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Research Paper

GC-MS Profiling of Palmarosa Essential Oil to Identify Bioactive Compounds and Develop Antiviral Metal Based Nano Emulsion for Chili Crop

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A B S T R A C T

Chili leaf curl virus causes yield loss of around 30% - 40% in chili crop. Present study looked at the alternative of pesticides to manage leaf curl disease with the help of nanotechnology and plant extract. Essential oil of Palmarosa leaves were extracted by hydro-distillation method using Clevenger apparatus yielded 1.2% of essential oil. GC-MS analysis of essential oil identified major bioactive compounds as Geraniol (47.82%), Linalool (12.09%), Caryophyllene (11.20%), Elemene (6.45%) and Geranyl acetate (4.60%) reported for antiviral, antimicrobial and pest repellent activities. Silver nanoparticles were synthesized by tri-Sodium Citrate reductant and particle size of silver nanoparticles obtained 59.8 nm. The essential oil (Concentration 0.1%, 0.2%, 0.3% and 0.4%) utilized to develop nano emulsion with silver nanoparticles and size of nano emulsion obtained 102.3 nm, 113.8 nm, 134.7 nm and 150.1 nm respectively. Zeta potential indicated better stability and solubility of nano emulsion after essential oil interaction with AgNPs. FTIR spectra of silver nanoparticles showed stretch which confirms presence of silver nanoparticles on to bioactive compounds. Scanning electron micrograph showed spherical shape of silver nanoparticles after interaction with bioactive compounds.

***Key words*:** Chili leaf curl virus, Essential oil, Nano emulsion, Silver nanoparticles

Chili leaf curl virus (Chclv) is an is an economically important plant pathogen of chili plant and caused huge amount of losses [1]. In modernization, many nanotechnological inventions proved a good performance for effective antimicrobials [2]. This phenomenon increased interest to develop a new beneficial, more effective and eco-friendly antiviral agents due to heavy economic losses by plant viral disease [3].

Cymbopogon martini is widely cultivated due to commercial importance of their essential oils. It is used in fragrances, Soaps, tobacco industries, cosmetics and other industrial products. It is comparatively easy to cultivate this crop due to its special quality to get adapated and well grown in different types of soils [4]. Essential oils from Palmarosa have different terpenoids like, geraniol, Citronellol and other which are used in perfume preparation. Palmarosa essential oil have so many important compounds which shows anthelmintic, antiageing, antimicrobial, antiviral, anti-inflammatory and pesticidal activities thus, are widely become composition of so many medicines and biopesticides [5]. Essential oils found active against viruses also. Palmarosa, Lemongrass and Roman chamomile showed a high level of inhibitory effects against bacteriophages. Different Eos and their extracted compounds in gaseous phase and liquid phase successfully showed antiviral activity. For example, citronellol and eugenol in gaseous form extracted from *Citrus bergamia* and *Eucalyptus globules* showed noticeable activity against Influenza virus (H1N1). Essential oils of *C. zeylanicum*, *C. bergamia*, *C. flexuosus* and *Thymus vulgaris* showed 100% antiviral activity. When 0.5 mg/ml of *Trachyspermum ammi* (ajwain) oil exposed to Japanese encephalitis virus (JEV), it gives 40% inhibition activity against these viruses [6].

Silver has identified as possessing great inhibition characteristics against broad spectrum of microorganisms [7]. Silver nanoparticles have attracted attention of researchers due to unique antimicrobial and other biological properties in different fields, even in plant pathogen management also. Silver nanoparticles have antiviral activity against human disease-causing viruses Hepatitis B virus [8], Herpes simplex virus, Human immunodeficiency virus and Influenza virus. There is some evidence against plant disease causing viruses are there for example spraying of silver nanoparticles on faba bean leaves can be reduce the disease symptoms of Bean yellow mosaic virus (BYMV). Spraying of 50 ppm colloidal solution of silver nanoparticles on cluster bean leaves infected with *Sunhemp rosette virus* (SHRV), potentially inhibited virus and complete curing of the disease [9].

