungicides, it was thought that a permanent and reliable solution of soil fertility and crop pathogens have been achieved but it has been proved that chemical fertilizers and pesticide application are not safe to the environment as the toxicants cause environmental pollution and have harmful effects on human beings (PAN, 2007). Chemical fertilizers may have harmful effects on the soil and its life, especially when they are very concentrated and water soluble (Smith *et al.*, 2008). Ammonium sulphate is a very strong biocide, hindering nitrogen fixation and killing nematodes and earthworms. Superphosphate has a negative effect on free-living nitrogen-fixing bacteria (Primavesi and Primavesi, 1990). Nitrate levels above 10 mg/L (10 ppm) in groundwater can cause 'blue baby syndrome' (acquired methemoglobinemia), leading to hypoxia (which can lead to coma and death if not treated) (Ward *et al.*, 2005; Fowler, 2006). Unfortunately to gain a target crop production with chemical fertilizers and pesticides, over 100 species of non target organisms are adversely affected (Alabouvette and Couteadier, 1992). Despite realization of adverse effects of chemical fertilizers and pesticides on plants, animals and environment, they are being applied indiscriminately (Eckert and Ogawa, 1988; PAN, 2007). So, we are constantly in taking these poisonous chemicals.

Keeping environment pollution and human health hazards in mind, we have tried to evaluate the soil fungal diversity in suburban municipality- Khardah, for indicating changes of soil health. The objective of this study includes to record the soil microflora of agricultural land, barren and garden land and fungal diversity comparison among the tree ecosystems of Khardah Municipality.

Materials and Methods

Study area

Khardah Municipality (Fig. 1) was taken as model study for fungal diversity. This is situated at Barrakpore subdivision of 24-Parganas (N) of West Bengal, India. It is surrounded in West by Holy Ganga (Hooghly River), North by Titagarh Municipality, South by Panihatı Municipality and East by some Panchayat areas and then Barasat Municipalities. It's out skirts all around are agrifields of some vegetables, rice and some barren lands. Within the town ship of municipality there are some play grounds, public gardens, municipality gardens, parks, ponds and other natural's undisturbed fields and some small – large industries are also present.

The 24-Parganas (N) district of West Bengal is situated in the tropical zone and extends from latitude 22°11' 6'' North to 23°15' 2'' North and from longitude 88°20' East to 89°05' East. It is bordered to Nadia by North, to Bangladesh (Kulna Division by North and east), to south 24 -Parganas and Kolkata by south and to Kolkata, Howrah and Hooghly by West. The elevation of the district is 2,134m (7001ft). It lies within the Ganga-Brahmaputra delta.

Annual rainfall is 1579 mm. The weather remains humid and dry during the most time of the year except the rainy seasons. It remains dry during the winter (mid-November to mid-February) and humid during summer. Temperature remains 41⁰C (maximum) in May and 10⁰C (minimum) in January. Relative humidity remains between 50% in March and 90% in July (http://famillpedia.wikia.com/wiki/north_24_pargana_district#climate; State of Forest Report 2003).

Collection of soil sample

Twenty (20) different soil samples were collected from different agricultural field (Brinjal, Tomato, Rice) and 9 samples were collected from nine different barren lands and also 9 soil samples were collected from 9 different garden lands.

Agricultural and barren lands were selected on all the directions along the outskirts of the city of Khardah but the gardens were situated in the city.

Agricultural soils were collected from rhizosphere of selected crops, barren & garden soils from 10-15 inch dept after discarding surface soil.

Preparation of the soil for isolation of fungi and Isolation and identification of fungi and fungal diversity index

Preparation of the soil for isolation of fungi

Collected soils were spread in dark and air dried. The dried soils are powdered and sieved under 1.0 mm mesh.

The powder sieved soil weight to 1 gm and they are used for soil microbes isolation, and they are preserved in 4⁰C temperature for further used also.

Isolation of fungi

Dilution plate method

Isolation of fungi from the soil samples were carried out by dilution plate method (Dhingra and Sinclair 1985; Warcup, 1950) using different synthetic and semi synthetic media.

Generally, isolation was done in potato-dextrose agar (PDA) medium at 28±2 °C following the methodology

recommended by Dhingra and Sinclair (1985). Rose Bengal agar medium (modified from Tsao, 1964) was used for the soil dilution method.

