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Innovative nano-solution: Biosynthesized nickel oxide nano-particles (NPs) protect carrot roots from root knot nematode, *Meloidogyne incognita* infestation

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Abstract— *Carrot (Daucus carota L.) is a crucial horticultural crop globally, valued for its nutritional benefits and economic significance. However, it faces substantial challenges from plant-parasitic nematodes, particularly Meloidogyne incognita, which significantly impact yield and quality. In this study, we examined the effect of Meloidogyne incognita (MI) infected carrot plants, that were treated separately with Ocimum santum leaves extract, Nickel sulphate salts and Nickel oxide nanoparticles (NiO-NPs) at different concentration (10, 50, 250, 500, 750 & 1000 ppm) as a pot set experiment. The result focused on the plant physiological, growth and nematode related parameters of MI inoculated plants that were treated with 1000 ppm verses the controlled untreated one. It shows significant plant growth in shoot length increased by 35.47%, root length by 266.4%, shoot fresh weight by 724%, root fresh weight by 631.2%, shoot dry weight by 819%, and root dry weight by 560.1% and the Physiological parameters shows improvement in total chlorophyll content 115% and carotenoid content decreased by 61.26% compared to the untreated control. Substantial reduction in the activity of nematode population per soil, gall formation and eggs per egg mass indicates promising applications of green-synthesized NiO NPs at 1000 ppm as sustainable agents for managing nematode infestations in carrot cultivation, there by contributing to enhanced crop productivity and sustainability in agriculture.*

Keywords— ***Biosynthesized nanoparticles, Meloidogyne incognita, Plant Parasitic Nematodes, Scanning Electron Microscopy***

I. INTRODUCTION

Carrot (*Daucus carota* L.), a member of the Apiaceae family, is known for its aromatic qualities, ornamental uses, and applications in herbal medicine. Approximately 20 species in the *Daucus* genus are distributed globally, the majority of subspecies are found in the western Mediterranean region. Accoding to Food and Agricultural Organization (FAO) Turnips and carrots global output reached 40.24 Mt from 1082967 ha in 2020 and approximately 41.67 Mt from 1096007 ha in 2021 (FAO STAT, hħps://www.fao.org/faostat/en/#data/QCL). China presently holds top position in the world for both carrot production and consumption, which represents about 45% of the worldwide market. The US, Ukraine, Uzbekistan, and Russia are the next in order. Carrots are grown on 97,000 hectares of land in India, producing 1648,000 million tonnes year [1] (Figure 1). Carrot requires cool to moderate temperature and typically have flowers with

flattened umbels and compound leaves. [2]. As a tap root vegetable it has good storage ability possess various health benefits because of their rich in minerals, dietary fiber, vitamins, carotenoids, carbohydrates and antioxidant. Due to β-carotene carrot gets the characteristic orange colour which act as the precursor of Vitamin A. It has the potential to resist the cardiovascular diseases and cancers [3, 4]. Carrots get affected by microorganisms like bacteria, fungi and nematodes and cause major diseases like Alternaria leaf blight by *Alternaria dauci*, C*ercospora carotae*, and *X. hortorum* pv. *carotae* [5,6,7]. Plant parasitic nematode (PPN) cause losses in 40 key crops of 12.3% every year on average; the losses are higher in developing countries (14.6%) than in developed countries (8.8%). The yearly economic crop output losses resulting from plant-parasitic nematodes in main crops have been estimated to be USD 173 billion based on this survey conducted globally [8, 9]. Many nematode species cause significant yield losses annually in agricultural crops, particularly genera such as *Meloidogyne* and *Heterodera*, which are economically impactful due to their sedentary endoparasitic nature. Carrots are severely affected by *Meloidogyne incognita*, and managing this nematode with pesticides or nematicides is challenging due to their environmental damage.

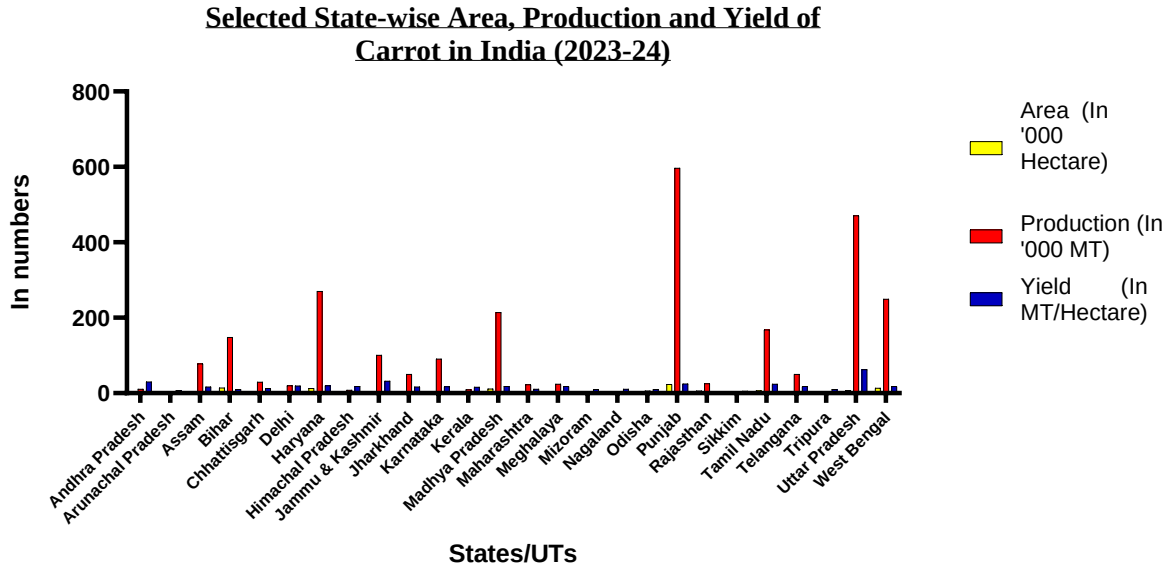


Fig.1: Carrot production states in India 2023-24, based on area (in hectare), production volume (in million metric tons) and yield (Source: https://www.indiastat.com/table/template/agriculture/selected-state-wise-area-production-yield-carrot-i/1455678)

