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Deciphering metabolite profiling in grafted *Solanum nigrum*: A comprehensive GC-MS and FTIR analysis

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Abstract

Vegetable grafting plays a significant role in modern agricultural practices, effectively managing abiotic and biotic stresses. Additionally, it offers the advantage of enhancing the phenotypic traits of the scion. This technique has gained widespread acceptance in commercial cultivation, particularly in crops like tomato, watermelon, melon, eggplant, *etc.*, but has not been reported in *Solanum nigrum*. The appeal lies in its swiftness compared to the traditional method of breeding vegetables with improved environmental stress tolerance. This study focused on identifying and studying the components present in the leaves and fruits of both grafted and ungrafted *Solanum nigrum*. The GC-MS analysis unveiled a multitude of bioactive compounds, some of which are well-known antioxidants and possess anti-inflammatory properties. These beneficial attributes make them potentially valuable for promoting health and well-being. In addition, Fourier-Transform Infrared Spectroscopy (FTIR) was employed to identify functional groups in the methanolic extracts. The FTIR findings confirmed the existence of diverse functional groups, such as alkanes, alkynes, carboxylic acids, aldehydes, and nitriles, within the selected grafted *Solanum nigrum* samples. The research outcomes suggest that the extracts could be valuable in managing fungal infections in crops, which may contribute to the successful grafting of *Solanum nigrum* onto wild rootstocks. The presence of bioactive compounds with antifungal properties in the extracts might enhance disease resistance, making a successful grafting process a viable solution for improved and extended production. This underscores the critical necessity for continued research, highlighting its potential benefits to various domains, including medicine and nutrition.

Key words*:* *Solanum nigrum*, antioxidant, bioactive compound, FTIR, methanolic extract

Introduction

In a rapidly changing world, where the emergence of new pests and the impact of climate change pose significant challenges, the future of food production is becoming increasingly daunting. Amidst these difficulties, plant grafting emerges as one of the most invaluable tools in vegetable production to combat soil-borne diseases and various stresses (Tsaballa *et al.*, 2021). The advantages offered by grafted plants extend far beyond disease resistance, encompassing benefits like enhanced yields even under adverse conditions, prolonged cultivation periods, reduced dependency on fertilizers and agrochemicals, utilization of a wide range of phytogenetic resources as rootstocks, and the elimination of the need for crop rotation. Embracing plant grafting techniques not only addresses immediate challenges but also sets the stage for a more sustainable and resilient future in agriculture (Lee *et al.*, 2010)

Grafting was initially introduced from East Asia to Europe during the 20th century, but it has experienced a surge in popularity over the last three decades. Nowadays, grafted plants have become a standard in the commercial cultivation of various crops, including tomato (*Solanum lycopersicum*), watermelon (*Citrullus lanatus*), melon (*Cucumis melo*), eggplant (*Solanum melongena*), cucumber (*Cucumis sativus*), pepper (*Capsicum annuum*) and numerous others. This widespread adoption of grafting demonstrates its effectiveness in improving crop productivity, disease resistance, and overall agricultural sustainability (Cao *et al.*, 2016)

Solanum nigrum (Makoi), locally called Mannathakali, belonging to the family Solanaceae, is a minor leafy vegetable with high medicinal value. The probable origin was Eurasia (Ogg *et al.*, 1981). Its berries are grown and consumed sporadically in India but not for commercial purposes. The leaves and berries are regularly eaten as food in Tamil Nadu after being cooked with tamarind, onion, and cumin seeds (Mukhopadhyay *et al.*, 2018)

Unlike all other vegetables, Makoi is not only primarily grown for its fruits but also for the tender green leaves, which are cooked and eaten directly to cure mouth ulcers; they fetch good prices in the local markets and for ayurvedic and medicinal uses. It is a pediatric plant used to treat a variety of disorders that cause infant death, including feverish convulsions, eye diseases, hydrophobia, and chronic skin ailments. In oriental medicine, *S. nigrum* is a commonly used plant that is thought to have antitumorigenic, antioxidant, anti-inflammatory, hepatoprotective, diuretic, and antipyretic properties (Jain *et al.*, 2011)

