

Available online at [GSC Online Press Directory](https://www.gsonlinepress.com/)

GSC Biological and Pharmaceutical Sciences

e-ISSN: 2581-3250, CODEN (USA): GBPSC2

Journal homepage: <https://www.gsonlinepress.com/journals/gscbps>

(RESEARCH ARTICLE)



Influence of microbial bioinoculants on the accumulation of new phytochemicals in *Oroxylum indicum* (L.) Benth. ex Kurz

Chandrima Debi ^{1*} and Vipin Parkash ²¹ Research fellow, Forest Research Institute, Dehradun, India.² Scientist E, Forest Research Institute, Dehradun, India.

Publication history: Received on 16 December 2020; revised on 23 December 2020; accepted on 25 December 2020

Article DOI: <https://doi.org/10.30574/gscbps.2020.13.3.0413>

Abstract

The seedlings of *Oroxylum indicum* were inoculated with plant growth promoting microbes (PGPMs) mainly, *Glomus mosseae*, *Trichoderma harzianum* and *Pseudomonas putida* both alone and consortium. The GCMS analysis of the methanolic root extract of inoculated seedlings of *O. indicum* showed that seedlings treated with mixed consortium of mycorrhizal fungi, bacteria and fungus showed the presence of maximum number of phytochemicals. The GC-MS analysis of control seedlings showed presence of 55 compounds where three new compounds were found i.e. 2-Cyclobutene-1-Carboxamide; Tetradecanoic Acid, 10, 13-dimethyl-, methyl ester; 1-methylene-2b-hydroxymethyl-3, 3-dimethyl-4b-(3-methylbut-2-enyl)-cy. 53 compounds were found in seedlings treated with mycorrhizae i.e., *Glomus mosseae*, and three new compounds were found i.e., 1-Ethyl-2-Hydroxymethylimidazole; Octadecanoic Acid, 11-Methyl-, methyl ester; 4-Methyl-1, 4-Heptadiene. The seedlings treated with bacteria i.e. *Pseudomonas putida* showed the presence of 52 compounds and three new compounds were found i.e. Meso-4, 5-octanediol; 1-ethyl-2-hydroxymethylimidazole; 2, 5-cyclohexadiene-1, 4-dione, 2, 5-dihydroxy-3-methyl-6-(1-methylethyl) - . A total of 56 compounds were present in seedlings treated with fungus i.e. *Trichoderma harzianum* and five new compounds were found i.e. 2-Cyclohexen-1-one, 2-Butyl-3-Methoxy; Methyl 12, 13-Tetradecadienoate; Methyl 6, 9, 12-hexadecatrienoate; 1, 9-Decadiyne; 1, 4-Naphthalenedione. The seedlings treated with dual consortium of mycorrhizae and bacteria showed the presence of 88 compounds and five new compounds were found i.e., N-(1-Methoxycarbonyl-1-methylethyl)-4-methyl-2-aza-1,3-dioxane; 1-ethyl-2 hydroxy methylimidazole; Methyl 8-methyl-nonanoate; Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl; Methyl 12,13-tetradecadienoate. 152 compounds were present in seedlings treated with dual consortium of mycorrhizal fungi and fungus and ten new compounds were found to be present i.e. 1,9-Decadiyne; 3,7,11-Trimethyl-3-hydroxy-6,10-dodecadien-1-yl acetate; 3-Heptyne, 7-chloro; 3-Methyl-4-(methoxycarbonyl) hexa-2,4-dienoic acid; Benzo[c]cinnolin-2-amine ; Tetradecanoic acid, 10,13-dimethyl-, Methyl ester; Cis,cis-4,6-octadienol; 2-Cyclohexen-1-one, 2-butyl-3-methoxy; Methyl 12,13-tetradecadienoate; 2-Aminopyridazino(6,1-b) quinazolin-10-one. A total of 36 compounds were present in seedlings treated with dual consortium of bacteria and fungi and two new compounds were found i.e. [1,4] Dioxino [2,3-b]-1,4-dioxin, hexahydro-2,3,6,7 ; 1-Ethyl-2-hydroxymethylimidazole. The seedlings inoculated with mixed consortium of mycorrhizae, bacteria and fungus showed the presence of 213 compounds and fourteen new compounds were found i.e. 3,7,11-Tridecatrienitrile, 4,8,12-Trimethyl; 1,9-Decadiyne; 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-Hexamethyl-, (ALL-E) ; 1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cy; 1,9-Decadiyne, Cyclobutane, 1,2-bis(1-methylethenyl)-, trans-, 3,7,11-Trimethyl-3-hydroxy-6,10-dodecadien-1-yl acetate, 5-Hydroxy-4-hydroxymethyl-1-(1-hydroxy-1-isopropyl)cyclohex-3-ene, 5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-z)-, 1-Cyclohexyl-2-buten-1-ol (c,t), 1-Oxetan-2-one, 4,4-diethyl-3-methylene-, Tetradecanoic acid, 10,13-dimethyl-, methyl ester, 2-Cyclohexen-1-one, 2-butyl-3-methoxy-, Methyl 12,13-tetradecadienoate, Heptacosanoic acid, 25-methyl-, methyl ester Hexadecanoic Acid, Methyl Ester; 2-Chloroethyl Linoleate; 9,12-Octadecadienoic Acid, Methyl Ester, (E,E); Butanoic

* Corresponding author: Dr. Chandrima Debi
Forest Research Institute, Dehradun P.O. New Forest- Dehradun (India)

acid, methyl ester; 4A,5,6,7,8,8A(4H) HexahydroBenzopyran-3-Carboxamide, 8A-Methoxy-4A-M;; Octadecanoic acid; Farnesene; Squalene; Myrcene; Naphthalene; Tetradecanoic Acid, Methyl Ester; Octadecanoic Acid, Methyl Ester; 1H-Cycloprop[E] Azulene, Decahydro-1,1,4,7-Tetramethyl-, [1AR-(1A).Alph ; Cyclohexane, 1-methyl-4-(1-methylethenyl)-, trans (Elemene); Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (s)- (Limonene); were found to be present in this treatment.

Keywords: *Oroxylum indicum*; *Glomus mosseae*; *Trichoderma harzianum*; *Pseudomonas putida*; phytochemicals; GCMS

1. Introduction

Plant growth is influenced by the presence of bacteria and fungi and their interactions are common in the rhizosphere of plants with high relative densities of microbes [1]. Rhizosphere interactions are not solely driven by roots but are highly integrated and influenced by residing organisms and local edaphic factors. Microbial populations react to the exudates released by plant roots making the rhizosphere interactions very dynamic which are altered by addition or loss of any microbe [2]. A strong interaction prevails between the group of microorganisms colonizing the rhizosphere region and plant roots. Microorganisms and their products also affect the roots in a variety of positive, negative and neutral ways [3]. The rhizosphere is therefore, a dynamic, system in which interaction and communication between the root and microorganisms play an important role in maintaining plant growth and productivity. The rhizosphere management may represent significant field for biotechnology improvement resulting in enhancement of the basic yield and biomass production with the application of minimum input of water, fertilizers and agrochemicals. This can be achieved by inoculating rhizosphere with selected beneficial microorganism or by engineering plants to modify the nature and level of exudate compounds. Diverse microorganisms are found in the rhizosphere which can produce substances that regulate plant growth and development and further contributing to plant immunity by producing elicitor molecules to counter these attacks, with the help of large set of defense responses [4].

Mycorrhiza is a symbiotic or mutualistic association between roots of about 90% of the vascular species of plants, including angiosperms, gymnosperms, pteridophytes and bryophytes [5] [6]. The arbuscular mycorrhizal fungi play a significant role in insuring the health of plantlets [7]. Moreover, the acclimatization period of micropropagated plants can be shortened by application of arbuscular mycorrhizal fungi [8]. Arbuscular mycorrhizal fungi is a symbiotic association essential for one or both partners, between a fungus (specialized for life in soils and plants) and a root (or other substrate-contacting organ) of a living plant, that is primarily responsible for nutrient transfer. In such associations, both the partners share mutual benefits. The importance of VA (vesicular arbuscular) mycorrhiza is that they have positive effect on plant nutrition, especially the immobile elements such as phosphorus. The external hyphae greatly increase the volume of soil and translocate the phosphorus to the roots. The transfer of polyphosphate occurs in presence of acid phosphatase during the life span on or senescence of arbuscule. In addition to stimulation of phosphorus uptake, mycorrhizal fungi stimulate rooting, growth, and survival of plants [9]. Moreover, the VA mycorrhizae also stimulates uptake of zinc, copper, sulfur, and potassium by the plant; enhances nodulation in legumes; decreases rots caused by fungal pathogen and root penetration and larval development of nematodes.