Over the decades the use of biosynthesized nanoparticles in sustainable farming has garnered significant attention [10]. These nanoparticles are typically spherical and polymeric, with sizes ranging from 10 to 100 nm and their properties are determined by their shape, dimensions, structure, and crystallinity [11]. NPs have been used to control nematodes because of large surface areas, which enhance their affinity for the target organism, such as *M. incognita* the RKN nematode [12]. Biological methods for synthesizing nanoparticles, such as those using plants, fungi, and bacteria, are gaining interest due to their eco-friendly nature and avoidance of toxic by-products. Among these, plant-based approaches are particularly advantageous for nanoparticle synthesis as they involve protocols free from harmful chemicals [13]. Tauseef et al., 2021 [14] reported that the use of magnesium oxide nanoparticles on *Meloidogyne* infected cowpea reduced nematode fecundity and the number of galls formed. When tomato plants were treated with biosynthesized silver NPs enhanced the growth parameters with notably reduction in gall formations, Number of eggs per egg mass and egg mass on the roots.

A study by Fabiyi et al., (2024) [15] demonstrates that biosynthesized nanoparticles show promising efficacy in controlling root-knot nematodes, specifically in reducing egg masses, gall index, and population of *Meloidogyne incognita* in cabbage fields, against the standard nematicide carbofuran. This all approach is advantageous due to the nanoparticles are available easily, capability for bulk preparation and handling, as well as their wide range of metabolites that contribute to their effectiveness [16]. However, there is a lack of study in research focusing on the biosynthesis and application of Nickel Oxide

nanoparticles (NiONPs) for controlling root-knot nematodes in carrot cultivation.

This study aims to address this research gap by biosynthesizing NiONPs from *Ocimum sanctum* leaves extract and characterizing the green-synthesized NiONPs. Furthermore, the study investigates the nematicidal activity of these NiONPs against *M. incognita* affecting *Daucus carota* L., with the primary objectives being: (a) biosynthesis of Nickel Oxide nanoparticles, (b) characterization of biosynthesized Nickel Oxide nanoparticles, and (c) evaluation of the nematicidal activity of NiONPs against M. incognita infecting carrots.

II. MATERIALS AND METHODS

2.1 Preparing and sterilising the mixture of soil

Sandy loam soil was collected from the Department of Botany at Aligarh Muslim University (27°52' N and 78°05' E), UP, India and passed through a 20-mesh size sieve. The soil was mixed with organic manure and river sand in 3:1:1 (v/v) ratio, and 9-inch diameter clay pots were filled with 2 kg of this mixture. The soil was pre-wet with 250 ml of water and then sterilized in an autoclave at 121°C under 20 lb pressure for 20 minutes [17]. After sterilization, the pots were cooled to room temperature before being used in the experimental study.

2.2 Raising of experiment plant

Carrot seeds from a certified seed bank in Aligarh were surface-sterilized in 0.01% mercuric chloride ($HgCl_2$) after rinsed with sterile water, and then sown in sterilized soil in 9-inch diameter clay pots. After germination, seedlings were thinned to retain a single seedling per pot [18]. The experiment followed a fully randomized block design with three replications per treatment, and untreated plants were used as control. The study was terminated 90 days after pathogen inoculation.

2.3 Preparation and maintenance of nematode inoculum

Nematode-infected eggplants were collected from fields near Vivekanand College of Technology and Management, Aligarh, and identified as *Meloidogyne incognita* based on perineal patterns [19]. Egg masses were handpicked, washed, and placed in mesh sieves mounted with cross double layered tissue paper over water in Petri dishes, where they were incubated at 25°C until hatching. After 48 hours, second-stage juveniles (J2) were emerged and their density was measured using a stereomicroscope. J2 concentration in water suspension was adjusted such that 200 ± 5 nematodes could be found in each ml.

2.4 Applying nematode inoculum to the test plant

The soil around the root of carrot seedlings was carefully removed and 10 ml of nematode suspension containing 2000 second-stage juveniles (*M. incognita*) was evenly applied. The roots were then immediately covered with the soil.

2.5 Nickel oxide nanoparticles (NiO NPs) synthesis

2.5.1 Preparation of the leaf extract

20 g fresh leaves of *Ocimum sanctum* from the Department of Botany, A.M.U., Aligarh, were washed with tap and distilled water (DW), then boiled in 100 ml of distilled water for ten minutes. The extract was filtered through Whatmann No. 1 filter paper, collected in an Erlenmeyer flask, and refrigerated at 4°C.

2.5.2 Preparation of nickel sulphate ($NiSO_4$) solution

NiO NPs were synthesized using Nickel sulphate ($NiSO_4$), which was purchased from the Thermo fisher Scientific Pvt. Ltd. Aqueous solution of $NiSO_4$ was prepared by adding 6.4 g of $NiSO_4$ in 1 litre

of double distilled water (DDW).

2.5.3 Concoction of $NiSO_4$ biosolution and biosynthesis of NiO NPs

The leaf extract (40 ml) was added to 160 ml of $NiSO_4$ solution to reduce the nickel ions (Ni^{+2}) to nickel nanoparticles (NiO). The contents were boiled for 20 minutes to complete the reaction. The composite mixture was almost transparent having NiO NPs in liquid form (Figure 2). This mixture was centrifuged at 4000 rpm for 10 min, and obtained nanoparticles were dried at 60 ºC for 24 h (Figure. 3; Liquid form of NiO NPs) [20].

2.6 Characterization and analysis of Biosynthesized NiO NPs

2.6.1 Ultraviolet- visible (UV-VIS) Spectroscopy analysis

The qualitative optical properties of biosynthesized NiO nanoparticles (NPs) were analyzed using UV-Vis spectroscopy, a non-destructive technique that measures absorbance across a wavelength range of 200 to 700 nm. A 1 ml sample of the nanoparticle suspension was compared to a 1 ml distilled water reference. The intensity of the reflected light was measured with a UV-Vis spectrophotometer (Schimadzer, Kyoto, Japan, UV-visible spectrophotometer, Model 1800) at a right angle to the light source.

