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Exposure effects of non-ionizing radiation of radio waves on antimicrobial potential of medicinal plants

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ABSTRACT

The high frequency electromagnetic (EM) waves have been proven as potent environmental polluters. The radio towers are considerably increasing to cater to the growing telecommunication requirements. The presented research focuses on the investigation of EM waves on antimicrobial potential of pharmaceutically important medicinal plants viz. Tulsi (*Ocimum sanctum* L.) and Brahmi (*Bacopa monnieri*). The similar work has not been reported till date at the best of our knowledge. The medicinal plants were exposed to EM waves of 900 MHz possessing a 1.9 mG magnetic field. Our former investigation on plant physiology gave deteriorative effects on selected plants physiology in terms of growth, chlorophyll content, protein and carbohydrate content. In the presented article, the antimicrobial activity of two plants was inspected through various bacterial and fungal species. In case of *B. monnieri*, investigations revealed raise in Zone of Inhibition (ZOI) for both the bacterial and fungal strains in the range of 50%, whereas for *O. sanctum* increase of 63.26% and 59.42% ZOI for bacterial and fungal strains were noted respectively upon 24 h of exposure. Minimum Inhibitory Concentration (MIC) also decreased for 24 and 48 h of exposure. The transient raise in antimicrobial activity indicated the induction of plant defense system which can be considered as positive influence of short term radiation on medicinal properties of plants. Both plants exhibited the highest antimicrobial potential for *E. coli* and *P. chrysogenum* showing a prominent decline after 72 h of exposure. A substantial decline in antimicrobial in MIC of *B. monnieri* reported was 211% against *E. coli* and 166% against *P. chrysogenum* upon 144 h of exposure compared to control plants. The antimicrobial potential of *O. sanctum* was also decreased upon radiation, which is having a 392% reduction against *E. coli* and a 225% reduction against *P. chrysogenum* upon 144 h of exposure. The noteworthy deteriorative effects of radio waves on antimicrobial potential have been observed for both the medicinal plants.

1. Introduction

The inevitable utilization of cellular mobile communication is continuously polluting the environment by hazardous electromagnetic (EM) waves in apical intent. EM waves have been proven as a potent mutagen for animal tissues. Current literature lacks in demonstration of ferocious biochemical effects of electromagnetic waves on plants and therefore it encourages analysis of the biological consequences of electromagnetic radiation exposure on plants. In India and other Asian countries, Ayurveda is a practiced on a large scale as an ancient system of medicine and health care. Amongst different approaches of therapies, natural products have vital importance and place in therapeutics. Natural plant extracts are one of the best options to utilize as starting material for the synthesis of drugs. These extracts also contain pharmacologically active compounds utilized directly as therapeutic agents, which potentially reduce the hazardous effects of oxidative stress [Phuyal et al., 2020]. The urgent necessity is arisen to preserve and nurture the normal flora of medicinal plants to deal with alarming the situation of antibiotic resistance [Parekh & Chanda, 2007]. Pathogenic microorganisms are evolving at a faster rate, they have become resistant to various antibiotics, and thus, investigation of medicinal plants and their conservation has become a prime requirement.

The plant leaves, branches, stems, and roots behave as imperfect dielectric materials. The wave, comprising of changing electric field and magnetic field, in these plant components, follows the dielectrics wave propagation. The ion oscillations or dipole relaxation within the plant

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dielectric material may give rise to modifications in biochemical structural behavior. The complex permittivity and permeability in dielectric material shall induce wave losses; this, in turn, may be converted into a significant increase in the temperature of the material under test. In other words, the dielectric loss tangent of plant components may be considered a significant phenomenon for possible biological changes in plant tissues. Apparently, following the Poynting Wave Power theorem, the higher intensity of Electric and magnetic fields shall induce high instantaneous power density in the plant tissues.

The microwaves are absorbed by the cells and tissues of plants. Primarily, electromagnetic waves or energy absorption by tissue is specified through means of Specific Absorption Rate (SAR). The Specific Absorption Rate is the increment in stored energy (dE) in dielectric material with respect to the rate of change of time, being absorbed in accordance with increment in differential mass (dM) contained in the differential volume (dV) [Barnes & Greenebaum, 2018]

$$SAR = \left(\frac{d}{dt}\right)\left(\frac{dE}{dM}\right) \quad [1]$$

The dielectric material density can be given as:

$$\rho = \frac{dM}{dV} \quad [2]$$

$$Specific Absorption\ \ Rate = \left(\frac{d}{dt}\right)\left[\frac{dE}{\rho(dV)}\right] \quad [3]$$

The equation of Specific Absorption Rate of varying electromagnetic fields as per the Poynting vector theorem [Barnes & Greenebaum, 2018]:

$$SAR = \left(\frac{\sigma}{2\rho}\right)|\overline{E}|^2 = \left(\frac{\omega\varepsilon_0\varepsilon^{''}}{2\rho}\right)|\overline{E}|^2 \quad [4]$$

where E^{--} = Peak value of internal electric field, σ = Tissue conductivity, ρ = Material Resistivity, ε_0 = free space permittivity and ε'' = imaginary component of permittivity which is related to the dielectric losses of the material.