Essential oil is immiscible in aqueous solution and separate with each other to form different phase. There are different surfactants available which reduced the surface

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tension and mix both phases. When surfactant is added in aqueous solution, it reduces the surface tension by decreasing the droplet size to make oil in water emulsion [10]. There are many benefits of nanoparticles due to their lesser size. Sedimentation method, Laser diffraction method and microscopic image analysis are different methods to analyze particles size [11].

The aim of study was to develop an alternative of toxic pesticides used in plant pathogen management. It is well known that silver nanoparticles and palmarosa essential oil both have good antiviral activity. Nano emulsion with lesser size and more stability can give better antiviral activity. The main purpose of this study is to make a better antiviral nano emulsion by mixing both substances in proper ratio. Silver nanoparticles were synthesized and essential oil of Palmarosa was extracted and characterized. Then different concentration of essential oil with Silver nanoparticles were tried to check that which ratio will give best and suitable oil in water emulsion.

MATERIALS AND METHODS

Chemicals were used of analytical grade from Merck Chemicals and HiMedia. The water was used of Milli Q grade to avoid any interference of ions in the synthesis of nanoparticles.

Extraction of essential oil from palmarosa leaves

Palmarosa essential oil is extracted from the Palmarosa leaves using hydro distillation process using Clevenger apparatus. Palmarosa leaves were collected and washed with tap water. 1.5 Kg Palmarosa leaves were crushed by homogenizer to get a paste and transferred into a 10 liter round bottom flask. Approximately 4 liter of distilled water was added and mixed well using glass rod. The crushed sample was allowed to soak overnight in distilled water.

Next day the sample was again mixed with glass rod and 4-5 porcelain pieces were added to avoid bumping. Then the round bottom flask was placed on heating mantle, the flask was connected to a Clevenger apparatus (lighter than water) and the Clevenger apparatus was connected to an inner coil condenser; where chilled water was continuously circulating.

Then temperature was raised to 380-400°C. Once the entire mixture starts boiling after approximately 30 minute the temperature was reduced to 300°C to 320°C and continued for 4-5 hours [12]. The condensate was collected in Clevenger which continuous transferred into separating funnel until the layer of oil and water separated. The separated oil phase was collected into test tube, which contain 0.5 gm. Anhydrous Sodium sulphate to remove moisture. Volatile oil was collected into a clean glass vial; vial cap was sealed with parafilm and stored under refrigeration at 2-8°C until use for analysis.

Qualitative identification of chemical constituents of essential oil of Palmarosa using GC-MS

The extracted essential oil samples were analyzed using Shimadzu GC-2010 system comprising an AOC-20i auto-sampler and interfaced to a Mass Spectrometer (QP Plus 2010) equipped with a DB-17 MS (50% -Phenyl-50% Methyl Polysiloxane, mid polarity fused a capillary column (30 × 0.25 µm ID × 0.25 µm df) [13].

For GC-MS detection, an electron ionization system was operated in Electron Impact (EI) mode with ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 1 µl was employed (a split ratio of 50:1). The injector temperature was maintained at 250°C, the ion-source temperature was 200 °C, the oven temperature was programmed from 40°C (isothermal for 3 min), with an increase of 6°C/min to 290°C (isothermal for 10 min). Mass spectra were taken at 70 eV; a scan interval of 0.5 sec and fragments from 50 m/z to 700 m/z. The solvent delay was 0 to 3.5 min, and the total GC/MS running time was 54.67 min [14].

Synthesis of silver nanoparticles and palmarosa essential oil incorporated silver nanoparticles

Silver nanoparticles were synthesized by using silver nitrate which was reduced by reductant Tri-Sodium Citrate. 1mM of silver nitrate solution was kept on hot plate with magnetic stirrer at 100°C. 1% Tri-Sodium Citrate was added drop wise with continuous stirring and kept for 10 min till solution turned into pale brown color [15].

Silver nanoparticles were subjected to interaction with extracted essential oil of palmarosa leaves. For that different concentration of essential oil was checked i.e. 0.1%, 0.2%, 0.3% and 0.4%. Oil and water are immiscible with each other so 10 mg of SDS was added per 0.1% oil as a surfactant.