Due to the high palatability eminence of the leaves and fruits, there is high demand in the market. To meet this, the farmers of South Tamil Nadu are growing several local cultivars which exhibit a

lot of variation in yield and other components. Despite this, *S. nigrum* plants are characterized by low resistance to various stress conditions. Grafting, besides extending the harvesting periods, progresses the resistance to soil-borne diseases and advances the yield within a small area, which indirectly raises the farmer's income (Melnyk and Meyerowitz., 2015)

Therefore, the current study aimed to explore the alterations in metabolites found in the leaves and fruits of successfully grafted plants, utilizing GC-MS and FTIR spectroscopy to identify functional groups. The results of this investigation offered valuable insights into the active constituents of *S. nigrum* grafted onto wild rootstocks, highlighting its potential value. This promising discovery paves the way for future research and opens up possibilities for harnessing the plant's beneficial attributes of nightshade (*S. nigrum*).

Materials and methods

Selection of rootstock and scion: The selection of the scion is determined by local preferences and the need for high-yielding varieties. Hence, CO 1 variety of *Solaum nigrum* was selected as a scion but it is highly vulnerable to fungal and nematode infections. The root-knot nematode, in particular, infects the roots of *S. nigrum*, causing changes in the plant's chemical properties and poor herbage yield (Castillo *et al.*, 2008). In this context, rootstocks are chosen based on their historical resilience and ability to resist soil-borne diseases, particularly in comparison to *S. nigrum*. *S. torvum*, *S. chrysotrichum,* and *S. mauritianum* were the wild species selected as rootstocks.

Plant material collection: The leaves of the plants were harvested from the crop raised in the campus orchard, Department of Vegetable Science at Tamil Nadu Agricultural University, from December 2022 to May 2023. The samples were thoroughly cleaned with distilled water. They were blotted with blotting paper and shade-dried for a week at room temperature before being roughly pulverized with an electric blender and kept in an airtight container before extraction for GC-MS analysis.

Soxhlet extraction: As an extractant, 120 mL of methanol (90%) was used in the Soxhlet extraction method. The solvent was poured into a 100 mL round-bottomed extraction flask, weighed, and placed on the heating mantle. The thimble containing the dried ground plant sample (10 g) was placed in the Soxhlet extractor's chamber. Finally, the condenser was placed on top of the extractor and secured vertically. The extraction was performed in three time intervals of 3, 6 and 9 h (Salisu *et al.*, 2019). A rotary evaporator was used to concentrate the extracts. An aliquot (2 µL) of each methanol-concentrated crude plant extract was injected into the split-less GC-MS apparatus for analysis.

GC-MS investigation: The methanolic extract was investigated phytochemically using gas chromatography (GC) and a Shimadzu mass spectrometer (MS) (GC/MS-TQ8040 NX SHIMADZU, Shimadzu Corp., Tokyo, Japan). An electron ionization energy system with an ionization energy of 70eV was employed for GC-MS detection. The carrier gas, helium gas (99.999%), was used with an injection volume of l µL and a constant flow rate of 1.51 mL/min. With a 33:1 split, the injection volume is 0.5 L, and the injection port temperature is 200° C. Ultra-high purity helium moving at 1.2 mL per minute (Constant Linear Velocity mode) serves as the carrier gas. The oven programme was: 240°C with a hold of 9.0 minutes, a first ramp of 70 °C/min to 280°C with 1min hold, a second ramp of 225 °C/min with a hold of 3.0 minutes, and a third ramp of 300 °C/min with a hold of 5.0 min with a run time of 55 minutes.

Compound identification based on molecular mass and structure: Data on the mass spectral range was provided *via* the Wiley Online Library database. The molecular names, sizes, and structures of the test material's compounds were determined by correlating them with the library. PASS online software was used to predict the biological activities after getting the SMILES for the biological activities using Open Parser for Systematic IUPAC nomenclature (OPSIN).