In other terms, the ratio of whole energy captivation to the mass of exposed living body or sample volume is defined as Specific Absorption Rate (SAR) [Vian et al., 2016]. The Specific Absorption rate is measured in W/kg. If plants absorb the radiation then their bio-molecules Viz. nucleic acids, proteins, different enzymes, and antioxidants shall certainly react. Once biological material absorbs the electromagnetic waves, the phenomenon of ion movement through deposition of negatively charged electrons on minerals is created, which are water-soluble and suspended in solution. The other effects involve an increase in membrane leakiness [Goldsworthy, 2007]. Goldsworthy [2007] had reported such effects, which include weak EM fields responsible for dislodging calcium from cell membranes. The electromagnetic field mainly acts on living organisms by acting on ions such as calcium and potassium. This leads to membrane leakiness. Membrane leakage of cell organelles like lysosomes damages DNA by releasing hydrolytic enzymes, including DNAase, which significantly damages DNA. It infers the serious injury happen to the DNA of various cell types of the organisms by microwave radiations. Panagopoulos et al., [2007] revealed that when *Drosophila melanogaster* was exposed to a mobile communication signal up to six days for 6 min per day, resulted in DNA disruption. Other than that majority of metabolic processes are regulated by cations and thus due to the electromagnetic wave radiation effect, extra calcium is influx and causes alterations in the metabolic program of the cells. The rate of metabolism is also significantly affected and altered by electromagnetic wave effects. The report of 'Reflex Project' at an online brochure titled as Health and Electromagnetic Fields forecasted by the European Commission also show that if animal cell including human if exposed for 24 h to radiofrequency waves in a discontinuous manner, leads to a similar fragmentation of DNA as radioactive isotopes emitting gamma rays [Goldsworthy et al., 2007]. The radiation effects of such non-ionizing radiation on plant cells is illustrated in Fig. 1.

It has been reported that along with the metabolic rate, morphogenesis and embryogenesis of organisms also affected significantly, by the deed of endogenous electromagnetic fields [Levin, 2003]. According to discreet studies, short term positive effects of microwaves on seed germination and growth were observed by Russello et al., [1996] whereas long term exposure of microwaves resulted in deterioration in young spruce and beech tree growth has been reported by Schumutz et al., [1996]. Effects of irradiation on active compounds of medicinal plants were documented by Thongphasul P., 2011.

Wolfgang Volkrodt [1988, 1991] recorded the serious detrimental and hazardous effects introduced by the radars installed in the German forest through several years whose radiations of high frequency resonates with the plasma membranes of tissues and it also causes disruption in the water circulation. The equilibrium of polar particles gets disturbed as well. According to the report, "the short waves are those that more damages to the trees" [Volkrodt, 1988 & 1991]. Various factors like the developmental stage of plants and exposure time get affected due to the electromagnetic wave exposure [Magone, 1996]. Electromagnetic radiation hazards of the Skrunda Radio Location Station due to constant EM wave exposure coming out from Radar signals on the physiologic and morphologic characteristics of the *Spirodela polyrhiza* (L.) was presented. Based on the observations, long-term exposure resulted in a deterioration in vegetative growth and morphological abnormalities especially the effects were more prominent in the immature developmental stage.

Biochemical analysis such as effects on various pigments and carbohydrates content revealed stimulatory effects upon short-term exposure, which afterward deteriorated upon long-term exposure. Short-term exposure effects can be considered positive because assimilatory pigments like chlorophylls as well as total sugar content were increased which indicated a higher photosynthetic rate, which was gradually deteriorated upon long-term exposure. The carotenoid like assimilatory pigments also increased with a decrement in chlorophyll, which indicates premature aging of leaves [Vian et al., 2016]. There are various abiotic stressors, which affect development and physiochemical properties of plants like water stress, extreme temperatures, salinity, heavy metal or other pollutant and radiation [Ncube et al., 2012]. Thus, electromagnetic radiation was already proven as a potent factor for plant health. It also notably affects the content of secondary metabolites mainly phenolic compounds like Flavonoids, Tannins and phenolic acid [Manukyan, 2013] which are responsible for antibacterial, antifungal as well as antiviral activities of the majority of medicinal plants and thus have become an area of widespread research of pharmaceutics [Gurib-Fakim, 2006; Burda & Oleszek, 2001].

Out of whole Electromagnetic spectra, Ultraviolet (UV) radiation was extensively investigated by various investigators viz. Katerova et al., [2012] and Phuyal et al., [2020] which directly affects cell metabolism including synthesis of secondary metabolites. UV radiation categorizes into three classes Viz. UV-A, UV-B, and UV-C based on the frequencies. The shorter wavelengths such as UV-B and C are proven hazardous for plant cell metabolism in terms of growth, immense synthesis of ROS that lead to oxidative stress within cells and tissues, deteriorative effects on biomolecules and lowering of cell viability [Kovacs & Keresztes, 2002; Schreiner et al., 2012; Takeuchi et al., 2007]. The gloomy properties of electromagnetic waves of shorter wavelength upon vegetation have identified by various researchers and are denoted in Table 1.

The medicinal plants are cultivated extensively to meet the need for herbal products. The presented research intended to investigate the consequences of electromagnetic waves of microwave frequency on antimicrobial potential of such two medicinal plants for examining prolonged effects.

