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Research Article

FABRICATION AND CHARACTERIZATION OF TEBUCONAZOLE LOADED PVA NANOFIBRE

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Abstract: Advancement of nanotechnology raises the scope and hope of the researchers to invent new technologies for sustaining the productivity and production in agriculture. Nano-fibre is a versatile technique and acts as a carrier to entrap bio-active molecules for sustained release and improved use efficiency. The objective of this study was to encapsulate the systemic fungicide tebuconazole in biodegradable polymer nano matrix for smart delivery at targeted site. Tebuconazole at 250 ppm concentration was loaded in polyvinyl alcohol (PVA) nano-fibres. The samples were further characterized by scanning electron microscopy (SEM), transmission electron microscopy (TEM), Energy-Dispersive X-ray Spectroscopy (EDAX), UV-Vis spectrophotometer and Fourier Transform Infra-Red spectroscopy (FTIR). Increased diameter of the nano-fibre in SEM and TEM morphological study suggest that the Tebuconazole loading alter the internal structure of the PVA polymer matrix. The results were supplemented with qualitative analysis done with UV-Vis and FTIR where the data indicated that the tebuconazole could be effectively entrapped in PVA nano-fibre by showing the peaks and functional groups of the fungicide at respective wave length and wave numbers. EDAX results added value to this by conforming the elemental composition in tebuconazole entrapped PVA nano-fibre. The study concludes that tebuconazole can be successfully loaded in e-spun PVA nano-fibre and certainly this would be an alternative, viable and effective method for sustained release of fungicide molecules against pathogens.

Keywords: *Electrospinning, PVA Nano-Fibre, Encapsulation, Tebuconazole*

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Introduction

Nanotechnology is one of the most promising technologies where we could build up précised products, materials and processes. In recent years, number of nano-materials has been developed with intend to promote the efficiency and sustainability of agricultural interventions requiring less input and generating less waste than conventional products and approaches. Nanotechnology has advanced techniques like nanovesicles, nanospheres, nanotubes and nano-fibres for encapsulation and sustained release of bio-active molecule for prolonged Period [1]. Electrospinning technique is a fascinating advanced technology and gained high attention in nanotechnology where polymer nano-fibre can be easily fabricated in diameters of submicrons or nanometers. High surface area to volume ratio and easy incorporation of active molecules has impelled researchers to investigate using electro spun nanofibers in agricultural application. Nano-fibre has been exploited for effective encapsulation of agricultural inputs, which offers protection and sustained and targeted delivery of inputs [2]. E-spun PVA and PVP nano-fibres have been exploited for successful loading of plant growth promoting horrmones as smart delivery through seeds to improve germination and seedling growth in cotton [3] and groundnut [4].The controlled release of nanofibres encapsulated agrochemicals helps in reducing pathogen infection and their emergence [5]. Considering the importance of nano-fibre in agricultural uses, this study aimed to fabricate a biodegradable polymer nano-fibre matrix for effective loading of systemic fungicide tebuconazole in order to protect, précised and extended delivery.

Materials and method

Polyvinyl alcohol alcohol (PVA) and Tebuconazole purchased from Sigma Aldrich Pvt., Ltd., constituted as study materials.

Fabrication of PVA e-spun nanofibre

Different concentrations (5%, 7%, 8%, 9%, and 10%) of Polyvinyl alcohol were prepared using double distilled water and subjected to e-spin at constant voltage of 20 kV & flow rate of 0.3 ml per hour with constant distance between collector and tip of the needle and characterized for SEM.

Encapsulation of Tebuconazole in PVA nanofibers

E-spin mixture for tebuconazole loading was developed by using optimized concentration of PVA polymer. 250 ppm of tebuconazole was prepared by dissolving the fungicide in acetonitrile organic solvent and the polymer and fungicide blend was achieved by constant magnetic stirring at room temperature with 450 rpm. The blended solution was added to 5ml plastic syringe with needle internal diameter of 0.55mm, and elctrospun at at constant voltage of 20 kV & flow rate of 0.3 ml per hour with constant distance between collector and tip of the needle [6]. The fungicide entrapped PVA nano-fibre was characterized for morphology and confirmation of the loading using SEM, TEM, UV-Vis and FTIR.