Fourier Transforms Infrared Spectrophotometer (FTIR): The Fourier Transform Infrared (FTIR) Spectrophotometer was utilized to detect and validate chemical bonds/functional groups in plant extracts. FTIR spectroscopy has been demonstrated to be a reliable and sensitive approach for detecting biomolecular composition. A crucial characteristic of chemical bonding is the absorption of light wavelengths. The infrared absorption spectra can be used to analyze the chemical bonds in the substance. For FTIR analysis, the KBr pellet method was employed (Karthishwaran *et al.*, 2010; Kumirska *et al.*, 2010). Translucent sample discs were created by encapsulating 10 mg of each *S. nigrum* leaf methanolic extract in a 100 mg *S. nigrum* KBr pellet.

Results and discussion

Rootstock-scion compatibility: Following the grafting process, one week was allocated for the plants to acclimate under the shade net. The compatibility between the rootstock and scion was evaluated by observing the emergence of new growth flushes approximately one week after grafting. Combinations that lacked compatibility displayed signs of rot or desiccation at the graft junction, ultimately causing the plant to topple over. Among the various rootstocks examined, it was observed that *S. torvum* displayed the highest compatibility with the scion (*S. nigrum*), closely followed by *S. chrysotrichum* and *S. mauritianum*. These rootstock-scion pairs exhibited excellent compatibility, resulting in enhanced plant performance. Consequently, they produced a greater number of cuttings compared to other combinations.

Bioactive phytochemicals in the methanolic extracts of grafted and ungrafted *S. nigrum* grafted over *S. torvum*: The existence of several metabolites has been demonstrated by the appearance of distinct peaks, each of which has a distinct relative abundance at distinct retention durations. The most prominent peak was seen in Fig. 1a after 31.64 min, 31.62 min in grafted leaves, and 31.54 and 31.51 min in grafted and un-grafted fruits, respectively. Table 1 lists and illustrates the different types of chemicals that were discovered. The variety of pharmacological activities of molecules highlights the significance of plant metabolites.

In the analysis of grafted leaves and fruits of *S. nigrum* using GCMS, a total of 70 and 65 metabolites were identified, respectively. Among the compounds studied, six were found to be the most abundant in leaves, namely: 4H-Pyran-4-one,2,3-dihydro-3,5-dihydro (9.212), oxirane (17.360), Dodecanamide N-hydroxy(21.272), 3,3-Diethylglutaric acid (22.320), Linoleic acid-TMS(27.321) and 9,12,15-Octadecatrienoic acid(31.626).