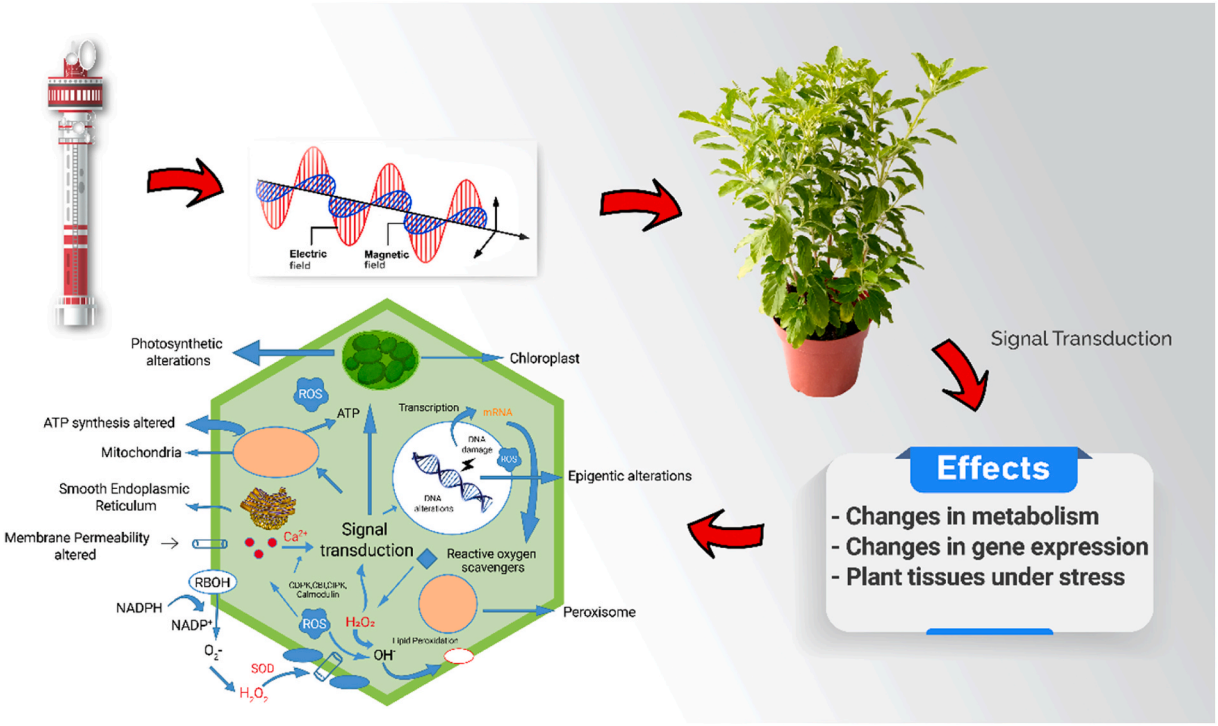


Fig. 1. Radiation on plant cells.

Table 1
Review of effect of EMF-r on plants.

Plant species	Frequency	Experimented characteristic effects	Author and year
Withania somnifera	900 MHz/ GSM	Reduction in antioxidant activity	[Upadhyaya et al., 2021]
Arabidopsis thaliana	1882 MHz/ GSM	Chloroplast deterioration, decline in plant growth, leaf thickness and biomass	[Stefi et al., 2016]
Solanum lycopersicon	900MHz/ GSM	1/3 decline in ATP concentration	[Roux et al., 2008]
Petroselinum crispum, Anethum graveolens, Apium graveolens	860–910 MHz/GSM 2.4 GHz/ WLAN	Reduction in stomatal conductance and assimilation	Soran et al. (2014)
Lemna minor	900MHz/ GSM	Increase in H_2O_2 and MDA concentration	[Radic et al., 2007]
Helianthus annuus	5.28 MHz	Decrease in Abcisic acid (ABA) hormone in exposed seeds	[Mildažienė et al., 2019]
Allium cepa	2100 MHz	H_2O_2 accumulation and elevation in super oxide ions	[Chandel et al., 2017]
Lens culinaris	1800 MHz	Root growth decline	[Akbal et al., 2012]
Vigna radiate	900MHz/ GSM	Rhizogenesis deterioration	[Singh et al., 2012]
Phaseolus vulgaris	915 MHz	Reduction in inflorescences	[Surducan et al., 2020]
Zea mays	1800MHz/ CDMA	Root Growth reduction	[Kumar et al., 2016]
Glycine max	900MHz/ GSM	Inhibition of epicotyl growth	[Halgamuge et al., 2015]
Rosa hybrid	900MHz/ GSM	Delayed growth of secondary branch axes	[Gremiaux et al. 2016]

2. Materials and methods

2.1. Raising plants for experiments

True-to-type plants of *Ocimum sanctum* L. and *Bacopa monnieri* were raised in botanical garden for limiting genetic variation. They were raised in the greenhouse of botanical garden by providing the similar environmental conditions in terms of soil, which was uniform, 0.5 mg/ lit fertilizer nutrients, 0.5 L per 3 days water content and 25 ± 2 °C temperature. Control plants were taken and kept in isolated locations. These control plants were away from Electromagnetic (EM) resonator set up having EM isolation of 15 dB compared to the exposed plant. The field strength was around 0.177 times compared to EM wave-exposed plants. The leaves of *Ocimum sanctum* L. and *Bacopa monnieri* plants were plucked periodically from the botanical garden. The antimicrobial activity of leaves of both plants was checked against five bacterial strains Viz. *Escherichia coli*, *Staphylococcus aureus*, *Sanlmonella typhi, Sanlmonella paratyphi*and *P. aerugenosa*and five fungal strains Viz. *Candida albicans*, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus* and *P. Crysogenum* were conserved on Nutrient agar and analyzed further for antimicrobial assays.

2.2. Electromagnetic dosimetry

Electromagnetic exposure treatment was given to one group of the plants by half-wavelength Dipole Antenna at 900 MHz Global System for Mobile Communication (GSM) frequency having an average 1.9 mG magnetic field surrounding the plants. The experimental setup and magnetic field strength measurements are illustrated in Fig. 2 and Fig. 3 respectively. A magnetic field strength of the half-wavelength dipole was deduced by Guss meter which remained constant at each angle of plant. The technical specifications of the device have been tabulated in Table 2.

A dipole antenna, having an electrical field strength of around 8 V/m and magnetic field strength of around 2 mG, was utilized. The E-Field was measured by means of high impedance E-field detector probe, an ATTEN make near-field radiation detector probe, AT8904. The probe

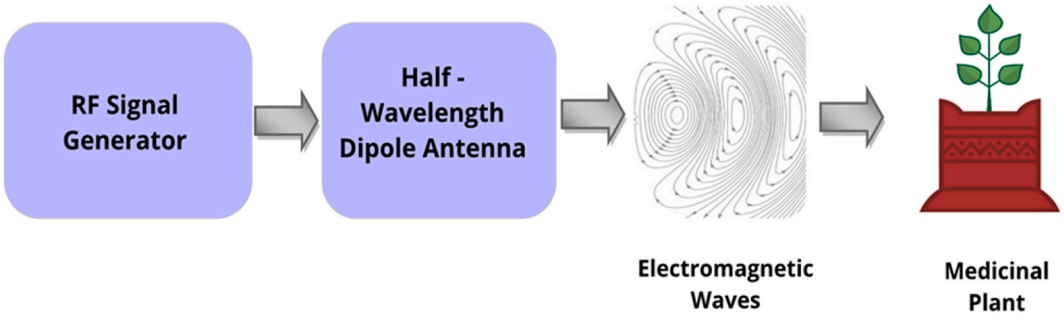


Fig. 2. An Experimental Setup for analysis of Electromagnetic Waves on Plants.