Scanning electron microscopy

Scanning Electron Microscope (Quanta 250, FEI, and Netherlands) was used to characterize the surface morphology of the fungicide loaded fibre. Samples of tebuconazole loaded PVA nanofibre were kept over the aluminium stub and the topography was observed at different magnifications.

Transmission Electron Microscope

The TEM FEI Technai Sprit was used to analyze structure of the nanofibre. Copper grid was placed over the horizontal collector and the fibre was projected over the copper grid for 5 min, and viewed with a W-source and an ultra-high resolution pole piece at different magnifications.

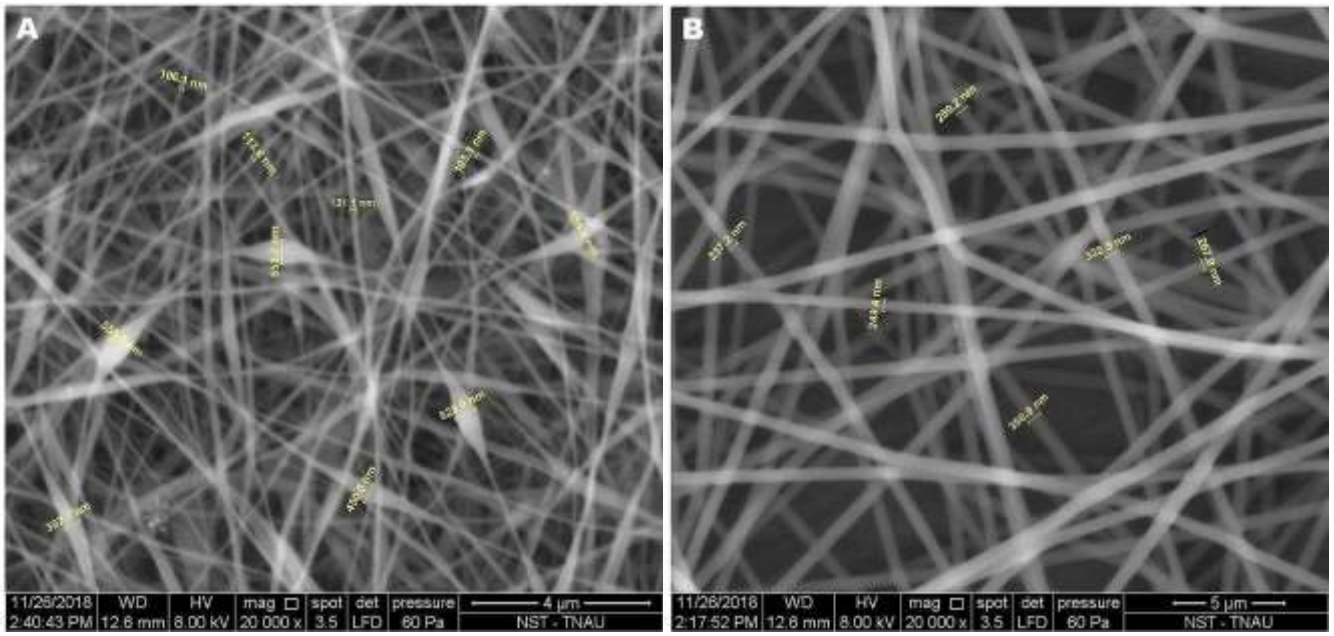

Fig-1A) Scanning electron microscope of 5% PVA nanofibre. B) 10% PVA nanofibre.