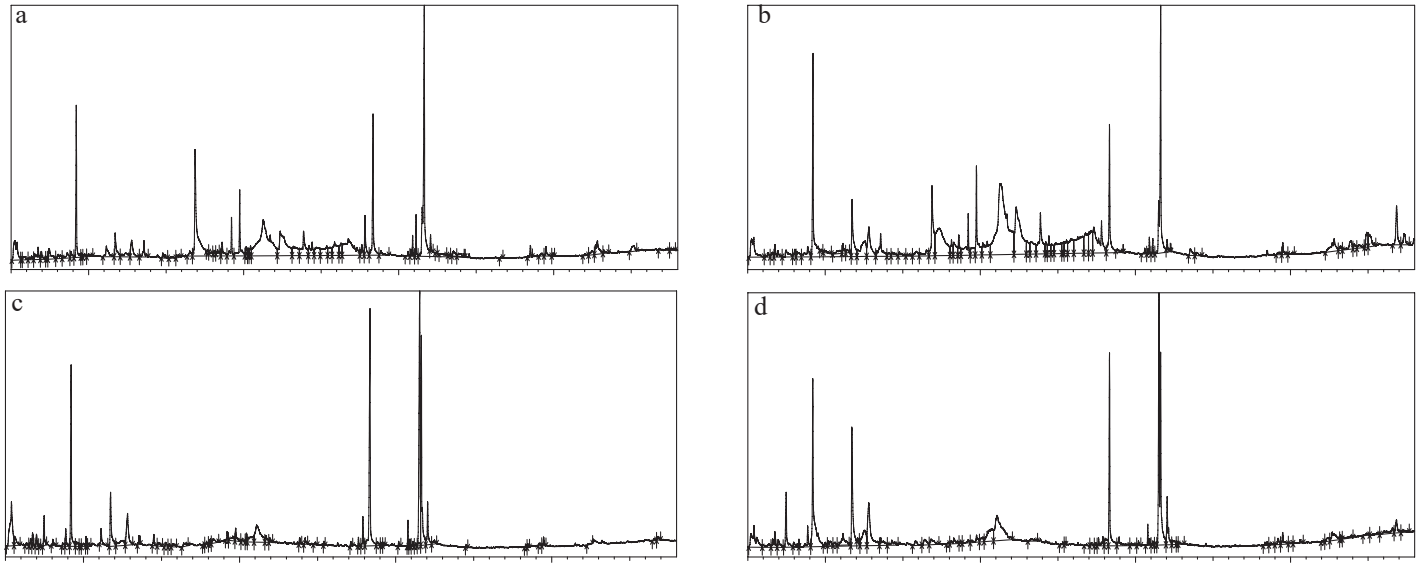

Fig. 1. Gas chromatogram of compounds from *Solanum nigrum*
a-Ungrafted leaf(control), b- Grafted leaf, c-Ungrafted fruit, d-Grafted fruit

In fruits, the identified compounds included Glycerin (5.209), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydro (9.190), Heptanoic acid, 6-oxo (12.804), 1,2,3,5-Cyclohexanetetrol (1 alpha, 2. beta) (21.078), Methyl butanoate (23.338), n-Hexadecanoic acid (28.323), and 10E,12Z-Octadecadienoic acid (31.510).

In ungrafted samples, the major compounds identified included 4H-Pyran-4-one, 2,3-dihydro (9.207), Dodecanoic acid 3-hydroxy (21.095), Scyllo-Inositol (26.765), n-Hexadecanoic acid (28.342), and 9,12,15-Octadecatrienoic acid (31.642) in both leaves and fruits. However, in grafted fruits, most of these compounds were present except for a few plants, such as oleic acid *etc,* which were not found in the leaves.

Using the GC-MS method, the constituents in methanol extracts of *S. nigrum*, grafted and ungrafted leaves and fruits were detected. Figs 1a,b,c,d show the chromatograms of the leaf and fruit extracts studied. Each peak reflects a different component with varying quantity and quality based on per cent. Some peaks show the same molecule but with varying retention durations, hence their area percentage compositions are additive. The discovered compounds' chemical formulae, molecular weight (MW), retention time (RT), and area percentage are presented in Table 1 for each extract.

GCMS analysis: The existence of various phytochemical substances in plants was revealed by GC-MS analysis of an methanolic plant extract prepared from *S. nigrum* grafted onto *S. torvum,* resulting in higher compatability (Table 1). The substances discovered belonged to many classes, including steroids, acids, phytosterols, alkaloids, ketones, and esters. Regardless of the amount or concentration (high or low) at which these compounds were revealed, practically all have been reported to exhibit some pharmacological or biological activity (Table 1. a, b, c, and d). Almost all of the compounds found have been shown to have antibacterial, antifungal, antioxidant, and antiviral properties against a variety of harmful bacteria, fungi, and viruses (Balasundaram *et al.*, 2016; Akhtar *et al.*, 2013; Soosairaj *et al.*, 2016; Ertas *et al.*, 2014). Results revealed that compounds such as Hexadecanoic Acid, Linolenic acid, Inositol, n-hexadecanoic acid and 4H-Pyran-4-one, 2,3-dihydro were identified to be present in all the extracts of *S. nigrum* and have been reported to possess potential antioxidant activity (Balasundaram *et al.*, 2016).

Fourier Transform Infrared investigation of grafted *S. nigrum* over wild rootstocks (FTIR): Based on the peak value in the infrared radiation portion of the FTIR spectrum, the functional group of the active components were identified. Table 2 displays the FTIR peak values and functional group data. Fig. 2 depicts the FTIR spectrum profile of *S. nigrum* grafted onto various rootstocks. Alkanes, alkynes, aldehydes, halo compounds, and ether were all detected in the methanolic extracts of all chosen samples according to the FTIR spectrum. The peak shows alkanes at 2846.93 and 2916.37cm^{-1} (H-C-H Asymmetric & Symmetric Stretch). Alkynes were detected at a peak of 3317.6 cm^{-1} (CC Stretch). The peak represents carboxylic acid at 1396.46 cm^{-1} (H-bonded O-H Stretch). A peak at 1095.57 cm^{-1} denotes the secondary alcohol (C=O Stretch). The peaks of 690-515 Nitriles (C≡N Stretch)