has a frequency range spanning from 100 kHz to 3 GHz for electric field detection. The distance of ~3 meter was kept between source and the medicinal plant. The pictorial view of λ/2-dipole is illustrated in Fig. 3 (c). The examination of the alterations in antimicrobial potential due to 900 MHz continuous wave exposure in closed laboratory vicinity has been carried out. The incident field strength measurement was carried out in the near-field/Fresnel region of the dipole antenna. Typically the distance is $< 2D^2/\lambda$ - where D is antenna length. The sensitive vector network analyzer and field meters were utilized to measure the field both in the botanical/herbal garden and within the laboratory test setup. The ambient field in the botanical/herbal garden was as low as 1.1–1.5 V/m. The radiation pattern of the half-wavelength dipole antenna is figure-of-eight, where the radiation is zero along the axis and maximum at the right angles of the antenna axis. The radiation pattern is illustrated below in Fig. 4.

2.3. Calculation of Specific Absorption Rate (SAR)

Specific Absorption Rate (SAR) was determined using the following heat transfer equation where the increase in temperature noted upon EMF exposure [Grémiaux et al., 2016]:

$$SAR = C \times \left(\frac{dT}{dt}\right)_{t\to 0} \quad [5]$$

where C is the heat capacity in J K−1 kg−1, (dT/dt) rate of change of temperature per unit time after the commencement of electromagnetic field exposure.

Measurement of SAR is uncertain for animals or plants [Berdinas--Torres, 2007]. Equation (5) provides better SAR estimation as the heat of specific material is independent of frequency. The temperature is more uniformly distributed compared to the electric field within the tissue material. SAR in case of animals and humans can be relatively easily determined using phantom models [Van Wyk et al., 2005] where special fluids having similar dielectric properties like biological fluids can be used. A similar approach is not feasible in the case of plant organs which has a higher ratio of surface area to volume [Vian, 2007]. Lutron surface temperature probe (Lutron Electronics Enterprise, Taipei, Taiwan) was utilized for the temperature measurement. The heat capacity of Tulsi was found to be 1552 J $K^{-1}kg^{-1}$ and Brahmi was found to be around 2280 J $K^{-1}kg^{-1}$. For the continuous exposure of electromagnetic field on the Tulsi plant for around 3 h, nearly 0.1 °C temperature was observed whereas for the same duration, Brahmi surface leaves temperature was increased nearly by 0.15 °C. The SAR value of Tulsi and Brahmi leaf was found to be in the order of 1.43×10^{-2} Wkg^{-1} and 3.16×10^{-2} W kg^{-1} respectively, with exposure of continuous wave (CW) electromagnetic field of 8 V/m.

2.4. Preparation of plant extracts

Sample plant leaves were cleaned, dried, and powdered with a grinder. A powdered 10 gm leaf sample was extracted with 100 ml of methanol and chloroform using Soxhlet apparatus [Shekhar & Prasad, 2015]. The extracts were evaporated under reduced pressure and dried which were reconstituted within one-fourth volume of solvent, Dimethyl sulfoxide to prepare concentrated extract and shelved at 4 °C for further assays.

2.5. Procedure of McFarland standards

Each of the tubes containing 9 ml sterile nutrient broth was inoculated with respected isolated colonies on nutrient agar slants and incubated at 37 °C for 24 h. The cultures were read at 600 nm for measuring optical density and adjusted to 0.5 McFarland standards optical density with sterile distilled water.

2.6. Preparation of inoculums of selected microorganisms

Bacterial inoculums were prepared by referring procedure presented by Golshahi et al. (2011). To fine-tune the count of bacterial cells in assay, the 0.5 McFarland standards equals to 1.5×10^8 CFU/ml, was taken as a reference. For, preparing fungal inoculums, loop full of fungal culture was transferred to a 10 ml normal saline-containing tube and mixed properly by sealing the tube for further use. By using these fungal cultures in order of 0.1 ml, the agar well diffusion method was followed in which 10 µl of fungal inoculums were individually transferred into Neubauer's chamber and the number of spores was counted under 40x magnifications.

For the calculations of spore count following expression was utilized:

$$\frac{Total\ \#cells}{\#Squares\ counted} x10^4 x Dilusion\ Factor = \#Cell \Big/ ml\ in\ original\ suspension \quad [5]$$

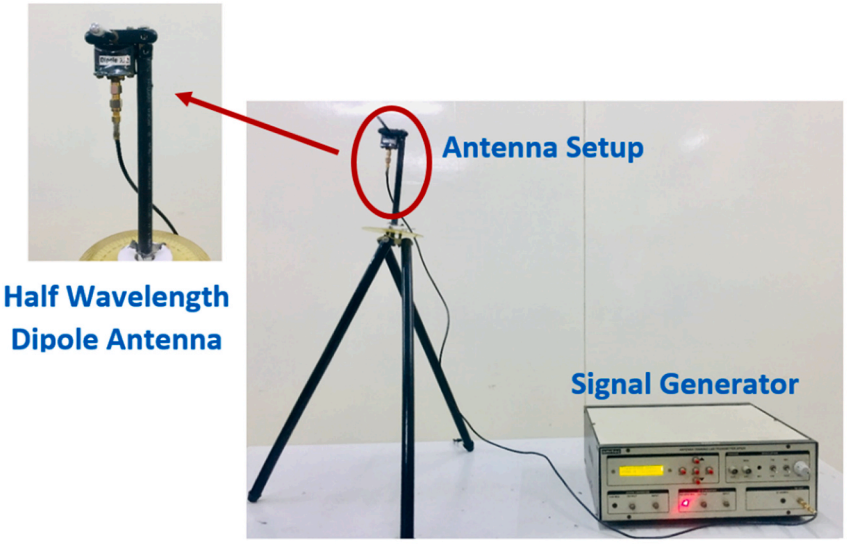
The susceptibility viewing of the chosen organisms to the extracts of *O. Sanctum* L. and *B. monneri* was governed via referring to documented methodologies (Akinpelu et al., 2008). For the antimicrobial activity test, the inoculums of each organism strain uniformly kept at 1.5×10^8 CFU/ml.