2.6.2 Fourier Transform Infrared Spectroscopy (FTIR) analysis

The Fourier transformed infrared (FTIR) spectrophotometer analyzes NiO nanoparticles by passing 100 to 10000 cm^{-1} infrared radiation through the sample, where absorbed radiation provides information on

molecular bonding through vibrational and rotational energy. The resulting spectrum, typically ranging from 400 cm^{-1} to 4000 cm^{-1}, serves as a molecular fingerprint for chemical identification. Peaks in the spectrum, compared with the Merck Infrared chart, indicate interactions and bond characteristics.

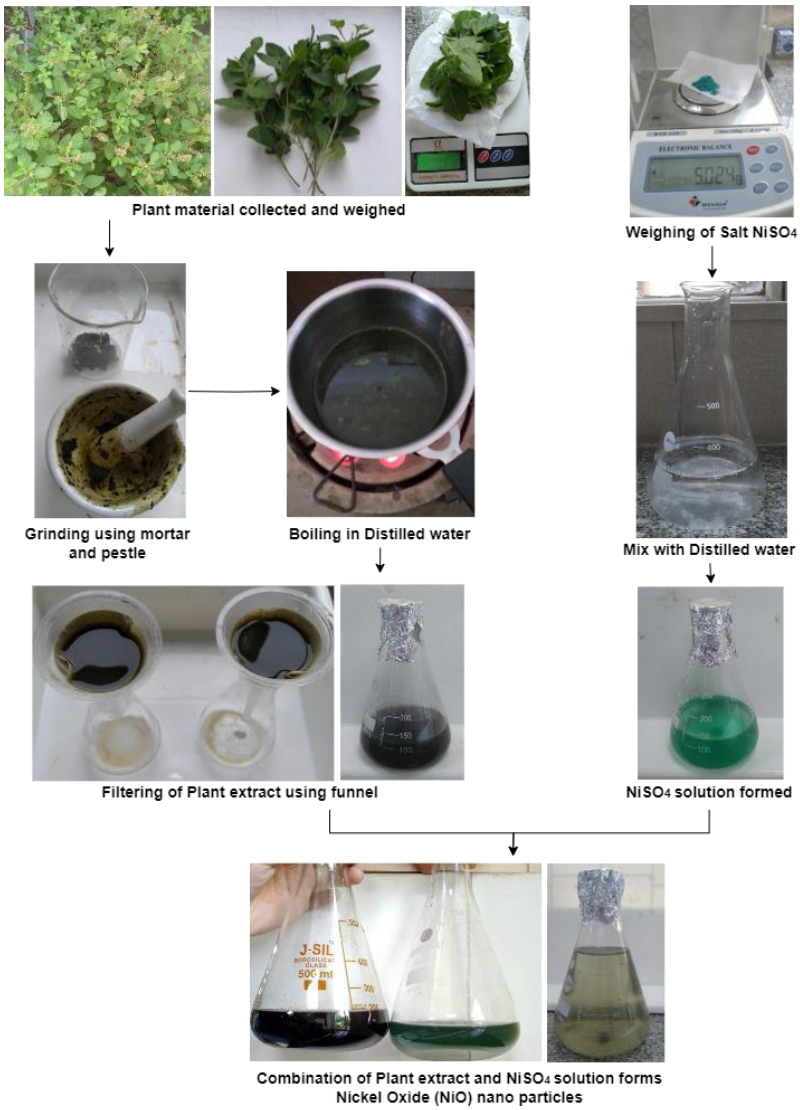


Fig.2: Preparation of Nickel oxide nanoparticles

2.6.3 Scanning Electron Microscopy (SEM) analysis

Scanning electron microscopy (SEM) was employed to examine the surface morphology of NiO nanoparticles. A small amount of the sample was applied to a carbon-coated copper grid to create thin film and excess solution was blotted off. Let the grid to dry under a mercury lamp. The dried grid was then analyzed using a scanning electron microscope (JSM6510 , JEOL, Japan).

2.7 NiO NPs inoculation to the test plant

Biosynthesized NiO nanoparticles (NPs) were applied to plants at concentrations of 10 ppm, 50 ppm, 250 ppm, 500 ppm, 750 ppm, and 1000 ppm. Soil around the roots was gently removed, and the NPs were introduced using a micropipette. The roots were then covered with soil, and the plants were regularly watered until the end of the experiment.

2.8 Experimental setup

The experiments were conducted during the winter season (November to January) using a completely randomized block design, with three variable treatments for each NiO NPs concentration and three replicates per treatment (Table 1). Pots were watered as needed and placed on glasshouse benches with temperatures ranging from 25-30 ^{o}C.

2.9 Plant growth parameters

After 90 days of nematode inoculation, plants were harvested and roots were carefully washed. Plants were cut above the root base for height and weight measurements,

while shoot and root lengths were recorded from the cut end to the top leaf and longest root, respectively. Fresh weights were recorded after blotting excess water, and dry weights were determined after drying in an oven at 60ºC for 72 hours.

Table 1: Treatments given to the testing plant at different concentration

Designation	Treatment
C	Control plant (untreated)
T1	$NiSO_4$ salt only
T2	*Meloidogyne incognita* (MI)
T3	MI + $NiSO_4$ salt
T4	MI + Plant extract
T5	MI + NiO NPs (10 ppm)
T6	MI + NiO NPs (50 ppm)
T7	MI + NiO NPs (250 ppm)
T8	MI + NiO NPs (500 ppm)
T9	MI + NiO NPs (750 ppm)
T10	MI + NiO NPs (1000 ppm)

2.9.1 Estimation of Chlorophyll content

Chlorophyll content in fresh leaves was estimated using Mackinney (1941) and Lichtenthaler and Welburn (1983) methods. Leaves were ground with 80% acetone, centrifuged, and the supernatant's absorbance measured at 645 nm and 663 nm. Chlorophyll content was calculated using Arnon's (1949) formula: Total chlorophyll (mg g-1 FW) = 20.2 (A645) + 8.02 (A663) x V/100 x W.

2.9.2 Estimation of Carotenoid content

Carotenoid content in fresh leaves was estimated using Mac Lachlan and Zalik's (1963) method, similar to chlorophyll extraction. Absorbance was measured at 480 nm and 510 nm using a spectrophotometer (EI 3305) with 80% acetone as a blank. Carotenoid content (mg g-1 FW) was calculated using the formula: 7.6 (OD 480) – 1.49 (OD 510) x V/D x W x 1000.