Most of these phytoconstituents have been reported to display remarkable biological activity against certain diseases, prevent many diseases, and have health-promoting properties (Aboul-Enein *et al.*, 2014). Unlike most fats, these essential fatty acids cannot be synthesized in the body but can be found in many vegetables in abundance in wild plants (Bhardwaj *et al.*, 2016). The presence of linoleic and linolenic acids in four out of the five selected wild edible vegetables in this study support earlier reports of Melariri *et al.* (2012) and Bhardwaj *et al.* (2016).

The outcome of the study has shed light on the active components present in *S. nigrum* leaves and fruits grafted onto wild solanaceous rootstock *S. torvum* which showed better performance in field conditions and detected chemical bonds/functional groups in plant extracts of all the graft treatments. Furthermore, the analysis of the methanolic plant leaf extract of *S. nigrum* has revealed the presence of various phytochemical substances, each possessing distinct biological properties. These findings emphasize the plant's therapeutic potential, indicating its suitability for further exploration and investigation in medical research. Identifying and characterizing these phytochemical compounds opens up new possibilities for harnessing the therapeutic benefits of *S. nigrum* and warrants continued study and potential application in various areas.

Table 1(a) Chemical composition of *Solanum nigrum* ungrafted leaf (Control) by GC-MS

S. No.	Compound name	Retention time (min)	Area %	Role	Molecular formulae	Molecular weight	+ve/-ve
1	4H-Pyran-4-one, 2,3-dihydro	9.207	4.24	Antifungal property	$C_5H_6O_2$	98.1 g/mol	+ve
2	C8H8O2	16.872	9.81	Antioxidant	$C_8H_8O_2$	136.15 g/mol	+ve
3	Dodecanoic acid,3-hydroxy-	21.270	12.91	Antioxidant, Anti-microbial and Anti-cancerous property	$C_{16}H_{32}O_3$	272.42g/mol	+ve
4	Scyllo-Inositol	26.765	5.41	Plant growth and adaptation	$C_6H_{12}O_6$	180.16g/mol	+ve
5	n-Hexadecanoic acid	28.342	4.90	Anti-inflammatory property	$C_{16}H_{32}O_2$	256.42g/mol	+ve
6	9,12,15- Octadecatrienoic acid, (Z,Z,Z-)	31.642	13.82	Insecticidal and antifeedant properties	$C_{18}H_{30}O_2$	278.4 g/mol	+ve

Table 1(b). Chemical composition of *Solanum nigrum* leaves grafted over *Solanum torvum* rootstock by GC-MS

S. No.	Compound name	Retention time (min)	Area %	Role	Molecular formulae	Molecular weight	+ve/-ve
1	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydro	9.212	6.96	Antifungal property	CH_6O_2	98.1g/mol	+ve
2	2-(Iso Butoxymethyl)oxirane	17.360	5.63	Skin irritant	$C_7H_{14}O_2$	130.18g/mol	-ve
3	Dodecanamide, N-hydroxy-	21.272	15.85	Antioxidant, Anti-microbial and Anti-cancerous property	$C_{12}H_{25}NO_2$	215.33g/mol	+ve
4	3,3-Diethylglutaric acid	22.320	6.39	Metabolite	$C_{11}H_{20}O_4$	216.27g/mol	+ve
5	Linoleic acid-TMS	27.321	3.58	Defense Against Abiotic Stress	$C_{21}H_{40}O_2Si$	352.6 g/mol	+ve
6	9,12,15-Octadecatrienoic acid, (Z,Z,Z-)	31.626	6.56	Insecticidal and anti-feedant property	$C_{18}H_{30}O_2$	278.4 g/mol	+ve