2.7. Agar diffusion method for analysis of antimicrobial potential

N-Agar was used for bacterial cultures whereas Potato Dextrose Agar (PDA) was used for fungal cultures. The plates contained with basal and top agar medium then utilized for antimicrobial potential check by inoculating each with respective culture organism along with stored plant extracts. The antimicrobial potential was determined by measuring the zone of inhibition after incubation at optimum growth condition of microbial strain and diffusion of plant extract (Faden., 2018).

Determination of Minimum Inhibitory Concentration (MIC) of electromagnetically unexposed and exposed plants of *Ocimum sanctum* and *Bacopa monneri* by Macro broth dilution method.

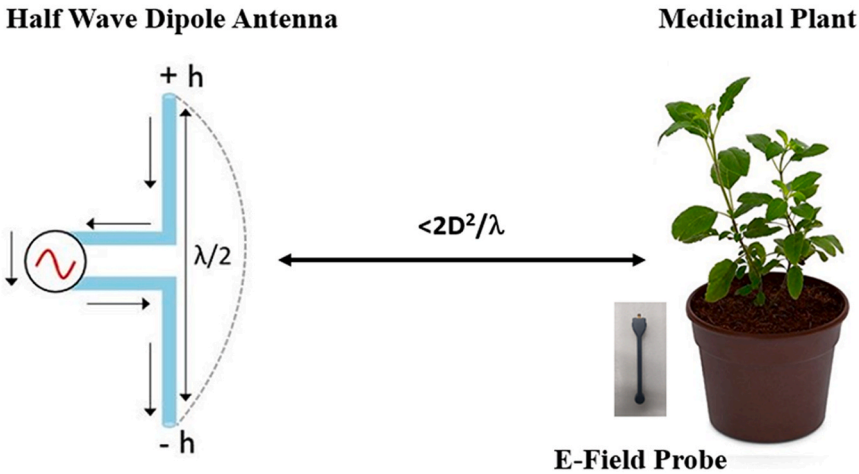
The MIC is considered as the fundamental laboratory measurement



(a)



(b)



(c)

Fig. 3. (a) Microwave transducer used for experiment (b) Signal producer display selected radio-wave frequency of 900 MHz (c) Pictorial view of E-field measurement.

Table 2
Technical specification of transmitting antenna system.

Parameters	Specifications
Transmitter Frequency Range	30 MHz to 4 GHz
Transmitted Frequency	900 MHz
Input Waveform	Sinusoidal
Communication Mode	Full-Duplex
Maximum Magnetic Field Strength	~4 mG
Frequency Resolution	<5Hz

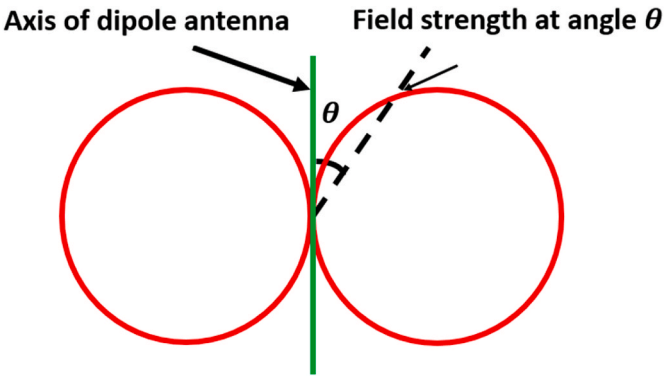


Fig. 4. Radiation Pattern of the Dipole antenna.

of an antimicrobial potential against selected microorganism. Plant extracts of both the plant leaves were prepared and used to check MIC against ten organisms five bacterial and five fungal strains by the micro broth dilution methods referred from Tardugno et al. (2019) with certain modifications. The dried extract was suspended in 10% DMSO to make a final concentration of 200 mg/ml. For MIC, inoculums consisting of 10^6 CFU/ml were used. For the assay, plant extract concentrations ranging from 50 to 0.097 mg/ml were prepared by serial dilutions in respective Nutrient broth and Sabouraud dextrose broth for bacterial and fungal strains. 100 µl of inoculum was transferred and tubes were incubated at 37 °C and 24 h for bacterial and 27 °C for 3–5 days for fungal strains.The MIC was deduced as the lowermost concentration of the extract which do not display observable growth of selected microbial strain.

3. Result and discussions

The presented investigation was carried out to check influence of non-ionizing electromagnetic waves on antimicrobial potential of selected plants. The methanolic extracts of leaf samples of *Bacopa monnieri* and *Ocimum sanctum* were investigated against five bacterial and five fungal strains to check their antimicrobial potential. The interpretation was made by comparing data of electromagnetically unexposed control plants with periodically exposed plants. Experimentation was performed by determination of zone of inhibition via agar disc diffusion method and MIC through Broth dilution method. Moreover, antibiotic and antifungal drugs were utilized as standard positive controls for comparative analysis in assay. The outcomes revealed that both the plants had good bactericidal and antifungal activity against all the ten microbial strains as illustrated in Figs. 3–6. Although, comparative study displayed higher activity of positive controls for all isolates than the plant extracts. The negative controls viz. methanol for ZOI and DMSO for MIC did not give any antimicrobial activity in presented assays.