Synthesized nanoparticles were characterized to check size, shape, stability and functional groups present on nanoparticles. Nanotrac wave instrument was used to check particle size and Zeta Potential value of nanoparticles. Scanning Electron Microscope (Zeiss) was used to check size and shape of nanoparticles. Fourier Transform Infrared Spectroscopy (8400 S Shimadzu) was used to check functional groups and effect of interaction on functional groups stretches.

RESULTS AND DISCUSSION

Essential oil of Palmarosa leaves can extract efficiently using hydro-distillation method. Essential oil was accumulated in a flask attached with Clevenger apparatus which was collected in a fresh vial. A pinch of Sodium Sulphate was added in vial and vertex rapidly. Then Sodium Sulphate was allowed to settle down and upper part of pure essential oil was transferred into another vial capped with parafilm and stored at -20°C for further analysis.

Extraction of essential oil from palmarosa leaves

The essential oil was obtained by conventional hydro distillation method from fresh leaves of palmarosa, which gives light yellow color oil with sweet rose like smell. Yield of the essential oil was calculated by dividing weight of biomass with extracted essential oil volume. It gave 1.2% (v/w) yields [16]. Different solvents were used to dissolve extracted essential oil but it is most soluble in 70% Ethanol. Different characteristics of extracted essential oil are depicted in (Table 1).

Table 1 Characteristics of extracted essential oil

Characteristic	Result
Color	Light yellow
Odour	Sweet, rose like
Solubility	70% Ethanol
Yield	1.2% (v/w)

Qualitative identification of bioactive constituents of essential oil of palmarosa using GC-MS

The GC-MS analysis of the essential oil gave identification of 18 compounds (Table 2). Essential oil contains mainly monoterpene hydrocarbons and other important bioactive constituents. Dominant Components were Geraniol (47.82%), Linalool (12.09%), Caryophyllene (11.20%), Elemene (6.45%) and Geranyl acetate (4.60%). All major compounds have their unique properties which are mentioned in (Table 2).

Table 2 Identified bioactive compounds from palmarosa essential oil

R.Time	Area (%)	Height (%)	Name	Properties
9.452	1.22	3.65	PINENE <BETA-> DB5-386	Antiviral and Antimicrobial activity
10.759	0.91	2.59	D-Limonene	Antiviral and Antimicrobial activity
11.315	1.32	4.75	OCIMENE <(E)-BETA-> DB5-519	Antifungal activity
11.743	6.45	9.48	ELEMENE <GAMMA->	-
14.195	12.09	5.95	LINALOOL DB5-632	Antiviral, repellent
20.906	47.82	15.44	GERANIOL	Antiviral
22.061	11.2	11.06	Caryophyllene	Antiviral, Nematicide
22.386	4.6	11.36	GERANYL ACETATE	Anti-inflammatory
23.001	1.28	3.35	HUMULENE <ALPHA-> DB5-1527	Antifungal activity
23.669	2.04	4.35	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2 -(1-methylethenyl)-, [2R-(2.alp)]	-
24.443	0.51	1.76	SELINENE <7-EPI-ALPHA-> DB5-1685	Antibacterial activity
25.112	2.13	5.15	NEROLIDOL <(E)-> DB5-1796 (= TRANS-NEROLIDOL)	Antimicrobial activity
27.334	0.5	0.83	HUMULENE EPOXIDE II DB5-1897	Antimicrobial activity
28.139	0.79	1.75	PHYTOL ACETATE <(E)-> DB5-3152	Nematicide
28.985	5.13	9.68	Isopulegol acetate	Antibacterial activity
29.924	0.05	0.16	(-)-Spathulenol	Antibacterial, repellent
30.746	0.88	3.9	SESQUILAVANDULYL ACETATE <(E)-> DB5-2197	-
32.167	1.08	4.78	LAVANDULYL ISOVALERATE DB5-1670	-