Table 1 (c). Chemical composition of *Solanum nigrum* ungrafted fruit by GC-MS

S. No.	Compound name	Retention time (min)	Area %	Role	Molecular Formulae	Molecular weight	+ve/-ve
1	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydro	9.201	6.07	Antifungal property	$C_5H_6O_2$	98.1 g/mol	+ve
2	1,2,3-Propanetriol, 1-acetate	11.739	4.12	Antiadipogenic	$C_5H_{10}O_4$	134.13g/mol	+ve
3	Dodecanoic acid, 3-hydroxy-	21.095	5.13	Antioxidant, Anti-microbial and Anti-cancerous property	$C_{16}H_{32}O_3$	272.42g/mol	+ve
4	n-Hexadecanoic acid	28.358	14.16	Anti-inflammatory property	$C_{16}H_{32}O_2$	256.42g/mol	+ve
5	10E,12Z-Octadecadienoic acid	31.540	15.35	Hardening soaps, softening plastics and in making cosmetics, candles and plastics	$C_{18}H_{32}O_2$	280.4g/mol	+ve
6	Oleic acid	31.646	11.50	An excipient in pharmaceuticals and as an emulsifying or solubilizing agent in aerosol products	$C_{18}H_{34}O_2$	282.5g/mol	+ve

Table 1(d). Chemical composition of *Solanum nigrum* fruit grafted over *Solanum torvum* rootstock by GC-MS

S. No.	Compound name	Retention time (min)	Area %	Role	Molecular formulae	Molecular weight	+ve/-ve
1	Glycerin	5.209	3.66	Stimulant, Moisture enhancer	$C_3H_8O_3$	92.09g/mol	+ve
2	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydro	9.190	11.25	Antifungal property	$C_5H_6O_2$	98.1 g/mol	+ve
3	1,2,3-Propanetriol, 1-acetate	11.720	7.28	Antiadipogenic	$C_5H_{10}O_4$	134.13g/mol	+ve
4	Heptanoic acid, 6-oxo	12.804	5.68	Synthesis of esters for products such as fragrances and artificial flavor preparations	$C_{20}H_{32}F_2O_5$	390.5g/mol	+ve
5	1,2,3,5-Cyclohexanetetrol, (1.alpha, 2.beta)	21.078	7.96	Antioxidant Antimicrobial Anti-inflammatory	$C_6H_{12}O_4$	148.16g/mol	+ve
6	Methyl butanoate	23.338	0.42	Solvent, fragrance and flavoring agent	$C_5H_{10}O_2$	102.13g/mol	+ve
7	n-Hexadecanoic acid	28.323	7.15	Anti-inflammatory property	$C_{16}H_{32}O_2$	256.42g/mol	+ve
8	10E,12Z-Octadecadienoic acid	31.510	17.75	Hardening soaps, softening plastics and in making cosmetics, candles and plastics	$C_{18}H_{32}O_2$	280.4g/mol	+ve