3.1. Antibacterial activity of plant extracts

Amongst the two plant extracts tested, *Ocimum sanctum* L. exhibited higher antibacterial activity against *E. coli*, S. *aureus* and *S. typhi* whereas *Bacopa Monnieri* revealed a higher bactericidal effect against *S. paratyphi* and *P. aeruginosa*. As illustrated in Fig. 5, *Ocimum sanctum* L. exhibited strong activity against *E. coli* (26.77 ± 0.55 mm ZOI and 0.44 ± 0.03 mg/ml MIC), moderate activity against *S. aureus* (17.86 ± 0.61 mm ZOI and 0.72 ± 0.03 mg/ml MIC), *S. typhi* (16.08 ± 0.14 mm ZOI and 1.06 ± 0.19 mg/ml MIC), *S. paratyphi* (14.67 ± 0.35 mm ZOI and 0.86 ± 0.05 mg/ml MIC) and weak activity against *P. aeruginosa* (8.67 ± 0.42 mm ZOI and 2.32 ± 0.23 mg/ml MIC). Whereas the *Bacopa monnieri* exhibited higher activity against *E. coli* (22.23 ± 0.83 mm ZOI and 0.65 ± 0.03 mg/ml MIC) amongst all strains, intermediate activity against *S. typhi* (14.57 ± 0.32 mm ZOI and 1.22 ± 0.24 mg/ml MIC), *S. paratyphi* (17.40 ± 0.56 mm ZOI and 0.86 ± 0.05 mg/ml MIC) and mild activity against *S. aureus* (11.07 ± 0.97 mm ZOI and 1.01 ± 0.17 mg/ml MIC) as well as *P. aeruginosa* (10.60 ± 0.82 mm ZOI and 1.86 ± 0.32 mg/ml MIC). The broth dilution assay outcomes were illustrated in Fig. 5 and it has been deduced that all the bacterial strains were sensitive to tested plant extracts and standards. The MIC value of *Ocimum sanctum* L. and *Bacopa monnieri* was in the range of 0.44 ± 0.33–2.32 ± 0.23 mg/ml and 0.65 ± 0.03–1.86 ± 0.32 mg/ml respectively.

Amongst all the tested bacterial strains, *E. coli* (MIC 0.44 ± 0.03 to 0.65 ± 0.03 mg/ml) was found as very sensitive to both the plant extracts and thus, both plants exhibited higher antibacterial potency against gram-negative bacteria which was analogous to outcomes obtained by Singh, D., & Chaudhuri, P. K. (2018). Whereas *P. aeruginosa* (MIC 2.32 ± 0.23 mg/ml) and *S. aureus* (MIC 1.01 ± 0.17 mg/ml) displayed a higher resistance to respective *O. sanctum* and *B. monnieri* extracts. Panghal et al. (2011) reported the significant resistance of *P. aeruginosa* for a range of antibiotics and antibacterial compounds which was reflected in obtained outcomes. Joshi et al. (2011) also investigated various medicinal plant antibacterial activity and obtained the outcome which shown the lower susceptibility of *P. aeruginosa* toward the majority of medicinal plants. MIC values of selected plant extracts were greater than control antibacterial drugs. The MIC of erythromycin and chloramphenicol was ranging from 0.054 ± 0.004–0.15 ± 0.03 mg/ml and 0.143 ± 0.040–0.247 ± 0.051 mg/ml respectively for selected bacterial strains.

3.2. Investigation of electromagnetic exposure effect on antibacterial potential of selected plants

In the presented investigation, leaf extracts of electromagnetically exposed test plants were analyzed after periodic exposure of high-frequency waves after 24 h of interval for their antimicrobial potential and compared with the unexposed control plants. The influence of high-frequency radio wave exposure on *O. sanctum* for 24 h exhibited an increase in ZOI in the range of 4.8–20.23% and consequent decrement in MIC of 18.18–39.60% for tested bacterial isolates. Whereas a similar increase in ZOI (6.84–33.47%) and consequent decline in MIC (7.06–43.78%) ensued after 48 h of exposure in case of *B. monnieri*. Such transient rise in the antimicrobial potential of both plants shown the stimulation of synthesis of more amount of secondary metabolites. The effects of abiotic stressors which induce the secondary metabolite synthesis is a vast area of research and literature has proven the fact that it happens due to the induction of a plant's own defense system (Borges et al., 2017). Accordingly, the presented outcome indicated that EM waves imposed positive effect on antimicrobial potential of selected medicinal plants upon short term exposure. The continuous exposure beyond 48 h led to a more significant drop in ZOI and consequent elevation in MIC for all bacterial strains. As illustrated in Fig. 5, the ZOI reduced in the range of 30.71–51.39% for *O. sanctum* also 32.58–50.69% for *B. monnieri* upon 48–144 h of exposure as compared to controls. There was a consecutive increment in MIC reported upon 72–144 h of exposure for both plant extracts against all selected bacterial strains and was ranging from 153.7 to 286% for *O. sanctum* and 141.8–261.6% for *B. monnieri.* The highest deterioration of antibacterial potential was