Other media such as potato carrot agar medium, Czapek Dox agar medium (Dox, 1910), and malt extract agar medium were also used.

Identification of fungi

The fungi were identified with the help of literature (Nagamani *et al.*, 2002; Barnett, 1998; Ellis, 1993; Gilman, 2001; Raper and Fennel, 1965; Thom and Raper, 1941; Subramanian, 1971, 2007).

Fungal diversity index

The number of soil sample taken, the number of fungal colony isolated, number of colony of individual fungus were recorded from three types of soils –agriculture, barren and garden. Their fungal diversity indices were calculated by Simpsons Diversity index (D): is a simple mathematical measure that characterizes species diversity in a community. It is calculated using the following formula.

Simpson's index

$$D = 1 - [\sum ni(ni-1) / [N (N-1)]]$$

Where,

n = No. of individuals in each species.

N = Total no. of individuals.

The D assumes value between 0 & 1. D = 0 indicates maximum diversity while

D = 1 represents the least diversity (Harrison *et al.*, 2004, <http://en.wiki.pedia.org/wiki/Diversity-index>)

Isolation, characterization and identification of yeasts

Isolation

Collected soils were spread in dark and air dried. The dried soils are powdered and sieved under sixty mesh. The powder sieved soil weight to 1 gm and they are used for soil microbes isolation, and they are preserved in 4⁰C temperature for further use also. Isolation of yeast from the soil samples were carried out by dilution plate method (Warcup, 1950) using MA medium. The petri dishes were incubated at 25⁰C for 3 days.

Single cell isolate of each type of yeasts was obtained by streaking loopful of cells on MA medium and transferring well isolated colonies to MA. The isolates were maintained on Malt-yeast-Glucose-Peptone –Agar medium of Wickerham *et al.*, (1951) at 28⁰C with monthly subculturing.

Identification

Identification of each isolate of yeast up to species level was carried on the basis of standard morphological, and physiological, biochemical tests presented by Barnett *et al.*, (2000), Kurtzman and Fell (2006), and Rose and Harrison (1987-1993).

Morphological and Microscopical investigation - The colonies were observed and described on MA and MYGPA medium. The isolates were also grown in MA and MYPGA broth for determination of their cultural characteristics (pellicle, sedimentation or ring formation). In certain cases, the isolates were grown on sterile slices of carrot for induction of ascospore formation.

Physiological or biochemical tests- For carbon and nitrogen assimilation, the basal medium of Barnett *et al.*, (2000) was used.

The results were determined after the 3th, 7th, 14th, 21th and 28th day.

The ability of some carbohydrates for anaerobic assimilation (fermentation) was determined by using Durham glass tubes after 3 weeks. The quantity of the tested carbohydrates was 2%.

For Diazonium blue –B (DBB) test, a ten day old culture on MYPG was held at 55^oC for three hours and then flooded with ice- cold DBB reagent. The reagent was prepared by dissolving diazonium blue salt (Sigma) in cold 0.5M-tris-HCL buffer pH 7.0 at 1 mg/ml the reagent was kept ice – cold and used within few minutes of pre parathion. Other additional tests such as starch formation, urea hydrolysis, cycloheximide (0.01% or 0.1%), were performed.

The isolated yeasts were stored by freeze-drying with protecting medium (sucrose 10%, gelatine 1.5% and agar 0.1%).

Results and Discussion

Diversity of soil fungi including yeasts from Khardah Municipality

The data in table 1 showed that total 563 colonies were isolated from the 38 soil samples collected from three different ecosystem types, namely agriculture field, barren land and garden soil. The colonies are distributed in 68 different species belonging to 31 genera. Maximum variation was observed in the agriculture soil, 350 colonies were isolated from 20 samples having 67 species and 31 genera. In all 54 species belonging to 28 genera were isolated from barren land samples. In the Garden soil samples, there were 123 colonies belonging to 30 genera and 66 species. Three genera (*Candida*, *Debaryomyces* and *Rhodotorula*) of yeasts were isolated and identified in three types of soil (Agricultural, barren and garden

soil) of this municipality and adjoining area. Mustaq *et al.*, (2004) similarly studied soil yeasts from Pakistan. The study reveals that *T. viride* exists in maximum per cent of occurrence. It is a good indication of that as *T. viride* is natural biocontrol agents against many plant pathogens (Alabouvette and Couteadier, 1992). The dominant genera in all the ecosystem types were also studied. *Aspergillus* was dominant in all the three types of soils followed by *Trichoderma*, *Fusarium*, *Alternaria*, *Penicillium*, and *Gloeosporium*. *Aspergillus* has 9 species, *Penicillium* has 6 species, *Curvularia* has 5 species. Kumar *et al.*, (2015) found that *Aspergillus* is dominant in different Populated Area of Bareilly City.