Plant growth-promoting bacteria (PGPB) occupy the rhizosphere of many plant species and have beneficial effects on the host plant. They may influence the plant in a direct or indirect manner. A direct mechanism increases plant growth by supplying the plant with nutrients and hormones. The release of carbon compounds from plants into the rhizosphere increases microbial biomass and activity. *Pseudomonas* sp. comprises a genus of ubiquitous Gram-negative bacteria that can live in several environmental niches in the rhizosphere. Although, a few *Pseudomonas* spp. are studied for their role as plant pathogens i.e., *Pseudomonas syringae* but there are many species such as *P. fluorescens*, *P. putida*, *P. aeaureofasciens* and *P. chloraphis*, which may act as plant beneficial bacteria by antagonizing plant pathogens and through the production of traits that directly influence plant disease resistance and growth (Venturi, 2006). Plant Growth Promoting Rhizobacteria (PGPR≈PGPB) are natural rhizosphere-inhabiting bacteria, which belong to diverse genera such as *Pseudomonas* and *Bacillus* species. These microorganisms have been isolated from a wide variety of wild and cultivated plant species such as *Arabidopsis* sp., barley, rice, canola, and bean [10]. Their contribution can be exerted through different mechanisms including root system architecture modulation and increased shoot growth by production of phytohormones such as auxins and cytokinin.

Fungi are usually more operational in spreading through the soil and rhizosphere therefore they have advantage over bacterial inoculants. The mechanism involved in plant growth promotion by fungi includes competition with fungal pathogens, antibiotic production and advanced defense responses. The rhizosphere is a narrow region of soil that is directly influenced by root secretions and associated microbial activity [11]. *Trichoderma* species belong to a class of free-living fungi beneficial to plants that are common in the rhizosphere. In addition to their mycoparasitic capabilities, many *Trichoderma* strains can colonize and grow in association with plant roots and significantly increase plant growth

and development. Colonization by *Trichoderma* sp. very rarely is detrimental to the plant or results in a pathogenic interaction [12]. In contrast, root colonization by *Trichoderma* sp. frequently is associated with induction of both local and systemic resistance, which depend on the production of a protein elicitor by the fungus designated Sm1 (small protein 1). Sm1 lacks toxic activity to plants and microbes. Instead, native, purified Sm1, triggers production of reactive oxygen species in rice and cotton seedlings and induces the expression of defense-related genes both locally and systemically [13]. The beneficial effects of *Trichoderma* sp. on plant growth and development may also depend on more direct mechanisms as a recent report has shown that certain species including *T. virens* and *T. viride* can produce indole-3-acetic acid (IAA) and other auxin-related compounds. In *Arabidopsis* sp., normal auxin perception is a prerequisite for growth enhancement when inoculated with *T. virens* [14].

Bioinoculants are artificially multiplied cultures of certain soil microorganisms that can improve soil fertility and productivity of the plant species. Bioinoculants have great potential to optimize productivity in sustainable manner. Inoculation of plants with bioagents can improve biomass production and can result in multiple effects which result in enhanced plant vigor, plant height, early bloom, and chlorophyll content, simultaneously alkaloid and flavonoid content of the plant species. Bioinoculants that can cater the different needs of growing plants acts as consortium along with the other microorganisms of the rhizosphere. Understanding the interactions between the consortium of microbial inoculants and plant systems will pave way to harness more benefits from the microbial inoculants for plant growth [15]. AMF inoculation can be a simple and useful method for obtaining higher content of phenolics, tannins and phenolic composition and have consequently increased antioxidant activity in *Valeriana jatamansi* Jones [16]. In India little information is available on the role of the bioinoculants on the tree species. Studies related to role of bioinoculants in increasing in growth, flavor content and yield, effect of bioinoculation on rice varieties of India, role of arbuscular mycorrhizal fungi as multibioinoculants in cotton plant growth, response of bioinoculants on growth, yield and fiber quality of cotton under irrigation, effect of bioinoculants on biomass productivity in agroforestry systems etc. are already done. Bioinoculation studies has been mainly carried on various aspects including the influence of bioinoculants on growth and mycorrhizal occurrence in the rhizosphere, plant growth stage, fertilizer management and bioinoculation of arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria. But there is no any reports on the influence of bioinoculation on the accumulation of phytochemicals on the target plant species i.e. *Oroxylum indicum* (L.) Benth. ex Kurz. Hence, the present study has been undertaken. Natural products including medicinal plants have a great significance due to their wide range of therapeutic potential to treat many ailments, so it becomes necessary to enhance their biomass production and their quality in order to fulfil the need of society.

Oroxylum indicum (L.) Benth. ex Kurz vernacularly known as 'Shyonaka' or 'Sonpatha', 'Bhatghila' in Assamese, is a medicinally important deciduous forest tree species belonging to the family Bignoniaceae. Also known as Tree of Damocles, this tree has long, flattened sword shaped pods containing layered flaky papery seeds with butterfly like wings [17]. The plant is used in many Ayurvedic preparations like, Shyonakapatpak and Bruhatpanchamulayadikwath, Dashmula and Chyawanprash, Rasayana, Amratarista, Dantyarista, Dhanwantara, Ghrita, Narayana Taila etc. [18]. The root and stem bark are also used in the treatment of diarrhoea, dysentery, erythema, gastralgia, hoarseness, infantile, measles, sore throat, urticaria, snakebite and scorpion-sting. Root bark is diaphoretic and used in rheumatism. Root bark also has antioxidant properties [19]. Dichloromethane extract of stem bark and root possesses antimicrobial, antifungal, anti-inflammatory and anti-cancerous properties [20] [21].

O. indicum is already reported to contain phytochemicals of high medicinal value. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, steroid, terpenoid [22] [23]. Phytochemicals are the natural bioactive compounds found in plants. These phytochemicals work with nutrients and fibers to form an integrated part of defense system against various diseases and stress conditions [24]. The seedlings were inoculated with Plant Growth Promoting Microbes (PGPMs) [25] were further analyzed for the qualitative as well as quantitative assessment of accumulated phytochemicals [26]. The present study shows the presence of new phytochemicals in the methanolic root extract of the bioinoculated seedlings of *O. indicum* through Gas chromatography mass spectrometry (GC-MS).

Gas chromatography mass spectrometry (GC-MS) is a key technological platform for secondary metabolite profiling in both plant and non-plant species [27] [28] [29]. The literature review revealed that ample studies has been done mainly on the screening of phytochemicals and antimicrobial activity of *O. indicum* from the extracts of bark, stem, seeds or roots on the target plant species but there is no report on the GCMS analysis of bioinoculated seedlings of *O. indicum*. The present study demonstrated the presence of many new phytochemicals in the methanolic root extracts of *O. indicum*. A detailed literature review on the plant in investigation has shown that so far there are no published reports worldwide, related to the possible chemical components of '*Oroxylum indicum*' due to bioinoculation. So, the present study was aimed to investigate the possible chemical components in the methanolic extract by subjecting it to GC-MS analysis.

2. Material and methods

An experiment was set up in the nursery of Rain Forest Research Institute, Jorhat to study the inoculation effect and the GCMS analysis was done in Guwahati Biotech Park of IIT, Guwahati. For this purpose, seeds from different seed sources were analyzed or seed germination ability [30]. The seedlings were raised and different treatments like single and combined/ synergistic/influential of plant growth promoting microbes (PGPMs) mainly, *Pseudomonas putida*, and *Trichoderma harzianum* *Glomus mosseae* were applied for the present investigation [31]. In control sets, no bioinoculant (inoculum) was added. The seedlings treated with bacteria (TB), fungus (TF) and mycorrhizae (TM), dual consortium of bacteria and fungus (TBF), bacteria and mycorrhizae (TBM), mycorrhizae and fungus (TMF), and mixed consortium of mycorrhizae, bacteria, and fungus (TMBF) were harvested after 270 days of inoculation.

2.1. Qualitative and quantitative analysis of phytochemicals present in *O. indicum*

The qualitative analysis of root extract was done to detect the presence of carbohydrates, protein, saponins, tannins, alkaloids, phenols, flavonoids, terpenoids and glycosides. The quantitative analysis of the root extracts was also performed [26].

2.1.1. Sample Preparation for GC-MS Analysis

About 5g of powdered material of plant was taken in a clean, flat-bottomed glass container and soaked in 25ml of 80% methanol. The container with its content was sealed and kept for a period of seven days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through Whatman filter paper. The filtrate (methanolic extract) obtained for the plant was evaporated under ceiling fan and in a water bath until dried.

2.2. Gas Chromatography and Mass Spectrometry (GC-MS) analysis of the plant samples

The GCMS analysis was carried out using a Clarus 500 Perkin- Elmer (Auto System XL). Gas chromatograph was equipped and coupled to a mass detector, Turbo mass gold – Perkin Elmer Turbo mass 5.2 spectrometer with an Elite - 5MS (5% Diphenyl /95% Dimethyl polysiloxane), 60.0m× 250µm. The instrument was set to an initial temperature of 1100 C for 3 min. The oven temperature was increased upto 2800C, at the rate of 50C/min increase and it was maintained for 10 minutes. Injection port temperature was confirmed as 2000C and Helium flow rate as 1.0 ml/min. The ionization voltage was maintained as 70 eV. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 45-600(m/z). The analysis of the data was done with the help of NIST library (MS data center).