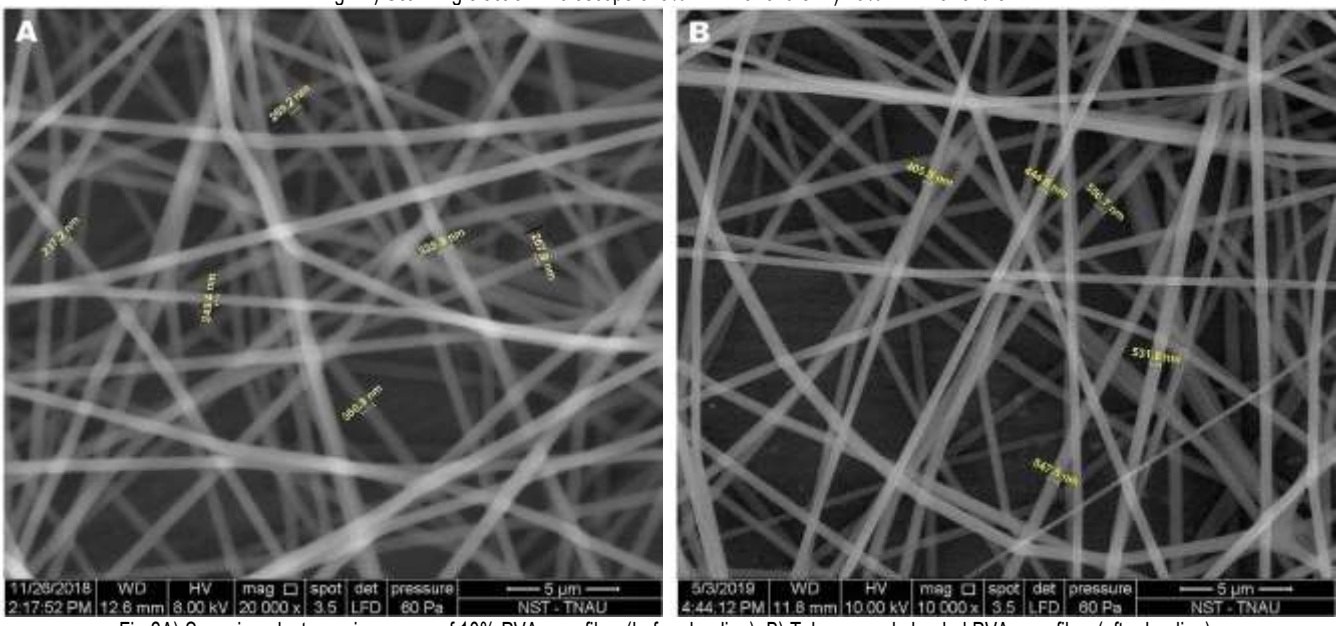

Fig-2A) Scanning electron microscope of 10% PVA nanofibre (before loading). B) Tebuconazole loaded PVA nanofibre (after loading)