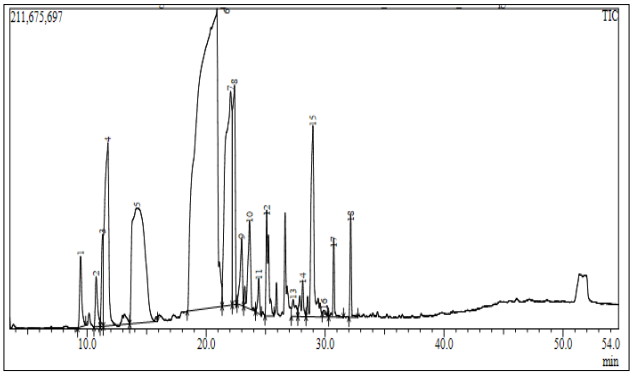

Fig 1 Chromatograph of GC-MS analysis of palmarosa essential oil

GC-MS analysis of essential oil showed geraniol as the main constituent (47.82%). The main reason of rose like sweet odour is higher content of geraniol. It imparts a floral sweet rose like smell to the oil [17]. Antiviral activity of geraniol was tested by many researchers and proved it. Next major constituent is linalool (12.09%). Linalool is widely used for the antiviral treatment against HSV-1. There are many reports on the antiviral activity of linalool and its synergistic effect with conventional antiviral drugs can increase antiviral activity [18]. Similarly, other major constituents identified in palmarosa essential oil like Caryophyllene (11.20%), Elemene (6.45%) and Geranyl acetate (4.60%) also having an antiviral activity [19].

The antiviral activities of monoterpene alcohols (including linalool, nerol, citronellol, and geraniol) probably are due to their solubility in the phospholipid bilayer of cell membranes and increased permeability of cells [20]. These results suggest that geraniol exhibits anti-coxsackievirus B1 activity, supporting its therapeutic potential for virus-associated disorders.

Effect of essential oil interaction with silver nanoparticles

Nanoparticles are synthesized in such a way that it can efficiently fulfill the purpose. Nanoparticles with desired characteristics like size, shape and surface properties can be used as protectants or more precisely target the pathogens. Nanoparticles with lesser size are suitable for all purpose like easily penetration from cell wall, encapsulation, adsorption and conjugation [21].

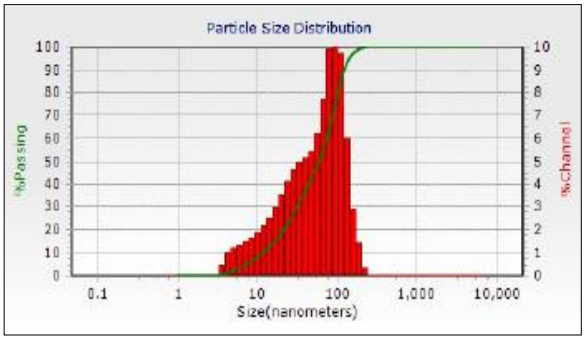

Fig 2 Size distribution micrograph of Silver nanoparticles

Effect on size of nanoparticles

Silver nanoparticles with smaller size had significantly higher antimicrobial and antiviral activity [22]. Silver nanoparticles were characterized by different instruments. Particle size analyzer shows size range of 59.8 nm ± 5 nm (Fig 2). Silver nanoparticles incorporated with palmarosa essential oil with different concentration i.e. 0.1%, 0.2%, 0.3%, 0.4% showed size range of 102.3 nm ± 5 nm, 113.8 nm ± 5 nm, 134.7 nm ± 5 nm and 150.1 nm ± 5 nm respectively (Fig 3–6). The smallest size was obtained in 0.1% of essential oil incorporated silver nanoparticles amongst these four concentrations.