2.9.3 Estimation of Phenolic content

100 mg (0.1 g) of fresh leaves were homogenized in 2 ml of 80% methanol and agitated at 70 ºC for 15 mins (Zieslin and Zaken, 1993). Homogenate was collected in a test tube and 2.5 ml of 10% Folin–Ciocalteu reagent (FCR) and 2.5 ml of 7.5 % sodium bicarbonate ($NaHCO_3$) solution were added to it. This mixture was thoroughly mixed before incubating at 45 ºC for 45 minutes. An absorbance was recorded at 765 nm in a spectrophotometer (Model: EI 3305) against blank (all the mixture content without leaf material). Total phenolic content subsequently calculated and expressed as mg gallic acid equivalent (GAE) per gram of dry extract (mg GAE/g dw) (Patel et al., 2010; Ashraf et al., 2021).

2.9.4 Estimation of Proline content

50 mg of leaf tissue were homogenised in 7.5 ml of sulphosalicylic acid (3% aqueous) (SSA). The homogenate was placed in a centrifuge tube and spun for five minutes at 5,000 rpm. The pellet was disposed of and the supernatant was collected. A test tube was filled with one ml of leaf extract, one ml of glacial acetic acid (GAA), and one ml of acid ninhydrin were added to it. This mixture was heated in boiling water bath for 1 h and the reaction was terminated by placing the test tube in an ice bath. After cooling, 2 ml of toluene was added to it. After shaking it for 30 sec, two heterogenous layers were formed. The upper layer was pipetted out carefully and absorbance was recorded at 520 nm. Toluene was used as blank [21].

2.10 Nematode Related Parameters

2.10.1 Nematode population per 250g soil

Soil in each pot was well mixed before the juveniles were extracted using the Cobb's sieving, and decanting method followed by Baermann funnel technique. The nematode suspension was homogenized and 5 ml was transferred to a counting dish. Nematode populations per 250 g of soil were calculated by counting under a stereoscopic microscope [22].

2.10.2 Number of galls and egg masses per root system

At the end of the experiment, harvested plant roots were washed with tap water, and gall counts were recorded visually. For egg mass assessment, roots were immersed in 20 ml of 2% NaOCl solution and agitated for 1 minute. After dyeing with acid Fuchsin, egg masses were counted under a stereoscopic microscope [23].

2.10.3 Fecundity

Fecundity, the number of eggs per egg mass, was assessed using the Chlorax method by shaking 1g 1-2cm cut pieces of root in a 1.0% NaOCl solution for 5 minutes and filtering through 200- and 500-mesh sieves. Then eggs were rinsed with cold water to remove NaOCl and counted under a stereoscopic microscope. The total number of eggs was determined by multiplying the count by the fresh weight of the root in each treatment [24].

2.10.4 Reproduction factor

Soil in each pot was thoroughly mixed and juveniles were collected by Cobb’s sieving and decanting method followed by Baermann funnel technique.

$$Rf = \frac{P_f}{P_i}$$

Where,

R_f = reproduction factor

P_f = final nematode population

P_i = initial nematode population

2.10.5 Root-knot gall index

The root-knot gall indices were determined on 0 to 5 scale, where 0 = no gall, 1 = 1 to 2 galls, 2 = 3 to 10 galls, 3 = 11 to 30 galls, 4 = 31 to 100 galls and 5 = >100 galls per root system [25, 26].

2.11 Statistical analysis

The whole data collected during the experiment was subjected to analysis of variance statistically. Least significant difference (LSD) was calculated at $p \leq 0.05$. Duncan's multiple range test (DMRT) was employed to indicate the level of significance between the treatments [27].

III. RESULTS

3.1 Biosynthesized NiO NPs: Characterization

3.1.1 Ultraviolet-visible (UV-Vis) Spectroscopy analysis

Ultraviolet-visible spectroscopy was utilized to analyze the optical characteristics of biogenically synthesized NiO nanoparticles using a Perkin-Elmer-Lambda 365 UV-vis spectrophotometer. The spectra of bio-synthesized NiO NPs have been examined in the 200 – 750 nm range, and outcomes are displayed in (figure 4). The presence of an excitonic peak in the ultraviolet range, coupled with absorption in the visible spectrum, clearly demonstrates the UV and visible light responsiveness of NiO nanoparticles. It is a useful material for photovoltaic applications because of its high transparency in the visible area and low transparency in the UV region. The good optical properties of the synthesised NiO NPs are indicated by the absence of other peaks in the spectrum.

3.1.2 Fourier Transform Infrared Spectroscopy (FTIR) analysis

FTIR analysis provides information about the vibrational characteristics of NiO nanoparticles. FTIR analysis provides information about the vibrational signatures of NiO nanoparticles. The peak observed at 702.84 cm^{-1} indicates the presence of Ni-O stretching vibrations. Other peaks within the range of 500–4000 cm^{-1} correspond to vibrations from molecules affixed to the surface of the NiO nanoparticles. The stretching vibration of the C=C bond in alkane groups is detected at 1633.80 cm^{-1}. Additionally, a peak at 3436.32 cm^{-1} signifies OH stretching vibrations, indicating the presence of absorbed CO_2 and molecular water on the nanoparticle surface (Figure 5).

3.1.3 Scanning Electron Microscopy (SEM) analysis

The morphology of the synthesized NiO nanostructure was analyzed using SEM images (figure 6). SEM micrographs at different magnifications, specifically (a and b) 10 µm, (c) 5 µm, and (d) 1 µm, clearly depict the formation of NiO nanosheets that are randomly distributed and have well-defined particle boundaries. These NiO nanosheets are uniformly shaped and have a thickness ranging from 15 to 40 nm.