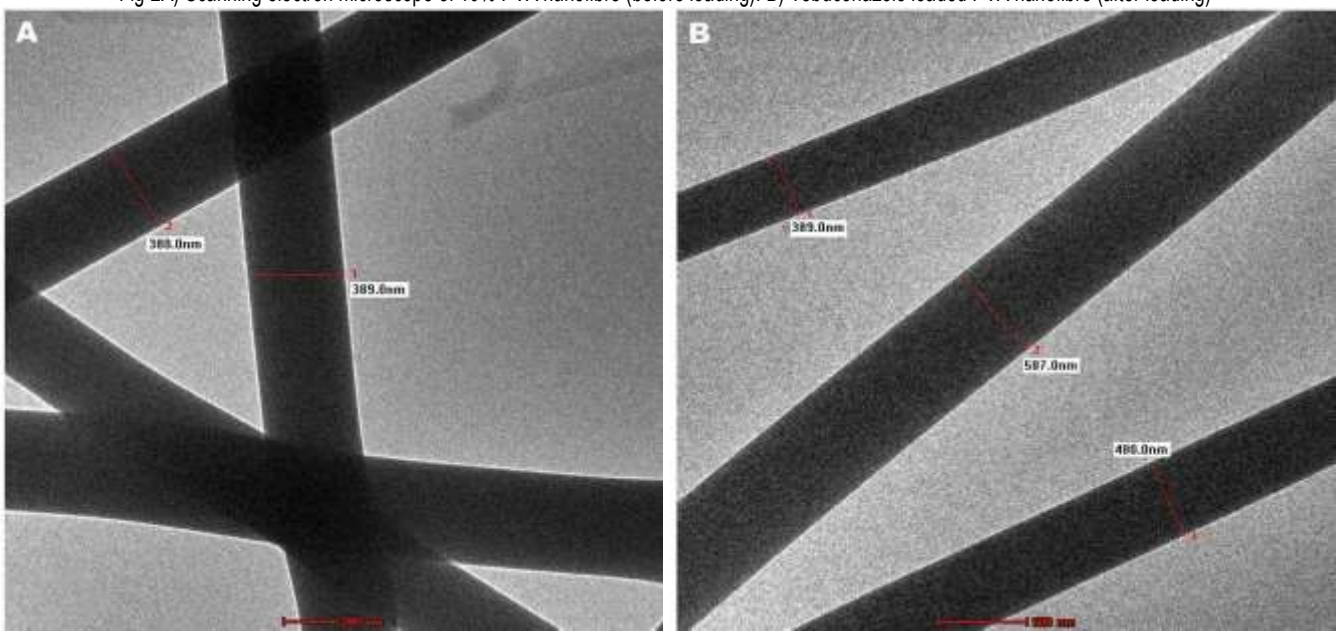

Fig-3A) Transmission electron microscope of 10% PVA nanofibre (before loading). B) Tebuconazole loaded PVA nanofibre (after loading)

Energy dispersive X-ray spectroscopy (EDAX)

Energy dispersive X-ray spectroscopy is an analytical technique used for the elemental analysis or chemical characterization of a sample. EDAX of tebuconazole loaded PVA nanofibres was done by FEI QUANTA 250 EDAX while characterizing them for surface morphology.

UV-VIS

Qualitative analysis

Tebuconazole presence or absence in the nanofibre were ensured by using UV-Visible spectroscopy (Model SPECORD plus 210 BU, Analytik Jena AG, Germany) through scan mode ranges from 200 nm to 800 nm at the rate of 5 nm per second. Acetonitrile was used as blank for setting the absorbance at zero level. Initially the absorbance of technical grade tebuconazole was noticed and used as reference for comparing the sample spectral value.

Quantitative analysis

Encapsulation efficiency of tebuconazole in the nanofibre was measured using UV-Visible spectrophotometry. Standard graph was prepared by dissolving technical grade tebuconazole in acetonitrile at 200, 400, 600, 800 and 1000 ppm and the absorbance was measured at 270 nm, and using absorbance data, a standard graph was prepared with R^2 value of more than 0.90. The quantity of tebuconazole encapsulated in PVA nanofibre was calculated using the given formula.

$$Encapsulation\ efficiency(\%) = \frac{Amount\ of\ fungicide\ in\ the\ PVA\ nanofibre\ measurement}{Amount\ of\ Fungicide\ initially\ loaded} \times 100$$

FT-IR

Fourier transform infrared spectroscopy (FTIR) is a technique used to confirm the encapsulated tebuconazole in PVA nanofibre through infrared spectrum of transmittance or emission from samples using FT/IR-6800 typeA equipped with Attenuated Total Reflectant Unit (ATR Pro One) sensor. Spectral data between 400 and 4000 cm^{-1} was collected with averaging 64 scan at a resolution of 4 cm^{-1}.

Results and Discussion

Standardization polymer concentration for the development of nanofibers

Biodegradable synthetic hydrophilic polymer PVA was used to develop fibre in nano scale diameter for encapsulating the systemic fungicide tebuconazole. The results of SEM images showed that at low concentrations, nano-fibres were started to develop at 5 and 6% with more of beads [Fig-1a]. The fibres with required characters were developed at 10 % (diameter ranges from 293.0 to 373.0nm) PVA concentration without a single beads [Fig-1b]. Beads development at low concentrations is owing to low viscosity. A mixture of beads and fibres at less concentration is little higher [7]. At higher concentration, overlapping of the polymer chains favours entanglement, which gives rise to a much stronger interaction and so leads to smooth fibres rather than particles [8]. During electrospinning, a solution with low viscosity possesses a low viscoelastic force, which is not able to match the electrostatic and columbic repulsion forces that stretch the electrospinning jet. Under the effect of surface tension, the high numbers of free solvent molecules in the solution come together into a spherical shape causing formation of beads.