3. Results and discussion

The preliminary phytochemical analysis of the root extract of the seedlings of *Oroxylum indicum* revealed the presence of carbohydrates, protein, saponins, Tannins, alkaloids, phenols, flavonoids, terpenoids, glycosides. Further quantitative analysis of the phytochemicals was also done [26].

The GC-MS chromatogram of the methanolic extract of *Oroxylum indicum* showed major peaks which were identified after comparison of the mass spectra with NIST library. These compounds were identified through mass spectrometry attached with GC. The present study shows the presence of many new compounds in the bioinoculated seedlings of *O. indicum* both alone and mixed consortia. The results showed that maximum number of compounds were present in seedlings treated with mixed consortium of mycorrhiza, bacteria, and fungus (T_{M+B+F}) (Table 1). These compounds were also previously reported to be present in *O. indicum*. It was found that minimum compounds were present in Control treatment while maximum compounds were present in seedling treated with mixed consortium i.e. T_{M+B+F} . 2-Furancarboxaldehyde, 5-(Hydroxymethyl); Hexadecanoic Acid, Methyl Ester (Palmitic acid); Tetradecanoic Acid, Methyl Ester were found to be present in control seedlings. The seedlings treated with mycorrhizae (T_M) showed the presence of three compounds mainly, 2-Furancarboxaldehyde, 5-(Hydroxymethyl)-, Hexadecanoic Acid, Methyl Ester (Palmitic acid), Tetradecanoic Acid, Methyl Ester. The seedlings treated with bacteria (T_B) showed the presence of 2-Furancarboxaldehyde, 5- (Hydroxymethyl)-. In seedlings treated with fungus (T_F) 4A, 5, 6, 7, 8, 8A (4H) HexahydroBenzopyran-3-Carboxamide, 8A-Methoxy4A-M and Naphthalene were present. The seedlings treated with dual consortium of mycorrhizae and bacteria (T_{M+B}) showed the presence of 5 compounds mainly, 2 -Furan carboxaldehyde, 5-(Hydroxymethyl) ; Hexadecanoic Acid, Methyl Ester (Palmitic acid); Tetradecanoic Acid, Methyl Ester; Butanoic acid, methyl ester and Naphthalene. Five compounds were present in seedlings treated with dual consortium of mycorrhizae and fungus (T_{M+F}), mainly, Hexadecanoic Acid, Methyl Ester (Palmitic acid); 2- Chloroethyl Linoleate; 4A,5,6,7,8,8A(4H) HexahydroBenzopyran-3-Carboxamide, 8A-Methoxy4A-M; Naphthalene; 1H-Cycloprop[E]Azulene, Decahydro-1,1,4,7- Tetramethyl-, [1AR-(1A. Alph. Two compounds were found in seedlings

treated with dual consortium of bacteria and fungi (T_{B+F}) i.e. 2-Furancarboxaldehyde, 5-(Hydroxymethyl)-, Hexadecanoic Acid, Methyl Ester (Palmitic acid). The seedlings inoculated with mixed consortium of mycorrhizae, bacteria and fungus (T_{M+B+F}) showed the presence of 13 compounds, mainly, Hexadecanoic Acid, Methyl Ester

Table 1 Compounds present in *Oroxylum indicum* along various treatments

Sl. No.	Compounds	Molecular formula	Molecular weight (g/mol)	Treatments								
				T _c	T _M	T _B	T _F	T _{M+B}	T _{M+F}	T _{B+F}	T _{M+B+F}	
1.	2-Furancarboxaldehyde, 5-(Hydroxymethyl)-	C ₆ H ₆ O ₃	126	+	+	+		+			+	
2.	2,4-Dihydroxy-2,5-Dimethyl-3(2H)-Furan-3-one	C ₆ H ₈ O ₄	144.12					+			+	
3.	Hexadecanoic Acid, Methyl Ester (Palmitic acid)	C ₁₇ H ₃₄ O ₂	270.5	+	+			+		+		+
4.	2-Chloroethyl Linoleate	C ₂₀ H ₃₅ ClO ₂	342.9							+		+
5.	9,12-Octadecadienoic Acid, Methyl Ester, (E,E) (Linoleic acid)	C ₁₉ H ₃₄ O ₂	294.47									+
6.	Butanoic acid, methyl ester	C ₅ H ₁₀ O ₂	102.13					+				+
7.	4A,5,6,7,8,8A(4H) HexahydroBenzopyran-3-Carboxamide, 8A-Methoxy-4A-M	--	--				+			+		+
8.	Farnesene	C ₁₅ H ₂₄	204.35									+
9.	Squalene	C ₃₀ H ₅₀	410.7									+
10.	Myrcene	C ₁₀ H ₁₆	136.23									+
11.	Naphthalene	C ₁₀ H ₈	128.17				+	+		+		
12.	Tetradecanoic Acid, Methyl Ester	C ₁₅ H ₃₀ O ₂	242.39	+	+							+
13.	Octadecanoic Acid, Methyl Ester	C ₁₉ H ₃₈ O ₂	298.50									+
14.	1H-Cycloprop[E]Azulene, Decahydro-1,1,4,7-Tetramethyl-, [1AR-(1A.Alph	C ₁₅ H ₂₆ O	222.36							+		+
15.	Cyclohexane, 1-methyl-4-(1-methylethenyl)-, trans (Elemene)	C ₁₀ H ₁₈	138.25									+
16.	Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (s)- (Limonene)	C ₁₀ H ₁₆	136.23									+

+ denotes the presence of compound along treatment

(Palmitic acid), 2-Chloroethyl Linoleate, 9,12- Octadecadienoic Acid, Methyl Ester, (E,E) (Linoleic Acid), Butanoic acid, methyl ester, 4A,5,6,7,8,8A(4H)HexahydroBenzopyran-3-Carboxamide 8A-Methoxy-4A-M (derivative of baicalein), Farnesene, Squalene, Myrcene, Tetradecanoic Acid, Methyl Ester, Octadecanoic Acid, Methyl Ester, 1H-

Cycloprop[E]Azulene, Decahydro-1,1,4,7-Tetramethyl-, [1AR- (1A.Alph , Cyclohexane, 1-methyl-4-(1-methylethenyl)-, trans (Elemene), Cyclohexene, 1- methyl-4-(1-methylenyl)-, (s)-(Limonene)

Besides the above-mentioned compounds many new compounds were found to be present in the methanolic root extracts of the bioinoculated seedlings of *O. indicum* during GCMS analysis. The GC-MS analysis showed the presence of 55 compounds in the methanolic root extract of the control seedling. Three new compounds (Table 2) were found during the analysis namely, 2-cyclobutene-1-carboxamide at RT 13.27, Tetradecanoic acid, 10,13-dimethyl-, methyl ester at RT 21.15, 1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cy at RT 23.09 (Fig.1).

Table 2 GCMS analysis of seedlings of control treatment of *O. indicum*

S.No.	Compound name	Molecular formula	Molecular weight	RT	Peak area %
1.	2-cyclobutene-1-carboxamide	C ₅ H ₇ NO	97	13.27	14.38
2.	Tetradecanoic acid, 10,13-dimethyl-, methyl ester	C ₁₇ H ₃₄ O ₂	270	21.15	0.609
3.	1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cy	C ₁₅ H ₂₆ O	222	23.09	8.056