Fig.3: NiONPs in liquid form

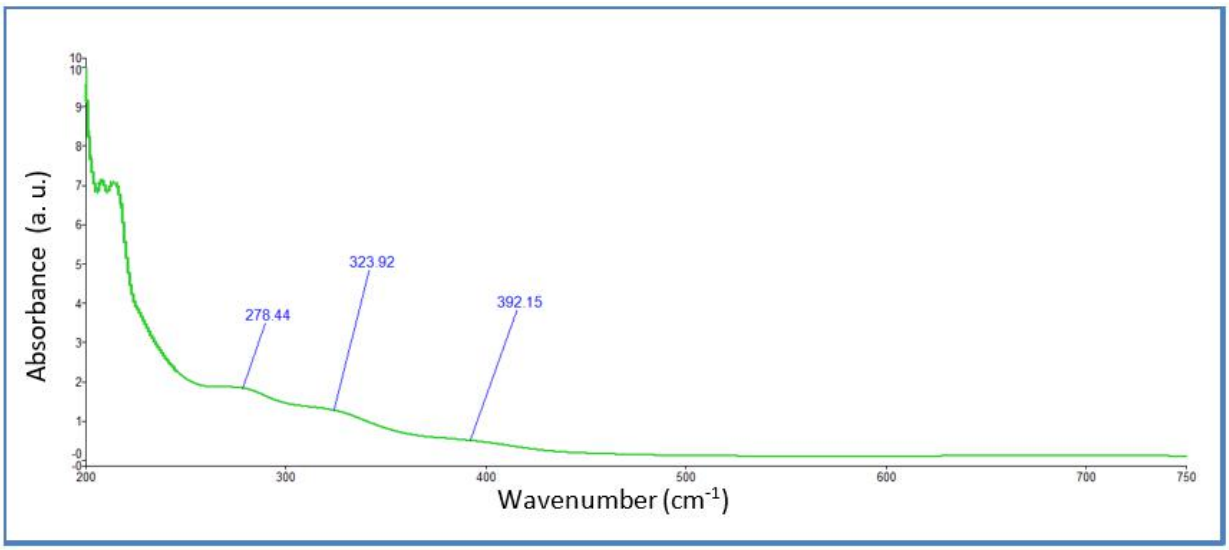


Fig.4: UV–Vis spectrum analysis of biosynthesized NiONPs

3.2 Plant growth Parameters

M. incognita led to a considerable reduction in all growth parameters of carrot plants. Inoculation with *M. incognita* resulted in a decrease of 30.26% in shoot length, 75.9% in root length, 83.42% in shoot fresh weight, 90.43% in root fresh weight, 89.8% in shoot dry weight, and 89.17% in root dry weight. The application of NiO nanoparticles at various concentrations significantly enhanced all plant growth parameters compared to inoculated plants. Among the treatments, plants treated with a 1000 ppm solution of NiO nanoparticles showed the greatest improvements: shoot length increased by 35.47%, root length by 266.4%, shoot fresh weight by 724%, root fresh weight by 631.2%, shoot dry weight by 819%, and root dry weight by 560.1% compared to inoculated plants.

Plants treated with a 10 ppm solution of NiO nanoparticles exhibited substantial enhancements in shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight, increasing by 18.49%, 190.5%, 101.3%, 216.9%, 71.

4%, and 163.8%, respectively as compared to plants that were only inoculated. (Table-3) (Figure 7.).

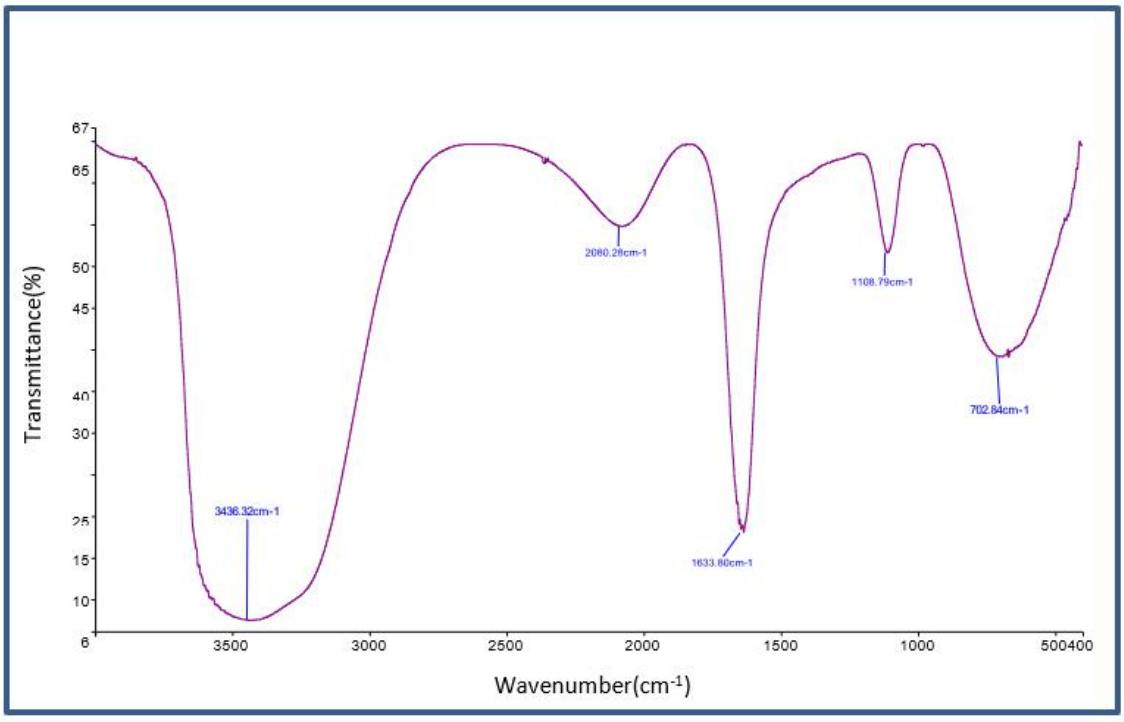
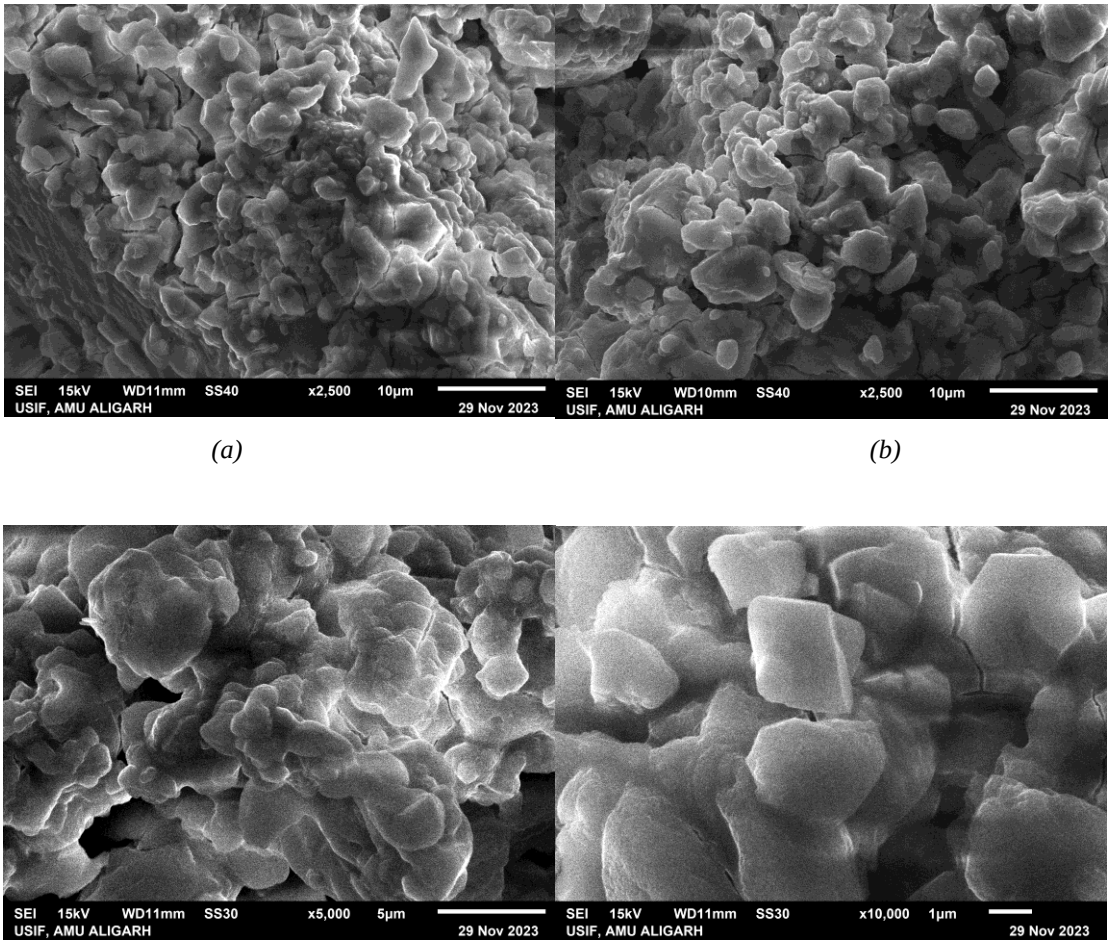