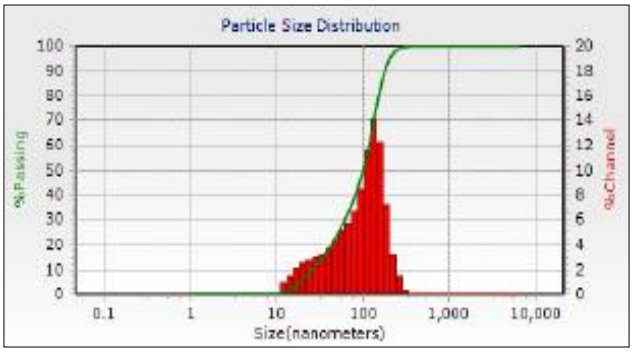

Fig 3 Size distribution micrograph of silver nanoparticles + 0.1% Essential oil

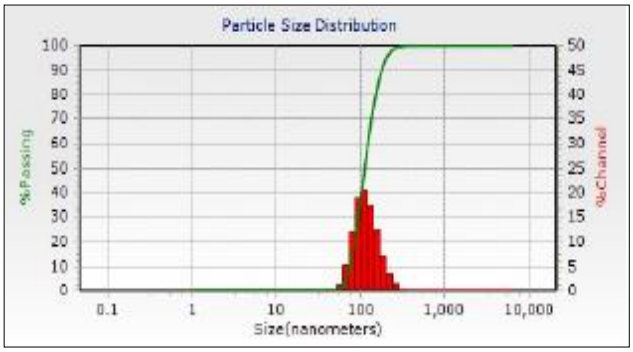

Fig 4 Size distribution micrograph of silver nanoparticles + 0.2% essential oil

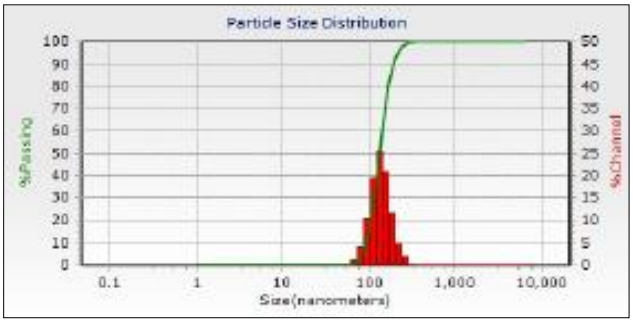

Fig 5 Size distribution micrograph of silver nanoparticles + 0.3% essential oil

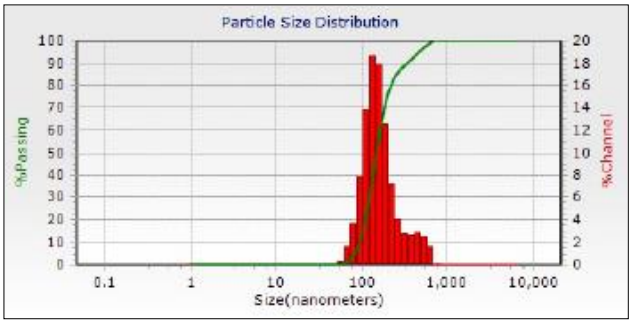

Fig 6 Size distribution micrograph of silver nanoparticles + 0.4% essential oil

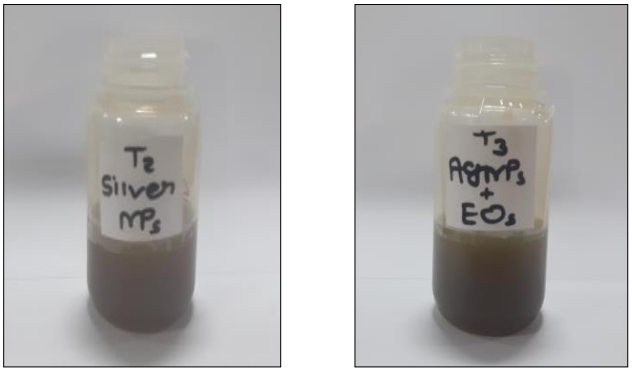

Fig 7 Synthesized silver nanoparticles and 0.1% essential oil incorporated silver nanoparticles

Effect on zeta potential of nanoparticles

Stability of colloidal silver nanoparticles was checked by zeta potential value, which shows the changes in surface charge due to interaction with palmarosa essential oil. It is widely used parameter to check the stability of colloidal metal nanoparticles. Higher value with a positive or negative zeta potential tend to repel more and cannot come closer to each other. Nevertheless, with lower value with positive or negative zeta potential value, colloidal nanoparticles attracted with each other and aggregate due to weaker repulsive force [23].