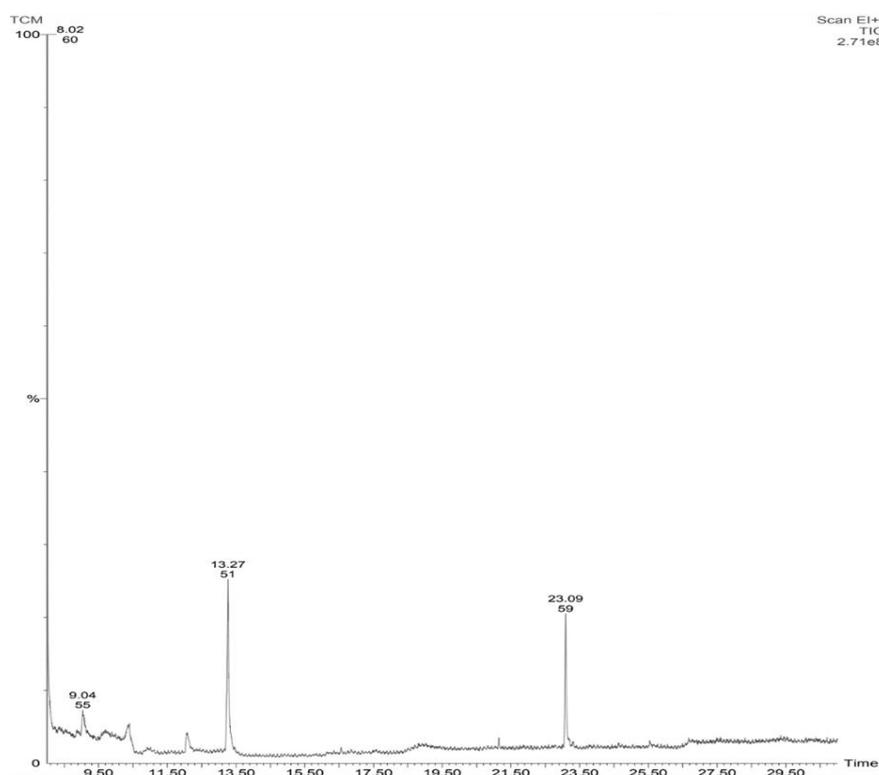
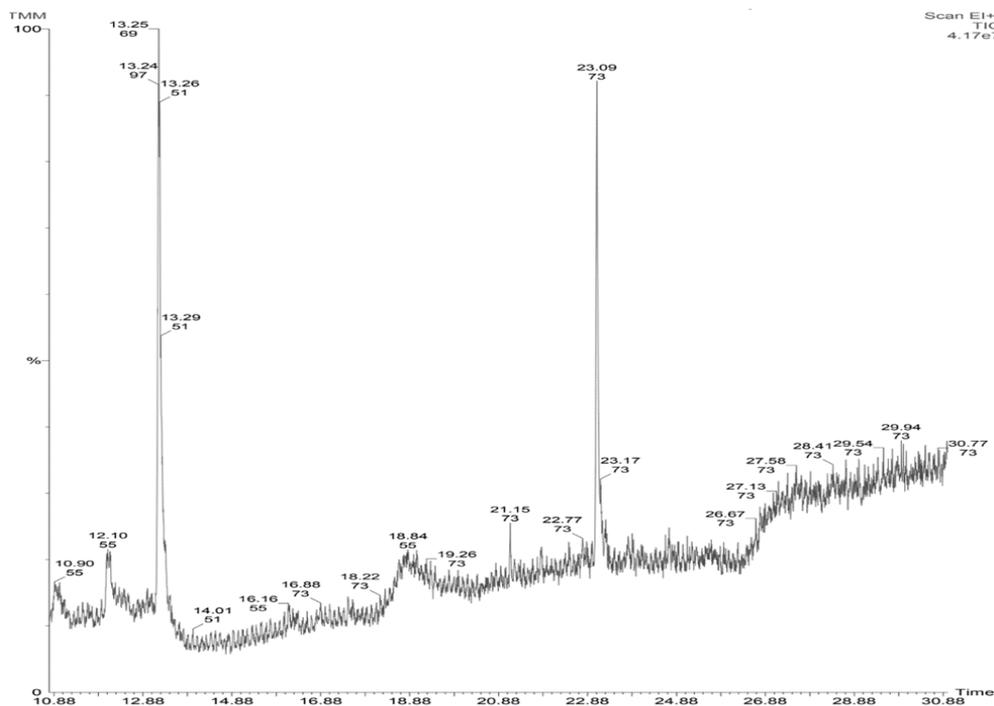


Figure 1 GC-MS chromatogram of methanolic root extract of control seedlings of *Oroxylum indicum*

53 compounds were found to be present in seedlings treated with mycorrhiza (TM), among which three new compounds were identified (Table 3) i.e., 1-Ethyl-2-Hydroxymethylimidazole at RT 13.25, Octadecanoic Acid, 11-Methyl-, methyl ester at RT 21.15 and 4-Methyl-1,4-Heptadiene at 23.09 RT (Fig.2).

Table 3 GCMS analysis of seedlings of *O. indicum* inoculated with mycorrhiza (TM)

S.No.	Compound name	Molecular formula	Molecular weight	RT	Peak area %
1.	1-Ethyl-2-hydroxymethylimidazole	C ₆ H ₁₀ N ₂ O	126	13.25	8.78
2.	Octadecanoic acid, 11-methyl-, methyl ester	C ₁₉ H ₃₆ O ₂	312	21.15	0.457
3.	4-Methyl-1,4-heptadiene	C ₈ H ₁₄	110	23.09	4.172

**Figure 2** GC-MS chromatogram of methanolic root extract of seedlings of *Oroxylum indicum* treated with mycorrhiza (*Glomus mosseae*)

The seedlings treated with bacteria (TB) showed the presence of 52 compounds. Three new compounds were identified (Table 4), namely, Meso-4, 5-octanediol at RT 12.08, 1-ethyl-2-hydroxymethylimidazole, at 13.28 RT and 2,5-cyclohexadiene-1,4-dione, 2,5-dihydroxy-3-methyl-6-(1-methylethyl)- at 21.84 RT (Fig.3)

Table 4 GCMS analysis of seedlings of *O. indicum* inoculated with bacteria (TB)

Sr. No.	Compound name	Molecular formula	Molecular weight	RT	Peak area %
1.	Meso-4,5-octanediol	C ₈ H ₁₈ O ₂	146	12.08	1.45
2.	1-Ethyl-2-hydroxymethylimidazole	C ₆ H ₁₀ N ₂ O	126	13.28	7.52
3.	2,5-Cyclohexadiene-1,4-dione, 2,5-dihydroxy-3-methyl-6-(1-methylethyl)-	C ₁₀ H ₁₂ O ₄	196	21.84	0.854

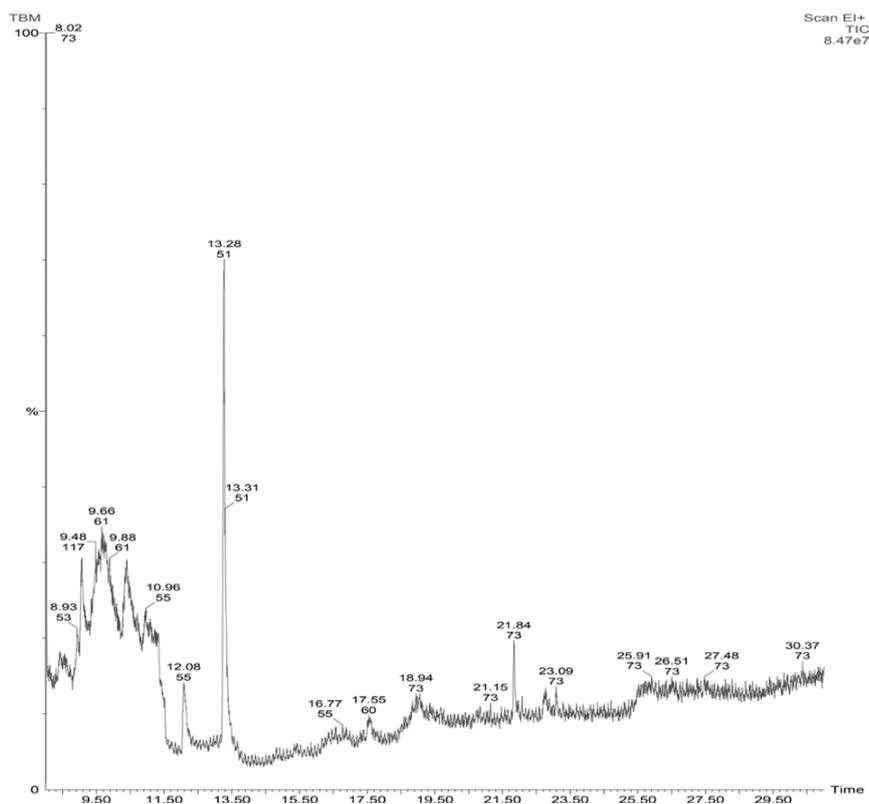


Figure 3 GC-MS chromatogram of methanolic root extract of seedlings of *Oroxylum indicum* treated with bacteria (*Pseudomonas putida*)

A total of 56 compounds were present in seedlings treated with Fungus (TF) under various retention times. Five new compounds were identified under this treatment (Table 5) namely, 2-Cyclohexen-1-one, 2-butyl-3-methoxy- at RT of 21.91, Methyl 12,13-tetradecadienoate at RT of 23.14, Methyl 6,9,12-hexadecatrienoate at RT of 23.73, 1,9-Decadiyne at RT of 26.55, 1,4-Naphthalenedione at RT of 27.47 (Fig.4).