Fig.5: FTIR spectrum of Biosynthesized NiONPs



(a) *(b)*

(c) *(d)*

Fig.6 (a-d): SEM images of biosynthesized NiONPs

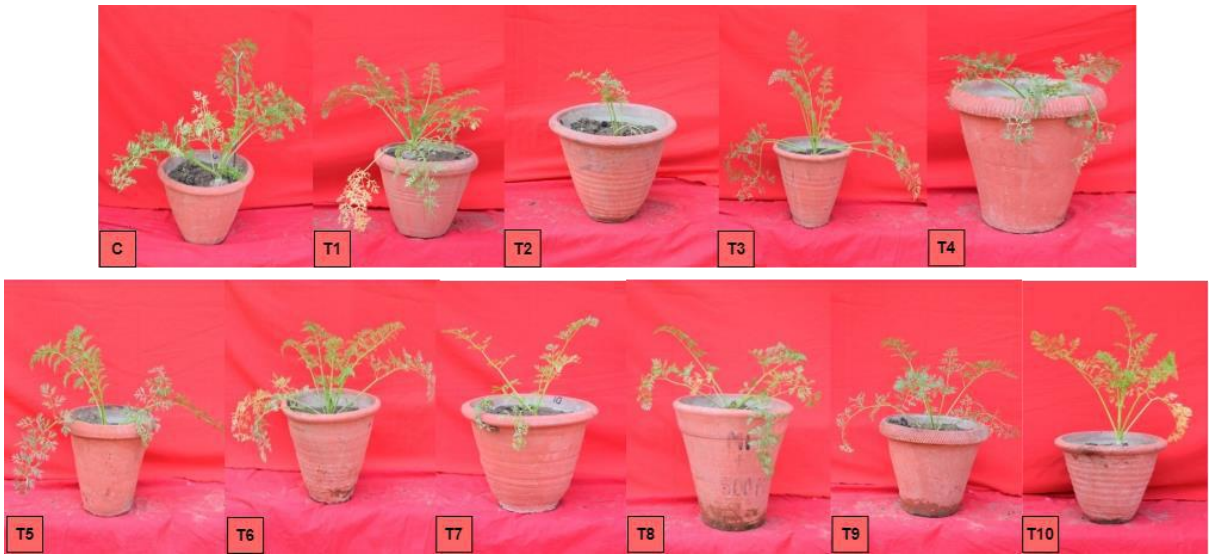


Fig.7: C = Control (untreated); T1 = $NiSO_4$ salt; T2 = Meloidogyne incognita (MI); T3 = MI + $NiSO_4$; T4 = MI + Plant extract; T5 = MI + 10 Ppm (NiO NPs solution); T6 = MI + 50 Ppm (NiO NPs solution); T7 = MI + 250 Ppm (NiO NPs solution); T8 = MI + 500 Ppm (NiO NPs solution); T9 = MI + 750 Ppm (NiO NPs solution); T10 = MI + 1000 Ppm (NiO NPs solution)

3.3 Physiological Parameters

3.3.1 Chlorophyll and carotenoid content

M. incognita caused substantial decrease in chlorophyll content by 53.55% as compared to untreated control. Application of NiO NPs solution caused considerable enhancement in chlorophyll content as compared to inoculated control. NiO NPs, at 1000 ppm, shows maximum improvement in total chlorophyll content by 115% as evaluating with inoculated control (Table-2). *M. incognita* caused pronounced decrease in carotenoid content by 61.26% as compared to untreated control.