Characterization of fungicide entrapped PVA electrospun fibre

SEM Morphology of fungicide loaded PVA electrospun fibre revealed that the diameter of fibre was increased due to entrapment of tebuconazole, and measured as ranges from 405.9nm to 556.7 nm after loading [Fig-2a &2b]. This result was further confirmed with TEM where there was increase in diameter of nanofibres observed after fungicide encapsulation (389.0 to 587.0nm) [Fig-3a &3b]. The EDAX spectra confirmed the elemental composition of tebuconazole encapsulated in PVA nano-fibre [Fig-4]. UV-Vis spectroscopy analysis also confirmed the presence of tebuconazole in PVA electrospun fibre as peaks appeared at 270 nm [Fig-5].

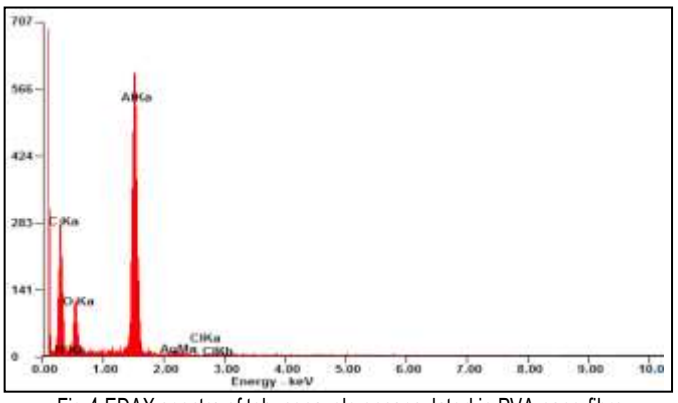

Fig-4 EDAX spectra of tebuconazole encapsulated in PVA nano-fibre

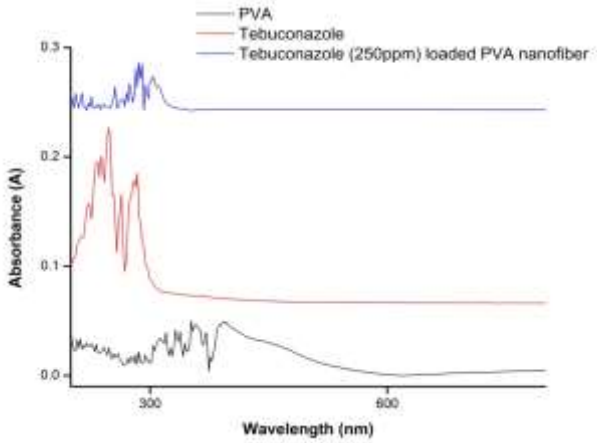

Fig-5 UV-Vis absorbance peaks of tebuconazole encapsulated in PVA nano-fibre

Table-1 *FT-IR transmittance peaks of tebuconazole encapsulated in PVA nano-fibre*

Functional group	PVA nanofibre (cm^{-1})	Tebuconazole compound (cm^{-1})	Nanofibre encapsulated Tebuconazole 250ppm (cm^{-1})
O-H stretch	3306	3287	3320
C-H stretch	2910	2915	2914
C=O stretch	1732	1732	1734
C-O stretch	1091	1087	1088
C=N stretch	-	1489	1487
N-N stretch	-	1508	1506