Table 5 GCMS analysis of seedlings of *O. indicum* inoculated with fungus (TF)

S.No.	Compound name	Molecular formula	Molecular weight	RT	Peak area %
1.	2-Cyclohexen-1-one, 2-butyl-3-methoxy-	C ₁₁ H ₁₈ O ₂	182	21.91	93
2.	Methyl 12,13-tetradecadienoate	C ₁₅ H ₂₆ O ₂	238	23.14	0.067
3.	Methyl 6,9,12-hexadecatrienoate	C ₁₇ H ₂₈ O ₂	264	23.73	0.067
4.	1,9-Decadiyne	C ₁₀ H ₁₄	134	26.55	0.120
5.	1,4-Naphthalenedione	C ₁₀ H ₆ O ₂	158	27.47	0.327

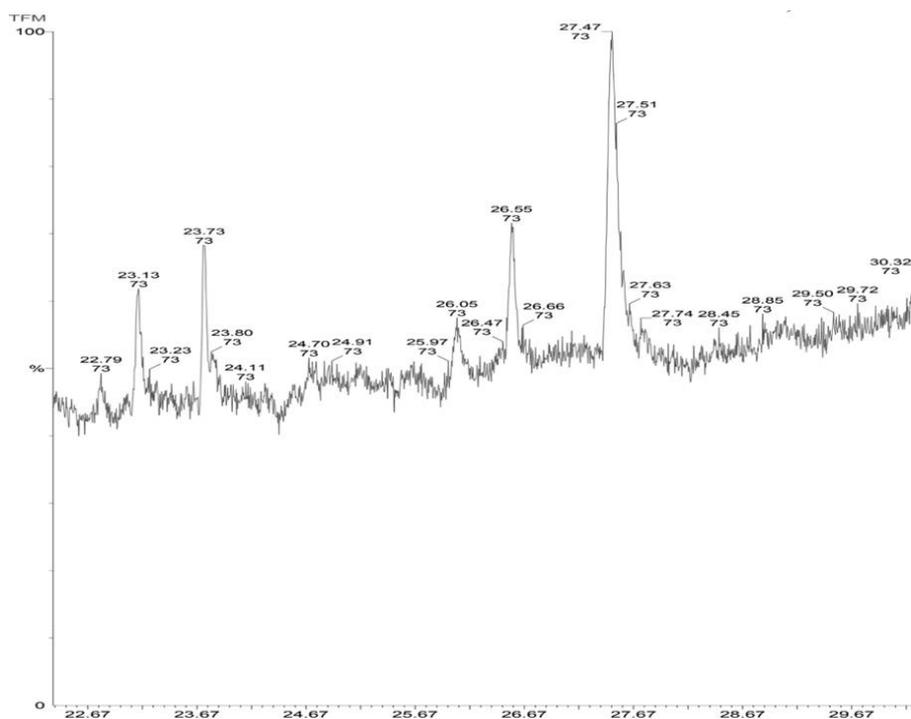


Figure 4 GC-MS chromatogram of methanolic root extract of seedlings of *Oroxylum indicum* treated with fungus (*Trichoderma harzianum*)

The seedlings treated with dual consortium of mycorrhiza and bacteria (TM+B) showed presence of 88 compounds at different retention times. Five new compounds were found to be present under this treatment (Table 6), namely, N-(1-Methoxycarbonyl-1-methylethyl)-4-methyl-2-aza-1,3-dioxane at 12.055 RT, 1-ethyl-2-hydroxymethylimidazole at 13.25 RT, Methyl 8-methyl-nonanoate at 21.14 RT, Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl- at 21.83 RT, Methyl 12,13-tetradecadienoate at 23.09 RT (Fig.5).

Table 6 GCMS analysis of seedlings of *O. indicum* inoculated with dual consortium of mycorrhiza and bacteria (TM+B)

S.No.	Compound name	Molecular formula	Molecular weight	RT	Peak area %
1.	N-(1-Methoxycarbonyl-1-methylethyl)-4-methyl-2-aza-1,3-dioxane	C ₉ H ₁₇ NO ₄	203	12.055	2.371
2.	1-Ethyl-2-hydroxymethylimidazole	C ₆ H ₁₀ N	126	13.25	22.31
3.	Methyl 8-methyl-nonanoate	C ₁₁ H ₂₂ O ₂	186	21.14	0.615
4.	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-	C ₁₅ H ₂₄	164	21.83	1.422
5.	Methyl 12,13-tetradecadienoate	C ₁₅ H ₂₆ O ₂	238	23.09	1.464

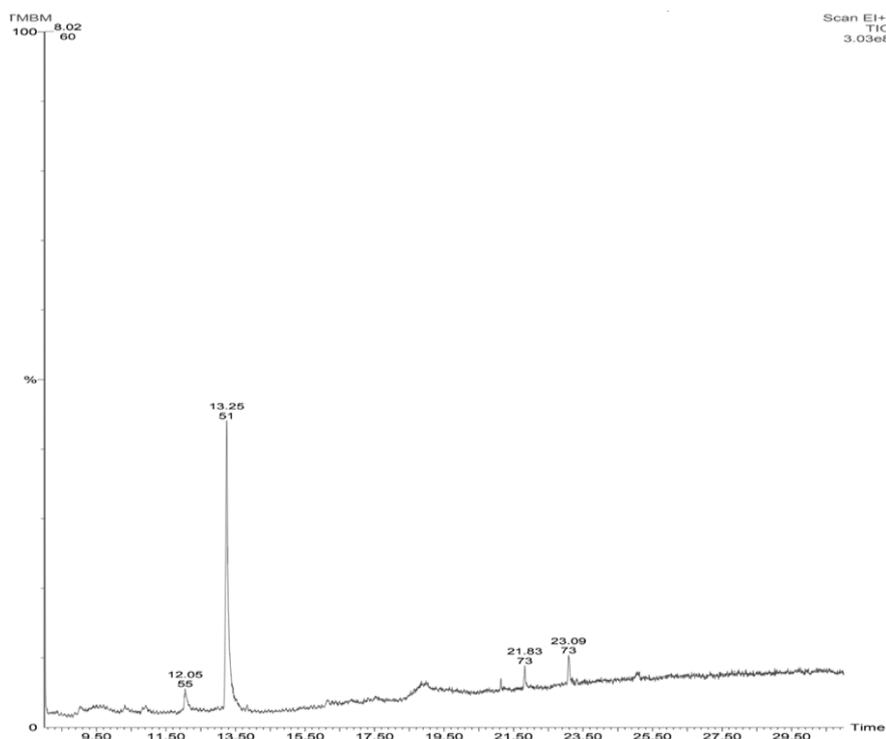


Figure 5 GC-MS chromatogram of methanolic root extract of seedlings of *Oroxylum indicum* treated with dual consortium of mycorrhiza and bacteria

152 compounds were present in seedlings treated with dual consortium of mycorrhiza and fungus (TM+F). A total of ten new compounds were identified under this treatment (Table 7) namely, 1,9-Decadiyne at RT of 15.85, 3,7,11-Trimethyl-3-hydroxy-6,10-dodecadien-1-yl acetate at RT of 17.33, 3-Heptyne, 7-chloro- at RT of 19.36, 3-Methyl-4-(methoxycarbonyl) hexa-2,4-dienoic acid at RT of 19.65, Benzo[c]cinnolin-2-amine at RT of 21.09, Tetradecanoic acid, 10,13-dimethyl-,methyl ester at RT of 21.13, Cis,cis-4,6-octadienol at RT of 21.24, 2-Cyclohexen-1-one, 2-butyl-3-methoxy- at RT of 21.87, 2-Aminopyridazino(6,1-b)quinazolin-10-one at RT 27.42 (Fig.6)

Table 7 GCMS analysis of seedlings of *O. indicum* inoculated with dual consortium of mycorrhiza and fungus (TM+F)

S.No.	Compound name	Molecular formula	Molecular weight	RT	Peak area %
1.	1,9-Decadiyne	C ₁₀ H ₁₄	134	15.85	8.36
2.	3,7,11-Trimethyl-3-hydroxy-6,10-dodecadien-1-yl acetate	C ₁₇ H ₃₀ O ₃	282	17.33	0.093
3.	3-Heptyne, 7-chloro-	C ₇ H ₁₁ Cl	130	19.36	0.177
4.	3-Methyl-4-(methoxycarbonyl) hexa-2,4-dienoic acid	C ₁₉ H ₁₂ O ₄	184	19.65	0.168
5.	Benzo[c]237innoline-2-amine	C ₁₂ H ₈ N ₂	195	21.09	0.652
6.	Tetradecanoic acid, 10,13-dimethyl-,methyl ester	C ₁₇ H ₃₄ O ₂	270	21.13	0.160
7.	Cis, cis-4,6-octadienol	--	126	21.24	0.253
8.	2-Cyclohexen-1-one, 2-butyl-3-methoxy-	C ₁₁ H ₁₈ O ₂	182	21.87	75.69
9.	Methyl 12,13-tetradecadienoate	C ₁₅ H ₂₆ O ₂	238	23.08	0.168
10.	2-Aminopyridazino(6,1-b)quinazolin-10-one	C ₁₁ H ₈ N ₄ O	212	27.42	1.194