Table 2 indicated that least diversity Simpson index (0.6139) is found in agriculture soil in comparison to barren (0.9763) and garden (0.950) soil. It means maximum diversity of fungi exists in the agriculture soil. Fungi get maximum and varied nutrient from agriculture soil. So agriculture soil supports maximum diversity of fungi. The diversity index of both garden and barren soil is near to 1. It indicates diversity of fungi in both garden and barren soil is poor. It may be due to lack of rhizosphere zone. This result was supported by other authors (Wahegaonkar, *et al.*, 2011). They showed the diversity of soil fungi in Aurangabad, M.S. India. They collected 23 soil samples of three eco-systems (agriculture, garden and barren lands) in and around the city of Aurangabad. Total 45 genera distributed in 85 species were isolated and identified. They reported maximum genera of fungi in agriculture soil. Their isolated dominant genera in all soil types were *Aspergillus*, *Alternaria*, *Trichoderma* etc. Similarly Saravanakumar and Kaviyarasan (2010) collected 48 soil sample from wet evergreen forest of Tamilnadu, South India and isolated a total of 76 taxa belonging to 25 genera. They also analysed the fungal diversity index of forest soil.

Table.1 Isolated fungi and their number of colony, % of occurrence in the agriculture, barren land and Garden soil

S. No	Fungal species	Agriculture Soil						Barren land soil		Garden Soil	
		Brinjal		Tomato		Rice		No.of colony	Occurrence	No.of colony	Occurrence
		No.of colony	Occurrence	No.of Colony	Occurrence	No. of colony	Occurrence				
1.	<i>Alternaria alternata</i>	5	4.16	3	2.41	4	3.77	2	2.29	4	2.0
2.	<i>Alternaria tenuissima</i>	3	2.5	3	2.41	2	1.88	2	2.29	3	1.5
3.	<i>Acremonium stromaticum</i>	3	2.5	2	1.61	1	0.94	0	0.00	4	2.0
4.	<i>Aspergillus niger</i>	3	2.5	4	3.22	4	3.77	1	1.14	4	2.0
5.	<i>A. flavus</i>	4	3.33	3	2.41	2	1.88	2	2.29	4	2.0
6.	<i>A. versicolor</i>	1	0.83	2	1.61	0	0.00	2	2.29	3	1.5
7.	<i>A. terreus</i>	2	1.66	3	2.41	2	1.88	0	0.00	3	1.5
8.	<i>A. fumigatus</i>	0	0.00	2	1.61	1	0.94	1	1.14	2	1.0
9.	<i>A. nidulans</i>	0	0.00	2	1.61	0	0.00	0	0.00	1	0.5
10.	<i>A. unguis</i>	1	0.83	0	0.00	0	0.00	2	2.29	3	1.5
11.	<i>A. ustus</i>	0	0.00	0	0.00	1	0.94	0	0.00	3	1.5
12.	<i>A. sydowii</i>	1	0.83	1	0.80	1	0.94	1	1.14	1	0.5
13.	<i>Ascochyta graminicola</i>	2	1.66	1	0.80	2	1.88	1	1.14	2	1.0
14.	<i>A. rabiei</i>	2	1.66	1	0.80	1	0.94	2	2.29	3	1.5
15.	<i>Beauveria bassiana</i>	2	1.66	2	1.61	2	1.88	2	2.29	2	1.0
16.	<i>Cladosporium lunata</i>	1	0.83	2	1.61	1	0.94	1	1.14	5	2.5
17.	<i>C. oxysporum</i>	2	1.66	2	1.61	2	1.88	2	2.29	5	2.5
18.	<i>C. herbarum</i>	1	0.83	1	0.80	2	1.88	0	0.00	1	0.5
19.	<i>C. elatum</i>	1	0.83	2	1.61	3	2.83	2	2.29	2	1.0
20.	<i>C. cladosporoides</i>	2	1.66	2	1.61	2	1.88	3	3.44	2	1.0
21.	<i>Curvularia lunata</i>	3	2.5	2	1.61	1	0.94	1	1.14	4	2.0
22.	<i>C. harveyi</i>	0	0.00	2	1.61	3	2.83	0	0.00	2	1.0
23.	<i>C. trifoli</i>	0	0.00	3	2.41	2	1.88	0	0.00	3	1.5
24.	<i>C. borrieriae</i>	3	2.5	1	0.80	1	0.94	2	2.29	2	1.0
25.	<i>C. verruculosa</i>	2	1.66	3	2.41	1	0.94	0	0.00	0	0.0
26.	<i>Fusarium oxysporium</i>	2	1.66	3	2.41	2	1.88	3	3.44	5	2.5
27.	<i>F. moliniform</i>	2	1.66	2	1.61	1	0.94	1	1.14	4	2.0
28.	<i>F. solani</i>	3	2.5	3	2.41	0	0.00	1	1.14	2	1.0