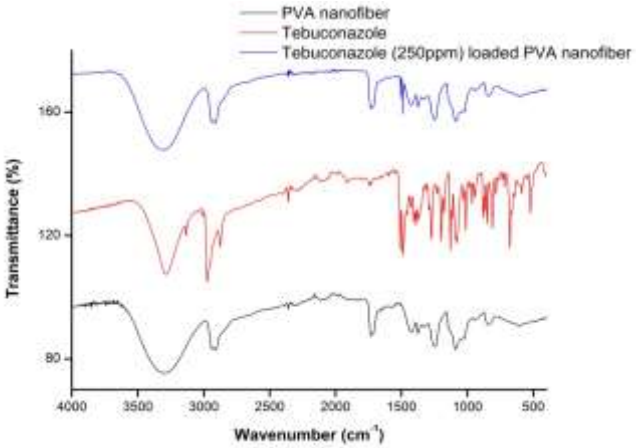

Fig-6 FT-IR spectra of tebuconazole encapsulated in PVA nano-fibre

The possible tebuconazole / polymer interaction was determined by infrared spectroscopy. The pure component of tebuconazole showed transmittance peaks at wavenumbers of 678 cm^{-1} (C-Cl stretch), 1087cm^{-1} (C-O stretch), 1489cm^{-1} (C=N stretch), 1508cm^{-1} (N-N stretch), 1732cm^{-1} (C=O stretch), 2915cm^{-1} (C-H stretch) and 3287cm^{-1} (O-H stretch). Similarly, the pure PVA registered the transmittance peaks at wavenumbers of 1091 cm^{-1} (C-O stretch), 1732 cm^{-1}(C=O stretch), 2910 cm^{-1}(C-H stretch) and 3306cm^{-1}(O-H stretch).

FTIR spectra of tebuconazole loaded PVA nanofibre had the transmittance peaks at wavenumbers of 1088cm^{-1} (C-O stretch), 1487cm^{-1} (C=N stretch), 1506cm^{-1} (N-N stretch), 1734cm^{-1} (C=O stretch), 2914cm^{-1} (C-H stretch) and 3320cm^{-1} (O-H stretch).The stretching of functional groups C=N and N-N was found absent in PVA nanofibre, while presence was noted in the tebuconazole loaded nanofibres as shown in [Fig-6] and [Table-1]. Thus, the fungicide compound loaded in the nanofibre was confirmed. Encapsulation efficiency of tebuconazole in PVA nanofibre was analyzed in UV-Vis spectroscopy and the results exhibited that about 30.0 per cent of the fungicide was effectively entrapped in PVA nano-fibre. The nutrients has UV absorbance peak at 326nm confirmed the loading of nutrients in PVA nanofibre [9]. The curcumin entrapment in cellulose acetate phthalate nano-fibres was 96.7% and UV-Vis spectra at 420nm demonstrated the successful encapsulation of curcumin in nano-fibres [10]. The amphotericin B molecule entrapment in the PLGA nanofibers was confirmed by FTIR as the typical peaks showed at 1600 and 1005 cm^{-1} shifted slightly, due to hydrogen bond formation [11]. The SEM morphology of PAN nano-fibres was altered due to fortification of eugenol as the average diameter of fibre increased from 127±21nm to 212±29nm after loading [2]. The topography of fluconazole loaded PVA electrospun nanofibres revealed that the diameter of fibre was increased due to loading of fluconazole. The EDAX spectra confirmed the elemental composition of fluconazole encapsulated in fibres and also confirmed the encapsulation and presence of fluconazole in electrospun fibre. The UV-Vis spectroscopy analysis also confirmed the presence of fluconazole in PVA electrospun fibre [12].The diameter of PVP nanofiber found swelling and loosening after the gelation of hydrogel loaded [13].

Conclusion

The study concluded that PVA polymer nanofibre matrix can be utilized for encapsulating tebuconazole in order to protect and sustain release of fungicide at the targeted site. The release kinetics and the bio-efficacy of fungicide entrapped nanofibres against the pathogen survivability would be studied in future for fine tuning the findings in this study.

Application of research: The fabricated nano-fibres can be used for smart delivery of tebuconazole fungicide to control pathogens through seed coating.

Research Category: Nano-fibre, Plant protection

Abbreviations:
PVA- Polyvinyl alcohol, PVP- Polyvinyl pyrrolidone
PLGA- Poly(lactic-co-glycolic acid)
SEM- Scanning Electron Microscope
TEM- Transmission Electron Microscope
EDAX- Energy- Dispersive X-ray Spectroscopy
FT-IR – Fourier Transform Infrared Spectroscopy
ATR – Attenuated Total Reflectant unit

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Cultivar / Variety / Breed name: Nil

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Ethical Committee Approval Number: Nil

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