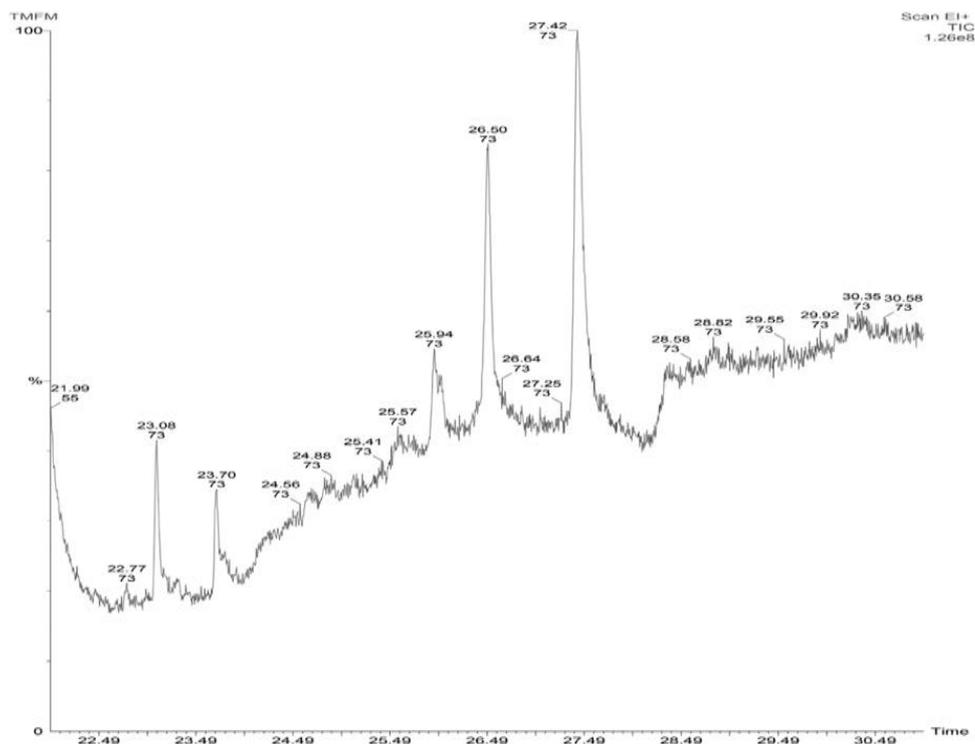


Figure 6 GC-MS chromatogram of methanolic root extract of seedlings of *Oroxylum indicum* treated with dual consortium of mycorrhiza and fungus

36 compounds were found in seedlings treated with dual consortium of bacteria and fungi (TB+F). Two new compounds (Table 8) were identified namely, [1, 4] Dioxino [2,3-b]-1,4-dioxin, hexahydro-2,3,6,7- at RT of 12.10 and 1-Ethyl-2-hydroxymethylimidazole was present at RT of 13.28 (Fig.7).

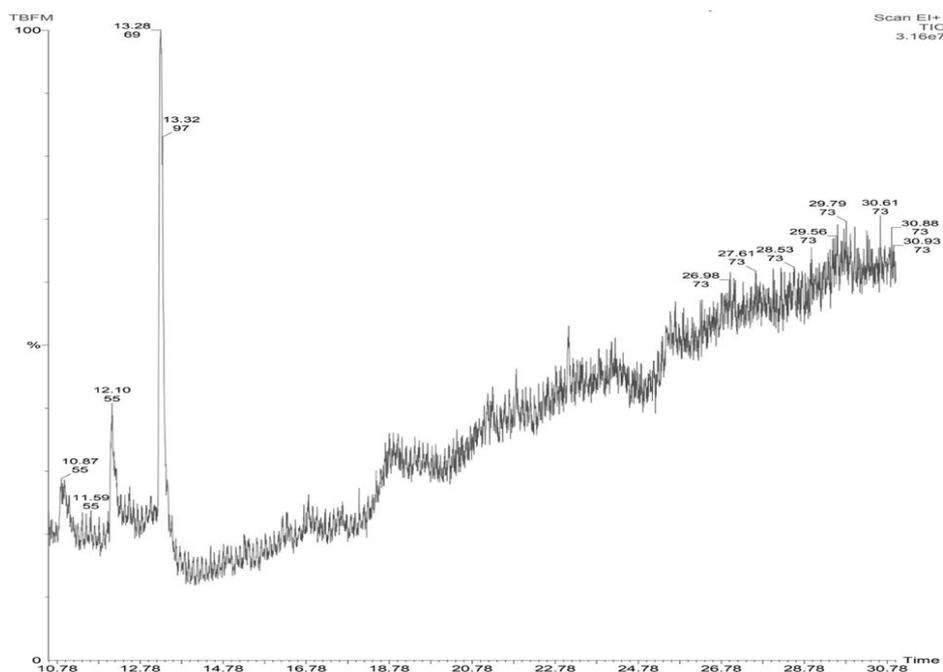


Figure 7 GC-MS chromatogram of methanolic root extract of seedlings of *Oroxylum indicum* treated with dual consortium of bacteria and fungus

Table 8 GCMS analysis of seedlings of *O. indicum* inoculated with dual consortium of bacteria and fungus (TB+F)

S.No.	Compound name	Molecular formula	Molecular weight	RT	Peak area %
1.	[1,4] Dioxino[2,3-b]-1,4-dioxin, hexahydro-2,3,6,7-	C ₆ H ₁₀ O ₄	202	12.10	1.430
2.	1-Ethyl-2-hydroxymethylimidazole	C ₆ H ₁₀ N	126	13.28	4.664

The seedlings inoculated with mixed consortium of mycorrhizal fungi, bacteria, and fungus (TM+B+F) showed the presence of 213 compounds at different retention times. Fourteen new compounds were identified under this treatment (Table 9), namely, 3,7,11-Tridecatrienenitrile, 4,8,12-Trimethyl- at RT of 15.80, 1,9-Decadiyne at RT of 15.87, 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-Hexamethyl-, (ALL-E)- at RT of 15.92, 1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cy at RT of 16.53, 1,9-Decadiyne at RT of 16.82, Cyclobutane, 1,2-bis(1-methylethenyl)-, trans- at RT of 17.18, 3,7,11-Trimethyl-3-hydroxy-6,10-dodecadien-1-yl acetate at RT of 17.35, 5-Hydroxy-4-hydroxymethyl-1-(1-hydroxy-1-isopropyl)cyclohex-3-ene at RT of 17.52, 5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-z)- at RT of 18.24, 1-Cyclohexyl-2-buten-1-ol (c,t) at RT of 19.03, 1-Oxetan-2-one, 4,4-diethyl-3-methylene- at RT of 19.06, Tetradecanoic acid, 10,13-dimethyl-, methyl ester at RT of 21.15, 2-Cyclohexen-1-one, 2-butyl-3-methoxy- at RT of 21.91, Methyl 12,13-tetradecadienoate at RT of 23.10, Heptacosanoic acid, 25-methyl-, methyl ester at RT of 23.31 (Fig.8)

Table 9 GCMS analysis of seedlings of *O. indicum* inoculated with mixed consortium of mycorrhiza, bacteria, and fungus (TM+B+F)

S.No.	Compound name	Molecular formula	Molecular weight	RT	Peak area %
1.	3,7,11-Tridecatrienenitrile, 4,8,12-trimethyl-	C ₁₆ H ₂₅ N	231	15.80	0.032
2.	1,9-Decadiyne	C ₁₀ H ₁₄	134	15.87	0.056
3.	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-Hexamethyl-, (all-e)-	C ₃₀ H ₅₀	410	15.92	0.034
4.	1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cy	C ₁₅ H ₂₆ O	222	16.53	0.030
5.	Cyclobutane, 1,2-bis(1-methylethenyl)-, trans-	C ₁₀ H ₁₆	136	17.18	0.008
6.	3,7,11-Trimethyl-3-hydroxy-6,10-dodecadien-1-yl acetate	C ₁₇ H ₃₀	282	17.35	0.067
7.	5-Hydroxy-4-hydroxymethyl-1-(1-hydroxy-1-isopropyl) cyclohex-3-ene	C ₁₀ H ₁₈ O ₃	186	17.52	0.021
8.	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-z)-	C ₂₁ H ₃₄ O ₂	318	18.24	0.026
9.	1-Cyclohexyl-2-buten-1-ol (c,t)	C ₁₀ H ₁₈ O	154	19.03	0.187
10.	1-Oxetan-2-one, 4,4-diethyl-3-methylene-	C ₈ H ₁₂ O ₂	140	19.06	0.064
11.	Tetradecanoic acid, 10,13-dimethyl-, methyl ester	C ₁₇ H ₃₄ O ₂	270	21.15	0.142
12.	2-Cyclohexen-1-one, 2-butyl-3-methoxy-	C ₁₁ H ₁₈ O ₂	182	21.91	95.703
13.	Methyl 12,13-tetradecadienoate	C ₁₅ H ₂₆ O ₂	238	23.10	0.477
14.	Heptacosanoic acid, 25-methyl-, methyl ester	--	438	23.31	0.023