Application of NiO NPs caused considerable enhancement in carotenoid content as compared to inoculated control. NiO NPs, at 1000 Ppm, shows maximum improvement in carotenoid content by 158% as compared to inoculated control. As compared to only inoculated plants, plants treated with 10 ppm solution of NiO NPs also showed significant improvement in chlorophyll by 10.8% and carotenoid content by 14.5%.(Table-2).

Table 2: Effect of different concentration of nickel nanoparticle treatments on plant growth parameters of carrot infected M.incognita.

Treatments	Length (cm)		Fresh weight (g)		Dry weight(g)		Total Chlorophyll (mg/g leaf tissue)	Total Carotenoids (mg/g)	Phenolic content (765nm) (mg/g)	Proline content (520nm) (mg/g)
	Shoot	Root	Shoot	Root	Shoot	Root	3.38±0.011^{a}	1.42±0.011^{a}	0.452±0.001^{d}	0.028±0.001^{i}
C	38±1.15^{a}	22±1.15^{a}	22.02±1.12^{b}	106.77±1.38^{a}	4.12±0.01^{a}	9.98±0.005^{a}	1.41±0.17^{f}	0.56±0.011^{d}	0.502±0.021^{c}	0.089±0.001^{g}
T1	27±1.15^{f}	10±1.15^{f}	6.38±0.02^{e}	25.36±0.023^{g}	0.45±0.02^{hi}	2.26±0.011^{i}	0.88±0.005^{g}	0.07±0.011^{c}	0.702±0.001^{a}	0.565±0.001^{a}
T2	26.5±0.23^{f}	5.3±1.52^{g}	3.65±0.03^{f}	10.21±0.017^{h}	0.42±0.01^{i}	1.08±0.023^{j}	1.57±0.011^{ef}	0.55±0.015^{d}	0.569±0.003^{b}	0.091±0.001^{g}
T3	30±1.73^{de}	13.5±1.53^{de}	7.06±0.01^{e}	26.68±0.02^{g}	0.55±0.01^{g}	2.55±0.023^{h}	0.92±0.011^{g}	0.54±0.011^{d}	0.577±0.001^{b}	0.468±0.001^{b}
T4	27.3±0.55^{ef}	12±1.3^{ef}	6.84±0.02^{e}	25.52±0.026^{g}	0.50±0.02^{gh}	2.28±0.011^{i}	1.67±0.011^{e}	0.59±0.017^{cd}	0.562±0.001^{b}	0.162±0.001^{c}
T5	31.4±0.7^{cd}	15.4±0.26^{cd}	7.35±0.02^{e}	32.36±0.017^{f}	0.72±0.02^{f}	2.85±0.023^{g}	1.74±0.011^{de}	0.63±0.115^{cd}	0.549±0.023^{b}	0.142±0.001^{d}
T6	31.7±0.78^{cd}	16±1.52^{cd}	12.74±0.02^{d}	37.04±0.011^{e}	1.74±0.01^{e}	3.40±0.115^{f}	1.92±0.015^{d}	0.70±0.017^{c}	0.509±0.001^{c}	0.120±0.001^{e}
T7	32.6±0.11^{cd}	16.8±0.32^{bc}	13.06±0.09^{d}	41.84±0.011^{d}	2.02±0.2^{d}	4.04±0.011^{e}	2.36±0.011^{c}	0.93±0.115^{b}	0.498±0.027^{c}	0.107±0.011^{ef}
T8	33.3±0.11^{bc}	17.1±0.35^{bc}	13.32±0.18^{d}	43.13±0.017^{d}	2.03±0.01^{d}	4.70±0.115^{d}	2.80±0.011^{b}	1.01±0.011^{b}	0.485±0.002^{cd}	0.102±0.001^{fg}
T9	33.5±1.72^{bc}	17.6±0.17^{bc}	16.43±1.24^{c}	68.15±0.476^{c}	2.58±0.02^{c}	6.55±0.015^{c}	3.33±0.115^{a}	1.04±0.015^{b}	0.458±0.001^{d}	0.060±0.001^{h}
T10	35.9±0.25^{ab}	19.4±0.23^{ab}	30.10±1.80^{a}	74.69±0.023^{b}	3.86±0.01^{b}	7.13±0.011^{b}	3.38±0.017^{a}	1.42±0.011^{a}	0.452±0.001^{d}	0.028±0.011^{i}
LSD	2.82	0.64	2.18	1.29	0.05	0.15	0.18	0.109	0.037	0.014

*Means with same letter are not significantly different according to Duncan Multiple Range Test (p0.05). Each value is a mean of three replicates.

**C= Untreated control; T1= $NiSO_4$ (1000 ppm); T2 =MI; T3= MI + $NISO_4$; T4 =MI+ Plant Extract (20 ml); T5 =MI +NiO NPs (10 ppm); T6 =MI +NiO NPs (50 ppm); T7 =MI +NiO NPs (250 ppm); T8 =MI +NiO NPs (500ppm); T9 =MI +NiO NPs (750 ppm); T10 =MI +NiO NPs (1000 ppm); LSD =Least Standard Deviation.

Table 3: Effect of different concentration of nickel nanoparticle on nematode related parameter of root-knot nematode, M. incognita infecting carrot plant.

Treatment	Total soil nematode population (250g)	Number of galls	Number of egg masses	Fecundity	Reproduction factor	RKI
C	0	0	0	0	0	0
T1	0	0	0	0	0	0
T2	900±7.63^{a}	108±1.15^{a}	85.8±1.94^{a}	311±1.15^{a}	2.53±0.03^{a}	5±2.3^{a}
T3	765±2.08^{b}	84±1.15^{bc}	74±1.15^{b}	241±1.52^{b}	1.87±0.02^{b}	4±1.15^{a}
T4	630±1.52^{c}	87±1.73^{b}	69.9±1.46^{c}	206±1.15^{c}	1.72±0.01^{c}	4±0.57^{a}
T5	585±2.51^{d}	81±1.73^{c}	60.3±1.19^{d}	194±2^{d}	1.64±0.01^{d}	4±1.15^{a}
T6	550±2.3^{e}	73±1.73^{d}	54±1.15^{e}	183±1.73^{e}	1.35±0.01^{e}	4±1.73^{a}
T7	471±3^{f}	65±2.88^{e}	51.8±1.15^{e}	177±2.08^{f}	1.12±0.01^{f}	4±2.51^{a}
T8	419±3.51^{g}	57±1.15^{f}	45.7±1.28^{f}	173±1.52^{f}	0.99±0.01^{g}	4±1.52^{a}
T9	390±1.52^{h}	48±1.15^{g}	43±1^{g}	165±1.52^{g}	0.94±0.01^{h}	4±1.52^{a}
T10	362±2.3^{i}	28±2.3^{h}	41±1.52^{g}	143±1.52^{h}	0.84±0.01^{i}	3±1.52^{a}
LSD	9.08	4.67	3.57	4.27	0.04	4.32

*****Means with same letter are not significantly different according to Duncan Multiple Range Test (p0.05). Each value is a mean of three replicates.