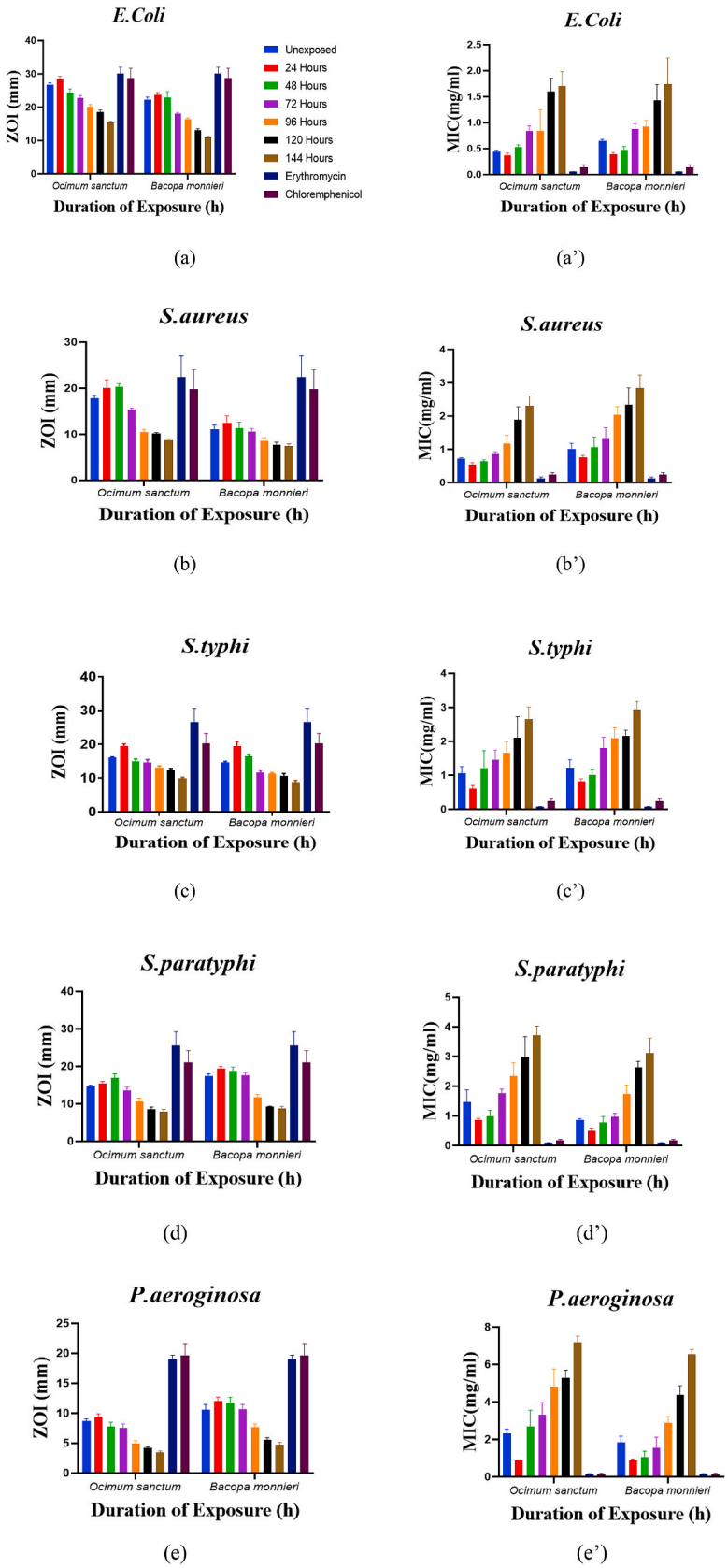


Fig. 5. Effect of radio waves on antibacterial activity of *O. sanctum* and *B. monnieri* plants by disc diffusion method (a) *E. coli*, (b) *S. aureus*, (c) *S. typhi*, (d) *S. paratyphi*, (e) *P. aeroginosa* and broth dilution method (a') *E. coli*, (b') *S. aureus*, (c') *S. typhi*, (d') *S. paratyphi*, (e') *P. aeroginosa* against five bacterial strains.