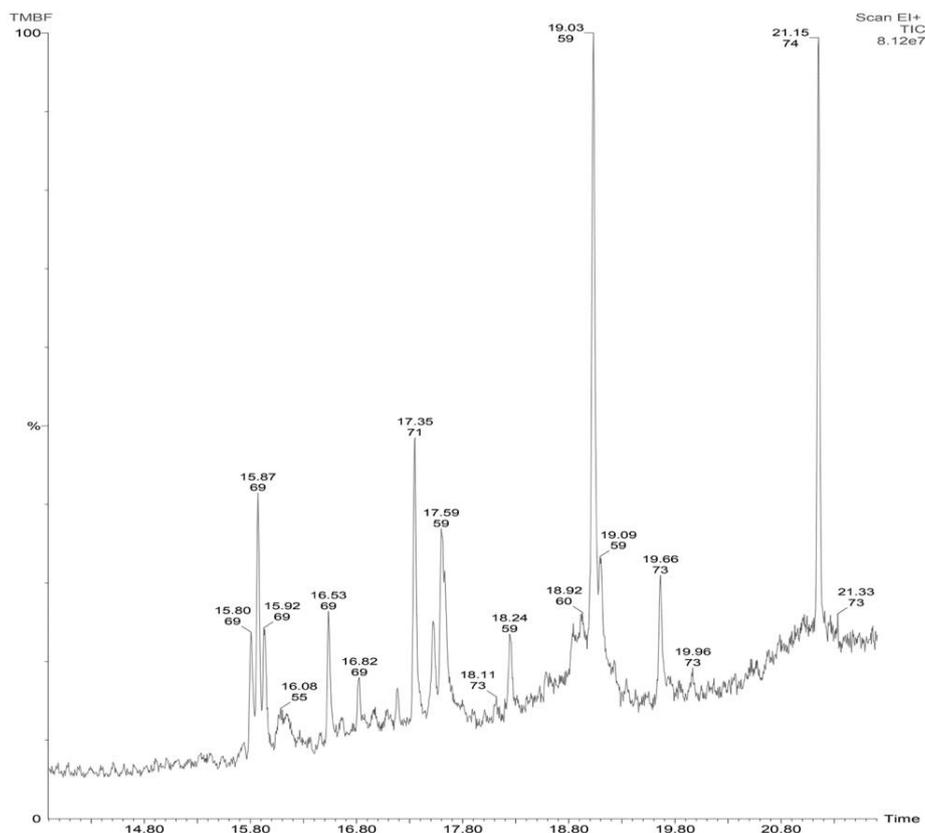


Figure 8 GC-MS chromatogram of methanolic root extract of seedlings of *Oroxylum indicum* treated with mixed consortium of mycorrhiza, bacteria, and fungus.

The GCMS analysis showed the presence of glycosides, flavonoids, phenols, terpenoids amino acids etc. in the methanolic plant root extract. Palmitic acid is the first fatty acid produced during fatty acid synthesis and is the precursor to longer fatty acids. Linoleic acid (LA) is a polyunsaturated omega-6 fatty acid. It is a colorless liquid at room temperature. Linoleic acid lipid radicals can be used to show the antioxidant effect of natural phenols. Methyl butyrate, also known under the systematic name methyl butanoate, is the methyl ester of butyric acid. Methyl butyrate has been used in combustion studies as a surrogate fuel for the larger fatty acid methyl esters found in biodiesel. The term Farnesene refers to a set of six closely related chemical compounds which all are sesquiterpenes. α -Farnesene and β -Farnesene are isomers. Squalene is a hydrocarbon and a triterpene and is a natural and vital part of the synthesis of all plant and animal sterols, including cholesterol, steroid hormones, and vitamin D in the human body. Myrcene, or β -Myrcene, is an olefinic natural organic hydrocarbon. It is more precisely classified as a monoterpene. Monoterpenes are dimers of isoprenoid precursors, and Myrcene is one of the most important. It is a component of the essential oil. From the results it was observed that maximum number of phytochemicals were present in the methanolic extract of inoculated seedlings as compared to control seedlings. It was found that seedlings treated with mixed consortium of mycorrhiza, bacteria and fungus (TMBF) showed the presence of maximum number of phytochemicals followed by seedlings treated with dual consortium of mycorrhiza and fungus (TMF), seedlings treated with dual consortium of mycorrhiza and bacteria (TMB) and seedlings treated with Fungus (TF). When literature was consulted to verify the presence of the phytochemicals present in *O. indicum*, again, major compounds known to be present in *O. indicum* were found present in seedlings treated with mixed consortium of mycorrhiza, bacteria, and fungus (TMBF) as compared to control seedlings. Dual or mixed consortium of seedlings showed more presence of phytochemicals as compared to seedlings treated with single treatments.

4. Conclusion

The results of the study are same with the earlier findings [32] [33] [34]. They reported that the mutualistic association was accounted for better colonization and plant growth due to interchange of carbon, phosphate and nitrogen between host fungi and bacteria. The Plant Growth-Promoting Microorganisms (PGPMs) and their inoculation in the rhizosphere of medicinal plants are particularly useful in increasing the growth of plants through nutrients uptake vis a vis phytochemical yield by active metabolism. The plant growth promoting microorganisms of the medicinal plants also

influence the quality and quantity of bioactive constituents. They also influence the metabolic activity and bioactivity of these medicinal plants. Numerous studies have showed that AMF can directly or indirectly influence the secondary metabolism of plants, causing changes in secondary metabolite levels [35] [36]. The symbiotic AM fungi can induce changes in the accumulation of secondary metabolites, including phenolics in roots and aerial parts and essential oil of host plants [37]. During the establishment of the AM symbiosis, a range of chemical and biological parameters is influenced in plants, including the pattern of secondary plant compounds. The accumulation of flavonoids [38], triterpenoids [39] in plants colonized by AM fungi has been reported. Many studies have shown that some bacterial species respond to the presence of certain AMF [40], suggesting a high degree of specificity between bacteria associated with AMF. The presence of phytochemicals indicates the medicinal importance of the plants and different phytochemicals have been found to possess a wide range of activities, which may help in protection against various diseases further, AMF inoculation not only promotes the growth of medicinal plants but also improves the productivity and quantity of chemicals [41]. The concept of improving the contents respectively the yield of plant secondary metabolites through AM is recent.

The GC-MS chromatogram of the methanolic extract of *Oroxylum indicum* showed major peaks which have been identified after comparison of the mass spectra with NIST library which shows the presence of many phytochemicals. The GCMS analysis showed the presence of glycosides, flavonoids, phenols, terpenoids amino acids etc. in the methanolic plant root extract. Based on the results, it can be concluded that mixed inoculation of *Glomus mosseae*, *Pseudomonas putida* and *Trichoderma harzianum* can be used in practice to produce improved seedlings of *O. indicum*. Understanding the natural dynamics of arbuscular mycorrhizal (AM) fungi and their response to global environmental change is essential for the prediction of future plant growth and ecosystem functions. The results suggest that these isolates can produce certain metabolites that can induce plant growth promotion. The application of PGP (Plant Growth Promoting) microbes for reducing chemical inputs in agriculture is a potentially important tool. At this juncture of the study, it appears that coinoculation of more than two growth promoting microbes can supplement each other effects. Secondly, results are more promising with coinoculation of native microbes. The production of higher yield and quality in medicinal plants, through conventional methods, often requires external inputs such as fertilizers and pesticides. In this context, use of mycorrhizal inoculation, a natural alternative to chemical fertilizers, is likely to boost the production of active ingredients. In this study growth and enhancement were recorded in morphological and phytochemical attributes, which indicate that AMF can be utilized for higher production as well as for the higher production of antioxidants and phenolics. The positive effect on the production of pharmacologically active compounds in medicinal plants through mycorrhization would mean a higher benefit and at the same time would contribute to a more sustainable practice of conservation of plant species [42]. The present investigation showed that maximum number of phytochemicals were found in combined synergistic treatment, which also showed growth effect on *O. indicum* seedlings. The indiscriminate collection, over exploitation, uprooting of whole plants, has posed threat to this plant in different parts of the Indian subcontinent. As the existence of *O. indicum* in natural population is in jeopardy [43], application of a synergistic interaction of *Trichoderma harzianum*, *Glomus mosseae* and *Pseudomonas putida* in the rhizosphere has positive influence on the growth and development of the *O. indicum* as well as accumulation of new phytochemicals. The bioactive components of medicinal plants among different habitats will pilot ways to unearth the relationship between plants, microorganism diversity and the bioactive compounds accumulation and pave way for future medical and industrial applications.

Compliance with ethical standards

Acknowledgments

Authors are thankful to Rain Forest Research Institute (Indian council of Forestry Research & Education), Jorhat, Assam for providing the all possible laboratory facilities and experimental field for carrying out the research work.

Disclosure of conflict of interest

The authors declare no competing interests

References

- [1] Berg G, Smalla K. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiol. Ecol.* 2009; 68: 1–13.
- [2] Badri DV, Weir TL, Lelie D van der, Vivanco JM. Rhizosphere chemical dialogues: plant-microbe interactions. *Current Opinion in Biotechnology.* 2009; 20: 642–650.