**C= Untreated control; T1= $NiSO_4$ (1000 ppm); T2 =MI; T3= MI + $NISO_4$; T4 =MI+ Plant Extract (20 ml); T5 =MI +NiO NPs (10 ppm); T6 =MI +NiO NPs (50 ppm); T7 =MI +NiO NPs (2 50 ppm); T8 =MI +NiO NPs (500ppm); T9 =MI +NiO NPs (750 ppm); T10 =MI +NiO NPs (1000 ppm); LSD =Least Standard Deviation.

Fig.8: C = Control (untreated); T1 = $NiSO_4$ salt; T2 = Meloidogyne incognita (MI); T3 = MI + $NiSO_4$; T4 = MI + Plant extract; T5 = MI + 10 Ppm (NiO NPs solution); T6 = MI + 50 Ppm (NiO NPs solution); T7 = MI + 250 Ppm (NiO NPs solution); T8 = MI + 500 Ppm (NiO NPs solution); T9 = MI + 750 Ppm (NiO NPs solution); T10 = MI + 1000 Ppm (NiO NPs solution)

3.3.2 Phenolic content

Compared to the untreated plants (control), the treated plants had noticeably higher phenol content. *M. incognita* caused the significant increase in phenol content by 25.8% as compared to untreated control. NiO NPs (1000 ppm) shown maximum increase in phenol content by 20.7% as compared to untreated control.

As compared to only inoculated plants, plants treated with 10 ppm solutions of NiO NPs also showed significant increase in phenolic content by 3.51 % (Table-2).

3.4 Nematode Related Parameters

Application of NiO NPs at 1000 ppm caused significant decrease in nematode population per 250 g of soil by 59.77%. When *M. incognita* was used alone, there were a lot of galls per root system and nematode proliferation (Table-3).

NiO NPs caused the significant decrease number of galls, the highest reduction being in case of 1000 ppm solution of NiO NPs in T10, by 74.07 %. Number of eggs per egg mass was found significantly decreased in plants treated with 1000 ppm solution of NiO NPs in T10; the highest reduction is by 54.65% (Figure 8). There was a decrease in fecundity by 54.01% and reproduction factor by 66.79%.

IV. DISCUSSION

Root knot nematodes, which are polyphagous sedentary endoparasites, pose a significant threat to global agricultural production, particularly in vegetable fields such as those growing carrots [28]. Nematicides are highly toxic and adversely affect the environment and may restrict their usage against plant parasitic nematodes. The interest in creating biological control strategies has increased due to the necessity for ecologically friendly control methods. Traditional methods of synthesizing nanomaterials involve the use of chemicals and solvents that pose hazards to the nature, human and animal health. In contrast, green synthesis utilizes organic compounds like plant extracts and solvents with minimal or no toxicity, promoting an environmentally sustainable approach. The synthesis of nanoparticles can use as an alternative due to their cytotoxicity, physicochemical traits, and biological properties [29, 30]. NiO nanoparticles fabricated using extracts from *Ananas comosus, Hordeum vulgare, Calotropis gigantea, Ocimum sanctum* and *Brassica rapa* leaf have shown consistent and trustworthy biochemical properties in vitro. The results demonstrate the usefulness of biogenic NiO NPs in agricultural and biomedical domains because of their intrinsic anti-pathogenic characteristics, controlled size, oxidative stress-generating capacity, and conducting nature [31, 32].

In this research, we have presented a sustainable method for synthesizing NiO nanoparticles using extracts from the *Ocimum sanctum* plant. NiO nanoparticles, appearing as an olive-green precipitate, were obtained after drying for 24 hours at 60°C. Nickel oxide is the second largest prevalent metal oxide, known for its affordability, safety, and straightforward preparation process.

Characterization involved UV-Vis spectrophotometry, FTIR analysis, and SEM analysis. According to Medda et al. (2015), nanoparticles typically exhibit absorbance spectra between 400-700 nm when synthesized from their respective salt precursors, showing a distinct and can perceive a prominent peak in the spectrum of visible light. This characteristic is often attributed to the close proximity of nickel nanoparticles' conduction and valence bands, facilitating electron movement and resulting in a pronounced surface plasmon resonance peak [33].This peak likely arises from nickel oxide band gap absorption, where electrons transition from the valence to the conduction band. UV-Vis spectrum analysis examined the spectra of biogenically synthesized NiO nanoparticles within the 200 – 750 nm range. FTIR analysis revealed a peak at 702.84 cm^{-1}, and SEM analysis indicated that the average thickness of NiO particles ranged from 15-40 nm, exhibiting a hexagonal shape.

V. CONCLUSION

The study utilized *Ocimum sanctum* leaf extract to biosynthesize NiO nanoparticles, characterized by FTIR, SEM, and UV-Vis techniques for revealing their shape, size, surface appearance, structural organization and purity of NPs and the presence of secondary metabolites in leaf extract, which served capping agents for the production of NPs. These nanoparticles contain specific phytochemicals and secondary metabolites that can provide resistance to pests and pathogens, making them a safer alternative to various pesticides. The green-synthesized NiO NPs effectively controlled *Meloidogyne incognita* in *Daucus carota*, enhancing plant growth and showing significant reduction in nematodal activity at various concentrations, particularly at 1000 ppm.

The nanoparticles, rich in phytochemicals, offer a safer and more eco-friendly alternative to conventional pesticides. Overall, this plant-based synthesis method is a promising, cost-effective and sustainable approach for managing plant pathogens and advancing agricultural research.

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