29.	<i>F. dimerum</i>	0	0.00	1	0.80	1	0.94	1	1.14	1	0.5
30.	<i>Fusarium Sp.</i>	2	1.66	2	1.61	1	0.94	1	1.14	5	2.5
31.	<i>Gigaspora giganteum</i>	2	1.66	0	0.00	1	0.94	1	1.14	3	1.5
32.	<i>Gigaspora remisporophora</i>	1	0.00	4	3.22	1	0.94	3	3.44	2	1.0
33.	<i>Glomus mosseae</i>	2	1.66	2	1.61	3	2.83	0	0.00	4	2.0
34.	<i>G. ambisporum</i>	1	0.00	2	1.61	4	3.77	0	0.00	2	1.0
35.	<i>G.formosanum</i>	3	2.5	1	0.80	2	1.88	1	1.14	2	1.0
36.	<i>Gleosporium</i>	1	0.00	0	0.00	1	0.94	2	2.29	5	2.5
37.	<i>Humicola grisea</i>	2	1.66	1	0.80	3	2.83	1	1.14	2	1.0
38.	<i>Unidentified</i>	0	0.00	3	2.41	1	0.94	0	0.00	4	2.0
39.	<i>Lasidiobolus heobramae</i>	2	1.66	1	0.80	2	1.88	1	1.14	2	1.0
40.	<i>Mucor sp.</i>	1	0.00	2	1.61	3	2.83	1	1.14	3	1.5
41.	<i>M. mucido</i>	1	0.00	2	1.61	2	1.88	2	2.29	3	1.5
42.	<i>M. hiemallis</i>	1	0.00	2	1.61	1	0.94	1	1.14	1	0.5
43.	<i>Macrophomina phaseolina</i>	1	0.00	2	1.61	1	0.94	2	2.29	4	2.0
44.	<i>Metarhizium anisopliae</i>	2	1.66	1	0.80	0	0.00	2	2.29	4	2.0
45.	<i>Nigrospora oryzae</i>	2	1.66	2	1.61	4	3.77	3	3.44	2	1.0
46.	<i>Neocosmospora sp.</i>	1	0.00	4	3.22	0	0.00	1	1.14	3	1.5
47.	<i>Penicillium rubrum</i>	1	0.00	2	1.61	3	2.83	2	2.29	5	2.5
48.	<i>P. tardum</i>	1	0.00	1	0.80	0	0.00	1	1.14	2	1.0
49.	<i>P. citrinum</i>	0	0.00	2	1.61	1	0.94	2	2.29	4	2.0
50.	<i>P. purprogenum</i>	1	0.00	0	0.00	0	0.00	0	0.00	1	0.5
51.	<i>P. puberrulum</i>	1	0.00	1	0.80	1	0.94	1	1.14	2	1.0
52.	<i>Phoma nebulosa</i>	2	1.66	1	0.80	2	1.88	1	1.14	4	2.0
53.	<i>P. humicola</i>	1	0.00	2	1.61	1	0.94	1	1.14	4	2.0
54.	<i>P. terricola</i>	3	2.5	1	0.80	1	0.94	0	0.00	3	1.5
55.	<i>Phycomyses Sp.</i>	2	1.66	1	0.80	1	0.94	2	2.29	2	1.0
56.	<i>Rhizopus oryzae</i>	2	1.66	1	0.80	1	0.94	1	1.14	2	1.0
57.	<i>R. arrhizus</i>	2	1.66	1	0.80	0	0.00	1	1.14	1	0.5
58.	<i>Sclerotium oryzae</i>	1	0.00	1	0.80	1	0.94	2	2.29	2	1.0
59.	<i>Unidentified (yeast)</i>	2	1.66	0	0.00	2	1.88	1	1.14	4	2.0