- [3] Broeckling CD, Broz AK, Bergelson J, Manter DK, Vivanco JM. Root exudates regulate soil fungal community composition and diversity. *Appl. Environ. Microbiol.* 2008; 74(3): 738-744.
- [4] Hammond KE, Jones JD. Resistance gene-dependent plant defense responses. *Pl. Cell.* 1996; 8: 1773–1791.
- [5] Cazres E, Trappe JM. Vesicular endophytes in the root of *Pinaceae*. *Mycorrhiza.* 1993; 2: 153-156.
- [6] William PG, Roser DJ, Seppely RD. Mycorrhizas of hepatics in continental Antarctica. *Mycol. Res.* 1994; 98: 34-36.
- [7] Gianazzi – Pearson V. Gianazzi S. Enzymatic studies on the metabolism of vesicular arbuscular mycorrhiza. *Physiol. Veg.* 1976; 14(4): 833-841.
- [8] Salamanca CP, Heera MA, Barea JM. Mycorrhizal inoculation of micropropagated woody legumes used in revegetation programmes for desertified Mediterranean ecosystems. *Agronomie.* 1992; 12: 869–872.
- [9] Parkash V, Aggarwal A, Sharma S, Sharma D. Effect of Endophytic Mycorrhizae and Fungal Bioagent on the Development and Growth of *Eucalyptus saligna* Seedlings. *Bullet. Nat. Instit. Ecol.* 2005; 15: 127-131.
- [10] Persello-Cartieaux, F, Nussaume L, Robaglia C. Tales from the underground: molecular plant-rhizobacteria interactions. *Pl. Cell & Environ.* 2003; 26: 189-199.
- [11] Bloemberg GV, Lugtenberg BJ. Molecular basis of plant growth promotion and biocontrol by rhizobacteria. *Curr. Opin. Plant Biol.* 2001; 4: 343-350.
- [12] Harman GE, Howell CR, Viterbo A, Chet I, Lorito M. *Trichoderma* species opportunistic, avirulent plant symbionts. *Natr. Revws. Microbiol.* 2004; 2: 43-56.
- [13] Djonovic S, Pozo MJ, Dangott LJ, Howell CR, Kenerley CM. A proteinaceous elicitor secreted by the biocontrol fungus *Trichoderma virens* induces plant defense responses and systemic resistance. *Mol. Plant-Microbe Interact.* 2006; 19: 838–853.
- [14] Contreras M, Cortés, López J. *Trichoderma virens* - A plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin-independent mechanism in *Arabidopsis* sp. *Pl. Physiol.* 2009; 149: 1579–1592.
- [15] Raja P, Una S, Gopal H, Govindarajan K. Impact of bioinoculant consortium on rice root exudates, biological nitrogen fixation and plant growth. *J. Biol. Sci.* 2006; 6(5): 815-823.
- [16] Jugran AK, Bahukhandi A, Dhyani P, Bhatt ID, Rawal RS, Nandi SK, Palni LMS. The effect of inoculation with mycorrhiza: AM on growth, phenolics, tannins, phenolic composition, and antioxidant activity in *Valeriana jatamansi* Jones. *J. Soil Sci. Plant Nutr.* 2015; 15(4).
- [17] Debi C, Parkash V. Seed source and habitat variation affect seed germination in *Oroxylum indicum* (L.) Benth. ex Kurz: An important threatened medicinal tree. *Int. J. Life Sc. & Tech.* 2015; 8(1): 1-9.
- [18] Vaidya BG. Some controversial drugs of Indian medicine. IX, *J. Res. Indian Med.* 1975; 10(4): 27.
- [19] Yang RY, Samson CST, Lee TC, Janhiu W, Hanson PM, Kuo G. Distribution of 127 edible plant species for antioxidant activities by two assays. *J. Sc. Food Agric.* 2006; 86: 2395-2403.
- [20] Warriar PK, Nambiar VPK, Ramankutty C. *Oroxylum indicum*. In: A Compendium of 500 Species, Indian Medicinal Plants, Vol IV. Madras, Orient Longman Ltd.; 1995. p. 186–190.
- [21] Ali RH, Houghton PJ, Amala R, Hoult JRS. Antimicrobial and anti-inflammatory activities of extracts and constituents of *Oroxylum indicum* (L.) Vent. *Phytomedicine.* 1998; 5(5): 375-381.
- [22] Grampurohit ND, Baichwal MR, Jolly CI. Chemical constituents of the roots of *Oroxylum indicum* (L.) Vent. *Indian J. Nat. Prod.* 1994; 10: 8-12.
- [23] Chen LJ, Games DE, Jones J. Isolation and identification of four flavonoids constituents from the seeds of *Oroxylum indicum* by high-speed counter-current chromatography. *J. Chromatogr. A.* 2003; 988: 95–105.
- [24] Koche DK, Shirsat RP, Syed I, Bhadange DG. Phytochemical screening of eight folk medicinal plants from Akola District (M.S) India, *Int. J. Pharm. and Bio. Sci.* 2010; 1(4): 256-261.
- [25] Debi C, Parkash V. Rhizospheric Inoculation Influence on Seedling Growth, Development and Biomass Yield in *Oroxylum indicum* (L.) Benth. ex Kurz. *International Journal Science and Research.* 2016 a; 5(9): 424-429.
- [26] Debi C, Parkash V. Bioinoculants influence accumulation of phytochemicals in *Oroxylum indicum* (L.) Benth. ex Kurz seedlings. *Journal of Medicinal Plants Studies.* 2016 b; 4(6): 124-131.

- [27] Robertson DG. Metabonomics in toxicology: A review. *Toxicology Science*. 2005; 85: 809-22.
- [28] Fernie AR, Trethewey RN, Krotzky AJ, Willmitzer L. Metabolite profiling: From diagnostics to systems biology. *Nature Review Molecular Cell Biology*. 2004; 5: 763-9.
- [29] Kell DB, Brown M, Davey HM, Dunn WB, Spasic I, Oliver SG. Metabolic footprinting and systems biology: The medium is the message. *Nature Review Microbiology*. 2005; 3: 557-65.
- [30] Debi C, Parkash V. Seed source and habitat variation affect seed germination in *Oroxylum indicum* (L.) Benth. ex Kurz: An important threatened medicinal tree. *Int. J. of Life Sci. and Tech*. 2015; 8(1):1-9
- [31] Debi C, Parkash V. The soil nutrient content alters arbuscular mycorrhizal association in *Oroxylum indicum* (L.) Benth. Ex kurz growing under different natural habitat in north east India. *Int. J. of Bot. and Res. (IJBR)*. 2018; 8(5): 1-18.
- [32] Rani P, Aggarwal A, Mehrotra RS. Growth responses in *Acacia nilotica* inoculated with VAM fungus *Glomus mosseae*, *Rhizobium* sp. and *Trichoderma harzianum*. *Indian Phytopathology*. 1999; 52(2): 151-153.
- [33] Gill TS, Singh RS. Effect of *Glomus fasciculatum* and *Rhizobium* inoculation on VA mycorrhizal colonization and plant growth of chickpea. *Journal of Mycology and Plant Pathology*. 2002; 32(2): 162-167.
- [34] Parkash V, Sharma S, Aggarwal A. Symbiotic and synergistic efficacy of endomycorrhizae with *Dendrocalamus strictus* L. *Plant Soil and Environment* 2011b; 57(10): 447-451.
- [35] Araim G, Saleem A, Arnason JT, Charest C. Root colonization by an arbuscular mycorrhizal (AM) fungus increases growth and secondary metabolism of purple cone flower, *Echinacea purpurea* (L.) Moench. *Journal of Agricultural and Food Chemistry*. 2009; 57: 2255-2258.
- [36] Yaghoub R, Weria W. Arbuscular mycorrhizal fungi associated with some aromatic and medicinal plants. *Bulletin of Environment, Pharmacology and Life Sciences*. 2013; 2(11): 129-138.
- [37] Rojas R, Bustamante B, Bauer J. Antimicrobial activity of selected Peruvian medicinal plants. *Journal of Ethnopharmacology*. 2003; 88(2-3): 199-204.
- [38] Morandi D. Occurrence of phytoalexins and phenolic compounds in endomycorrhizal interactions, and their potential role in biological control. *Plant Soil*. 1996; 185: 241–251.
- [39] Akiyama K, Hayashi H. Arbuscular mycorrhizal fungus-promoted accumulation of two new triterpenoids in cucumber roots. *Biosc. Biotech. Biochem*. 2002; 66: 762-769.
- [40] Andrade G, Mihara KL, Linderman RG, Bethlenfalvay GJ. Bacteria from rhizosphere and hyphosphere soils of different arbuscular-mycorrhizal fungi. *Plant and Soil*. 1997; 192(1): 71-79.
- [41] Karthikeyan B, Joe1 MM, Jaleel CA. Response of some medicinal plants to vesicular arbuscular mycorrhizal inoculations. *J. Sc. Resc*. 2009; 1(2): 381–386.
- [42] Parkash V, Aggarwal A, Bipasha. Rhizospheric effect of vesicular arbuscular mycorrhizal inoculation on biomass production of *Ruta graveolens* L.: A potential medicinal and aromatic herb. *Journal of Plant Nutrition*. 2013; 4(9): 1386-96.
- [43] Ravikumar K, Ved DK. 100 Red listed medicinal plants of conservation concern in Southern India. *Foundation for Revitalization of Local Health Traditions, Bangalore, India*. 2000.