Zeta potential value indicates the stability of synthesized nanoparticles according to their surface electrical charge. Silver nanoparticles synthesized by above method have higher zeta potential value of -100 mV. It is a good sign of stable colloidal solution. However, interaction with essential oil lowers the zeta potential value. As the concentration of essential oil increases, value of zeta potential decreases. Silver nanoparticles with 0.1% gave higher zeta potential value compared with other concentrations. Zeta potential values are depicted in (Table 3).

From particle size and zeta potential result, it is explained that silver nanoparticles and silver nanoparticles with 0.1% incorporated palmarosa essential oil will be suitable for further application due to its lesser size and longtime stability. Both treatments were further characterized by Scanning Electron Microscopy and FTIR.

Table 3 Zeta potential value of silver nanoparticles and Essential oil incorporated silver nanoparticles

Nanoparticles	Zeta potential value (mV)
Silver nanoparticles	-100.8
Silver nanoparticles + 0.1% Eos	-52.4
Silver nanoparticles + 0.2% Eos	-40.8
Silver nanoparticles + 0.3% Eos	-28.7
Silver nanoparticles + 0.4% Eos	-16.9

Effect in shape of nanoparticles

Scanning Electron Micrographs shows size and shape of synthesized nanoparticles. Size range of silver nanoparticles was 40.38 nm to 60 nm and spherical in shape (Fig 8A). When these nanoparticles were incorporated by 0.1% essential oil, Size of nanoparticles is slightly increased. Size range of silver nanoparticles after 0.1% incorporation of essential oil was 68 nm to 120 nm (Fig 8B).

Effects on functional groups

FTIR analysis reveals functional groups of synthesized nanoparticles. Analysis also revealed that changes occurred in peaks stretching of bioactive compounds present in incorporated essential oil. (Fig 9A) shows the FTIR spectrum of silver nanoparticles, absorption frequency of nitro compound falls at 1383 cm^{-1} of strong symmetrical stretch. The stretch between 1370-1390 cm^{-1} exemplifies the N=O symmetry stretching which is typically present in nitro compounds. It suggests dissociation of starting material Silver Nitrate ($AgNO_3$). Absorption band of nitro group proves precipitation of silver ions from $AgNO_3$ producing colloidal silver nanoparticles [24].

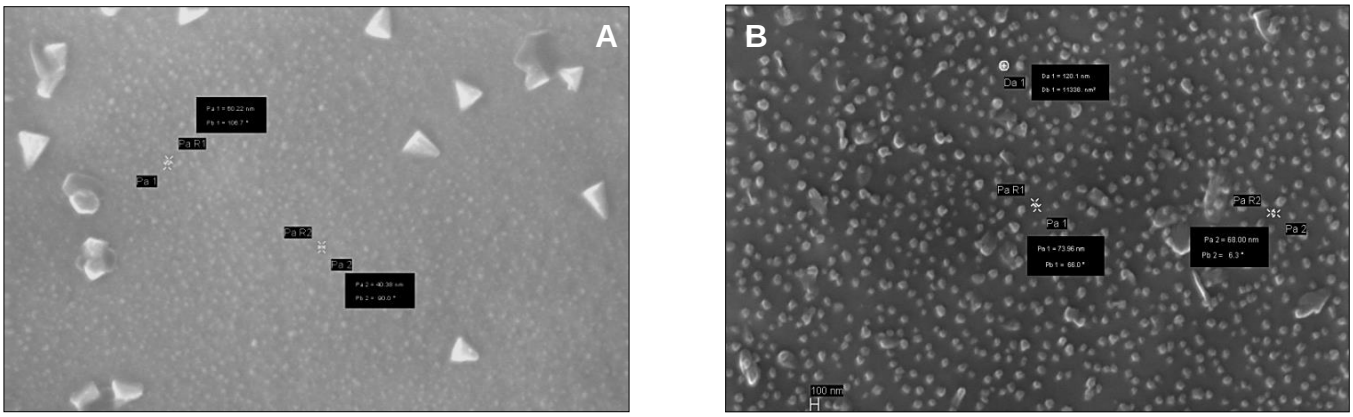

Fig 8 Scanning Electron Micrograph of (A) Silver nanoparticles (B) 0.1% essential oil incorporated silver nanoparticles