60.	<i>Scutellospora pellucid</i>	1	0.00	2	1.61	0	0.94	1	1.14	3	1.5
61.	<i>S. gregarea</i>	2	1.66	2	1.61	1	0.94	1	1.14	2	1.0
62.	<i>Sphaeronema sp.</i>	1	0.00	1	0.80	1	0.94	2	2.29	3	1.5
63.	<i>Trichoderma viride</i>	8	6.66	5	4.03	6	5.66	3	3.44	4	2.0
64.	<i>T. harzianum</i>	3	2.5	4	3.22	4	3.77	2	2.29	5	2.5
65.	<i>T. lignorum</i>	2	1.66	1	0.80	2	1.88	0	0.00	4	2.0
66.	<i>Verticillium Sp.</i>	1	0.00	2	1.61	0	0.00	2	2.29	2	1.0
67.	<i>Candida famata</i>	3	2.5	1	0.80	0	0.00	2	2.29	2	1.0
68.	<i>Rhodotorula Sp.</i>	2	1.66	2	1.61	2	1.88	0	0.00	3	1.5
69.	<i>Debaryomyces Sp.</i>	3	2.5	0	0.00	1	0.94	3	3.44	5	2.5
Total colony		121		123		106		87		126	

Table.2 Diversity analysis of microfungi including yeasts in three different ecosystems -Agriculture, Barren and Garden land and their comparison

Soil type(Ecosystem type)	No. of Samples collected	Total no. of colonies isolated	Average No. of colonies isolated	No. of identified genera	No. of identified Species	Simpson's Diversity index 'D'
Agriculture land	20	350	17.5	31	67	0.6139
Barren land	08	87	10.87	28	54	0.9763
Garden land	10	126	12.6	30	66	0.950
Total	38	563	40.97	89	187	----

Fig.1 Map of the Khardah Municipality



Although here other factors of soil such as the soil pH, organic content and water are not taken, but these factors affecting the fungal population and diversity (Yu *et al.*, 2007). Isolation and Identification of soil mycoflora from different Populated Area of Bareilly City were done by Kumar, *et al.*, (2015). During the investigation period 161 fungal colonies of 8 fungal species were observed (Kumar, *et al.*, 2015). They recorded that the maximum fungal species belongs to Ascomycotina and Zygomycotina were observed. In their findings, *Aspergillus niger* (13.04%), *Aspergillus flavus* (13.04%), *Aspergillus fumigates* (13.6%), *Aspergillus terreus* (10.5%), *Aspergillus fumigates* (13.6%), *Aspergillus niger* (13.04%), *Aspergillus flavus* (13.04%), *Aspergillus terreus* (10.5%) were the dominant species, and other few of them were *Aspergillus nidulance* (9.3%), *Botrytis cineria* (5.59%), *Rhizopus oryzae* (8.07%), and *Trichoderma viride* (8.07%) were found in their respective percentage. In this experiment the diversity of soil fungi have same trends. The diversity of yeasts in different natural resources including fruits in 24-Parganas, West Bengal were analysed by us (Ghosh *et al.* 2013, 2015). Moreover, we (Pal and Ghosh 2015) have recorded the diversity of fungi in agricultural fields and gardens in North 24 Parganas, West Bengal. Out of thirty-one fungal species isolated with 19 found in agricultural soil and 28 in garden soil. In agricultural soil, the dominant fungi were *Aspergillus niger*, *Rhizopus oryzae*, and *Penicillium expansum*, and the dominant fungi of garden soil were *A. niger* and *Fusarium moniliforme*. Simpson's diversity index indicated that garden soil had more fungal diversity than agricultural soil (Pal and Ghosh 2015). But in this experiment diversity of fungi in agricultural soil is higher than garden soil; it may indicate that farmers of Khardah and adjoining applied less fungicide in comparison to agrisoil of the whole district of 24-Parganas (N) keeping the soil health good.

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