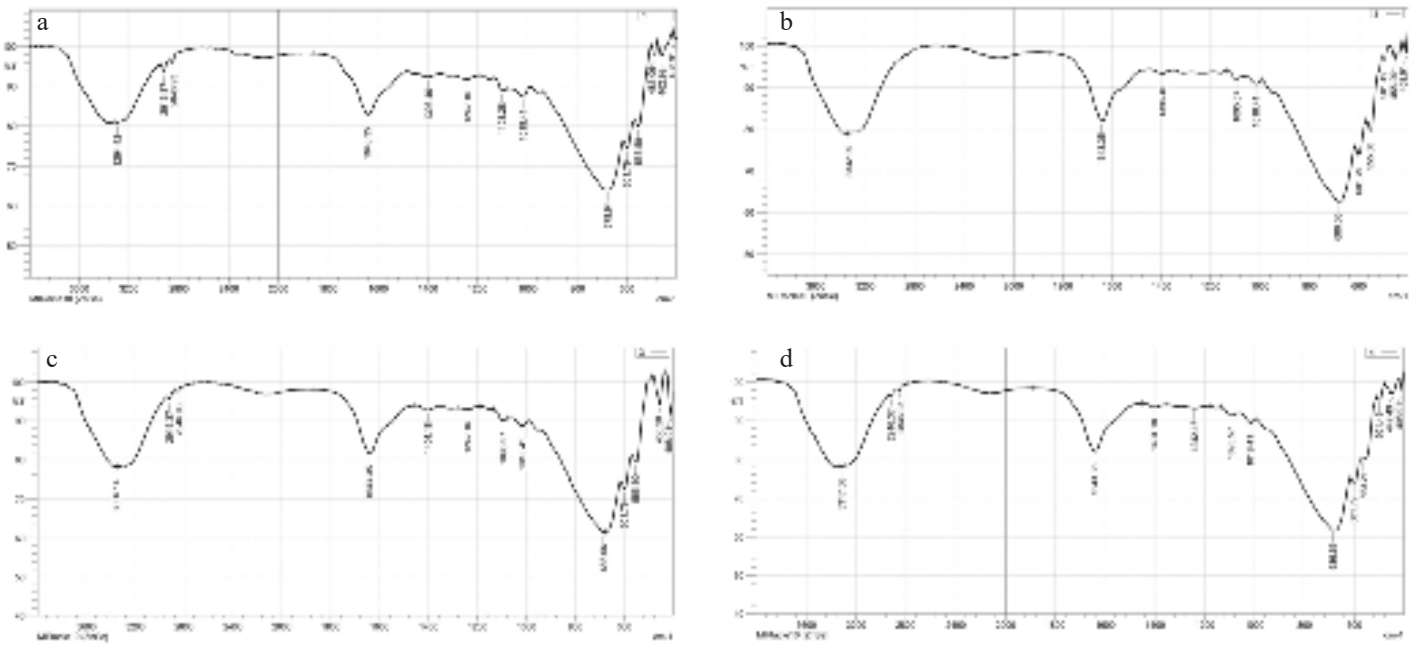

Fig. 2. Fourier transforms infrared spectrophotometer graph. 1. Control (Un-grafted), 2. Grafted onto *S.chrysotrichum*, 3. Grafted on to *S. mauritianum*, 4. Grafted onto *S.torvum*

Table 2. Functional groups and modes of vibration in the spectrum of grafted *Solanum nigrum* over wild rootstocks

Graft combination	Absorption wavelength (λ)	Functional Group	Appearance	Compound Class
Control, (Ungrafted)	678.94	Strong	C-Br stretching	Halo Compound
	1410-1380	S=O stretching	strong	Sulfonyl Chloride
	1396.46	O-H bending	medium	Carboxylic Acid
	1643.35	Medium	C=C stretching	Conjugated Alkene
	3000-2840 (2846.93, 2916.37)	Medium	C-H stretching	Alkane
	3294.42	Strong, Sharp	C-H stretching	Alkyne
Grafted onto *Solanum chrysotrichum*	501.49	Strong	C-I Stretching	Halo Compound
	690-515	Strong	C-Br Stretching	Halo Compound
	686.66	Strong	C=C Bending	Alkene
	1095.57	Strong	C-O Stretching	Secondary Alcohol
	1396.46	Strong	S=O Stretching	Sulfate
	1643.35	Strong	C=C Stretching	Alkene
	3332.99	Strong, Sharp	C-H Stretching	Alkyne
Grafted onto *Solanum mauritianum*	690-515	Strong	C-Br Stretching	Halo Compound
	686.66	Strong	C=C Bending	Alkene
	1095.57	Strong	C-O Stretching	Secondary Alcohol
	1242.16	Strong	C-O Stretching	Alkyl Aryl Ether
	1404.18	Strong	C-O Stretching	Alkyl Aryl Ether
	1643.35	Strong	C=C Stretching	Alkene
	2846.93	Medium	C-H Stretching	Alkane
	3356.14	Medium	N-H Stretching	Aliphatic Primary Amine
Grafted onto *Solanum torvum*	690-515 (601.79, 686.66)	Strong	C-Br Stretching	Halo Compound
	1095.57	Strong	C-O Stretching	Secondary Alcohol
	1242.16	Strong	C-O Stretching	Alkyl Aryl Ether
	1404.18	Strong	C-O Stretching	Alkyl Aryl Ether
	1643.35	Strong	C=C Stretching	Alkene
	2846.93	Medium	C-H Stretching	Alkane
	2916.37	Medium	C-H Stretching	Alkane
	3317.56	Strong, Sharp	C-H Stretching	Alkyne

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