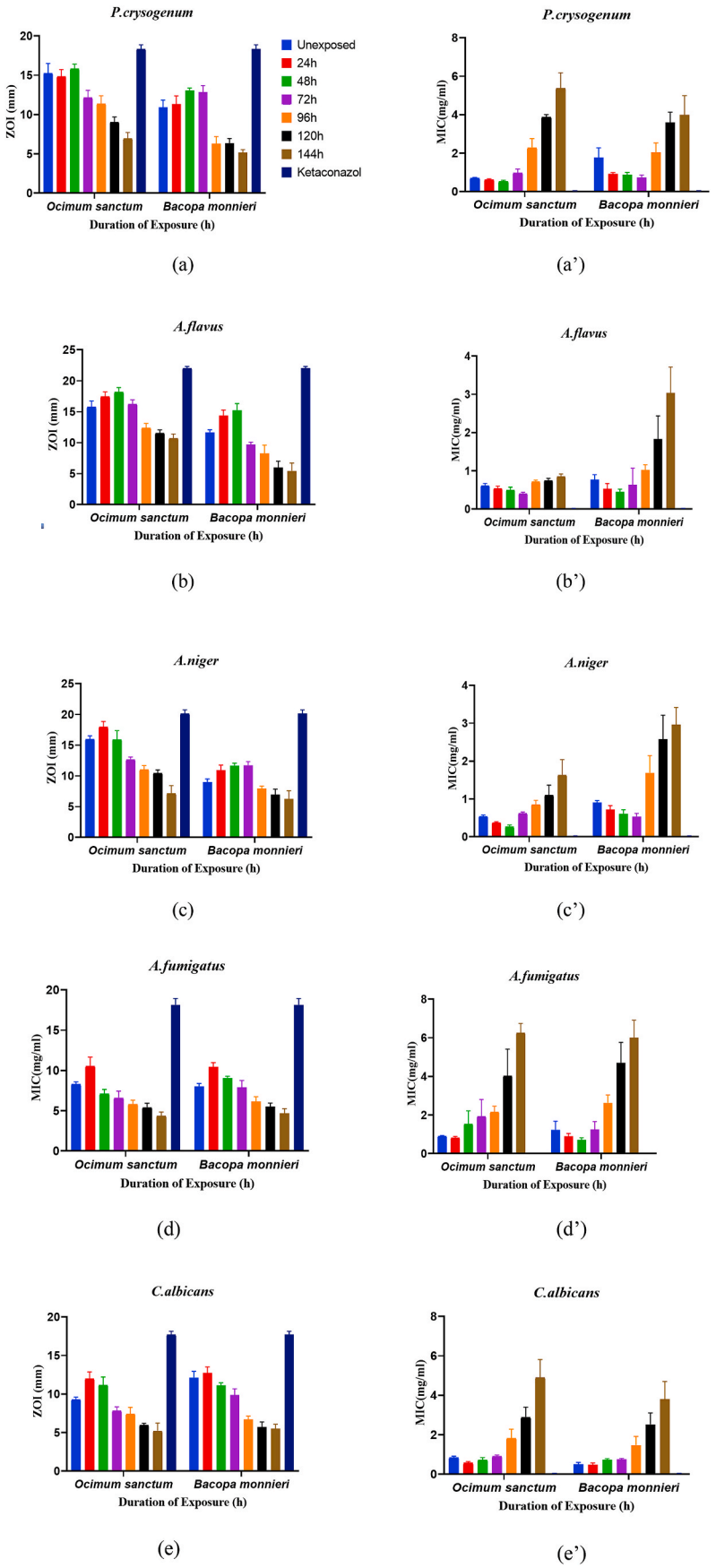


Fig. 6. Effect of radio waves on antifungal activity of *O. sanctum* and *B. monnieri* plants by disc diffusion method (a) *P. chrysogenum*, (b) *A. flavus*, (c) *A. niger*, (d) *A. fumigatous*, (e) *C. albicans* and broth dilution method (a') *P. chrysogenum*, (b') *A. flavus*, (c') *A. niger*, (d') *A. fumigatous*, (e') *C. albicans* against five fungal strains.

obtained in case of *O. sanctum* and *B. monnieri* against *P. aeroginosa* and *S. paratyphi* isolates respectively upon exposure of 144 h. The MIC is inversely proportional to ZOI which was appropriately reflected from the presented outcomes and thus, was proof of detriment of antibacterial activity of both the plants upon exposure of high-frequency non-ionizing radiation.

3.3. Antifungal activity of plant extracts

Antifungal potential of both the plants versus standard antifungal compound ketoconazole, revealed higher efficacy (ZOI ranges from 17.70 ± 0.44–22.00 ± 0.30 mm and MIC ranges from 0.023 ± 0.003–0.05 ± 0.006 mg/ml) of the ketoconazole than the extracts. The plant extracts of both *O. sanctum* and *B. monnieri* revealed antifungal activity against all selected fungal strains. The antifungal activity results denoted that both *Oscimum sanctum* and *Bacopa monnieri* revealed the highest antifungal effect against *Penicillium chrysogenum* strain whose respective ZOI and MIC were 15.3 ± 1.2 mm and 0.73 ± 0.15 mg/ml for *O. sanctum* whereas 12.13 ± 0.80 mm and 0.50 ± 0.10 mg/ml for *B. monnieri*. *O. sanctum* showed moderate antifungal effect toward *A. niger* (ZOI 15.76 ± 0.96 mm and MIC 0.61 ± 0.05 mg/ml), *A. flavus* (ZOI 15.30 ± 1.2 mm and MIC 0.70 ± 0.003 mg/ml) and *A. fumigatous* (ZOI 9.30 ± 0.30 mm and MIC 0.85 ± 0.06 mg/ml) whereas *B. monnieri* gave moderate antifungal activity against *A. niger* (ZOI 10.93 ± 0.90 mm and MIC 1.76 ± 0.51 mg/ml), *A. flavus* (ZOI 11.67 ± 0.41 mm and MIC mg/ml) and *C. albicans* (ZOI 9.00 ± 0.50 mm and MIC 0.91 ± 0.05 mg/ml). The *Candida albicans* was the most resistant to *O. sanctum* plant (8.30 ± 0.30 mm ZOI and mg/ml MIC) as it gave the least activity against it. While, *B. monnieri* had the lowest activity for *Aspergillus fumigatous* 8.03 ± 0.35 mm ZOI and 1.24 ± 0.44 mg/ml MIC which is illustrated in Fig. 6. Bharthi et al. (2014) and Saranya et al. (2019) investigated antifungal potential of various medicinal plants whose outcomes revealed that *O. sanctum* extract was highly effective against *P. crysogenum* which was comparable to the presented outcome.

3.4. Investigation of electromagnetic exposure effect on antifungal potential of selected plants

The antifungal activity of electromagnetically treated test plants was compared with the unexposed control plants and outcomes were denoted as variation in ZOI and MIC upon periodic exposure of non-ionizing radiation. The exposure effect of 24 h upon the antifungal activity of both plants was similar to bacterial strains that revealed transient raise. The positive effect of short term exposure of high frequency waves on antifungal property of plants was reported against all isolates. The range of variation in ZOI was 11.04–27.34% in the case of *O. sanctum* and 4.94–30.50% in case of *B. monnieri* revealed induction of plant defense. In addition, MIC was elevated along with a decline in ZOI and in the similar range. Consequently, it denoted the raise in the synthesis of secondary metabolites responsible for antifungal activity. The exposure effects beyond 48 h were deteriorative and revealed a significant ($p < 0.01$) decline in ZOI in the range of 30.77–55.25% and successive raise of MIC ranging from 39.34 to 670% for all isolates.

The prominent deterioration of antifungal potential of both plants was started upon 72 h of exposure and was 57.95% in the case of *A. niger* and 109.78% in the case of *C. albicans* strains. Beyond 72 h of exposure, the continuous decline in antifungal potential obtained up to 144 h and was in the range of 41.46–55.25% decrease in ZOI and 39.44–670.00% raise in MIC and illustrated in Fig. 6.

4. Conclusion

The high-frequency electromagnetic wave effects on the antimicrobial potential of both medicinal plants revealed a very similar pattern of modulation as compared to the electromagnetically unexposed plants. The raise in antibacterial as well as antifungal activity of *Oscimum sanctum* and *Bacopa monnieri* upon 24–48 h indicated the induction of plant defense system and transient positive effect which can be utilized further for getting benefits of controlled exposure on medicinal plants. A significant and persistent decline of 30.71–51.39% of antibacterial and 30.17–49.35% of antifungal activity was reported for *O. sanctum* upon 72–144 h of exposure as compared to control plants. While for *B. monnieri*, deterioration of 32.58–50.69% of antibacterial and 31.67–55.25% of antifungal potential was determined upon long-term exposure of 144 h. Thus, the antimicrobial potential of selected plants was significantly harmed by high-frequency electromagnetic waves upon 72 h–144 h exposure. Such experimental results indicated the stress response of high frequency radio waves on medicinal plants that adversely affect the antimicrobial potential of the plants.

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