(Fig 9 B) shows the FTIR spectrum of Silver nanoparticles incorporated with 0.1% essential oil of Palmarosa leaves. Absorption frequency fall at 857.39 cm^{-1} and 866.07 cm^{-1} indicates presence of Pinene (Beta) [25]. Absorption frequency fall near 1493 cm^{-1} indicates presence of caryophyllene [26]. There is a drastic change in the functional group's presence after the interaction with essential oil with silver nanoparticles.

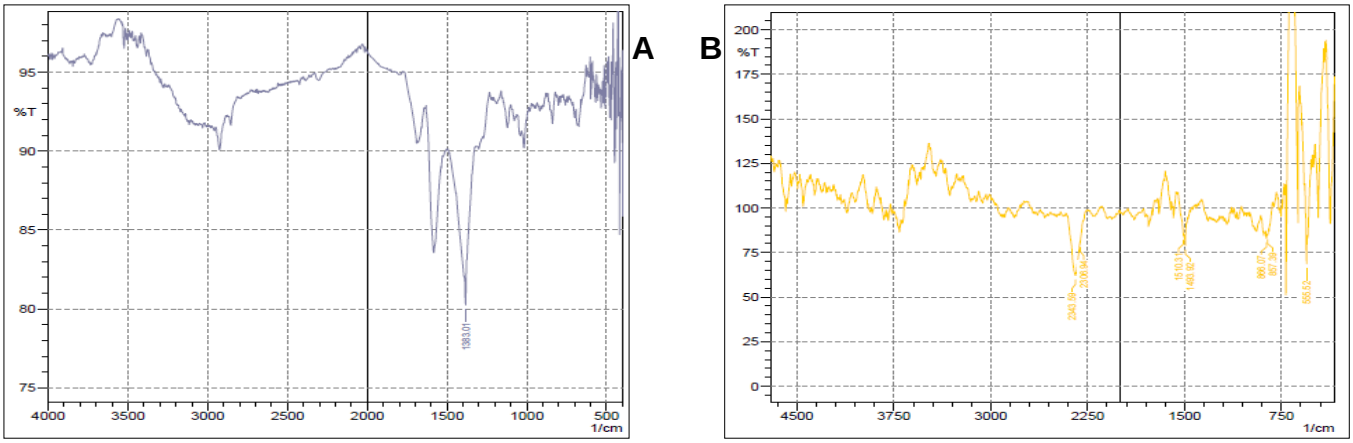

Fig 9 FTIR Spectrum of (A) Silver nanoparticles (B) 0.1% essential oil incorporated silver nanoparticles

CONCLUSIONS

The problem of virus mediated diseases in plants is very dangerous for economical loss. Chili leaf curl viral disease causes a huge economic loss in chili crop. The inefficacy of current pesticides has made necessary to find a novel antiviral formulation to control this disease. Essential oil possesses important bioactive constituents with different bioactivities including potential antiviral and antimicrobial activities. GC-MS analysis of palmarosa essential oil proved that it contains many potent antiviral constituents. The promising antiviral activity of Silver nanoparticles can be increased with combination of palmarosa essential oil. The main important characteristics are size and stability of synthesized nano emulsion. From the above study we can conclude that Silver nanoparticles with 0.1% palmarosa essential oil gave better antiviral nano emulsion due to lesser size and more stability. The interaction of essential oil with nanoparticles increases the chemical stability and solubility. Moreover, the rapid degradation and evaporation of bio active constituents are minimized. Nano emulsion concept supports their controlled and slow release, which increases the bioavailability and effect against virus pathogen. Thus, nano emulsion is a reliable strategy to facilitate its application as antiviral agent.

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