

Original Research Article

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Rapid Identification of Active Ingredients in Commercial Grade Fungicides by Fourier Transform Infrared Spectroscopy

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ABSTRACT

The determination of fungicides particularly the carbendazim and mancozeb could not be done satisfactorily using chromatograph techniques as they undergo fast decomposition when dissolved in solvents for sample preparation. Hence the suitability of the Fourier Transform Infrared Spectroscopy (FTIR) was tested for the rapid identification of fungicide active ingredients viz., carbendazim, mancozeb, copper oxychloride and sulphur in commercial formulations. This was achieved through targeting the characteristic wavenumbers, functional groups and vibration types of each molecule through measuring FTIR spectra over 4000-400 cm^{-1} range at the spectral resolution of 8 cm^{-1} with the 1024 scans. The FTIR spectrum of each compound was compared with the online database to confirm their identity. The present technique is found to be a true, alternative over lengthy and repetitive chromatograph method, which are commonly recommended for the quality assurance of market-accessible formulations. The present approach is rapid and offers green technology as it involves less sample, solvent, energy and waste generations.

Keywords

FT-IR, Commercial fungicides, Wavenumbers, Functional groups

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Introduction

Fungicides are pesticides that execute or forestall the development of parasites and their spores. They can be utilized to control growths that harm plants, including rusts, moulds and mildews. Fungicides work in an assortment of ways, yet the majority of them

harm contagious cell layers or meddle with vitality creation inside parasitic cells. Most farming and agricultural fungicides are applied as showers or dust. Seed fungicides are applied as a defensive covering before germination. Systemic fungicides, or chemotherapeutants, are applied to plants, where they become circulated all through the

tissue and act to kill existing malady or to ensure against conceivable sickness. The purpose of fungicide use could be achieved only if consumers have access to quality products. Many times the assurance of quality products to the farmers becomes a challenging task for the quality control personnel due to adulteration of the registered commercial formulations during transit or storage. The biggest downside is that the analysis of such substances typically requires multiple techniques for sample preparation and assessment using volumetry or chromatograph beside needs more time and resources.

As indicated by the CIPAC, the techniques used for the routine analysis of pesticide formulations are now focused on gas and liquid chromatography. Nonetheless, the potential of infrared spectrometry approaches as an appropriate alternative methodology for addressing this research demand was shown in recent studies. For emulsifiable concentrate formulations, pyrethroids such as cypermethrin and deltamethrin were tested by FTIR spectrometry following separation by thin-cell chromatography and re-dissolution in CCl_4 (Sharma *et al.*, 1997)

These days, there is a progressing interest in the advancement of quick and non-dangerous methods with no sample preparation or manipulation, which can be valuable for the quality control of manufactured items or crude materials. In this sense, vibrational spectroscopy-based procedures with a chemometric treatment of the information give significant apparatus to tackle the issue in other modern territories (Salari and Young, 1998).

Fourier transform infrared (FTIR) is a fast and micro-destructive spectroscopy method widely applied in the measurement of solid (Post *et al.*, 1995), liquid (Van de Voort *et al.*,

2004), and gas (Esler *et al.*, 2000). This technique is used to identify the functional groups of the compounds through different strategies and FTIR is working at a different range of spectra. Mid-infrared absorption bands (MIR) are representative of the bonds and functional groups of a molecule. Such bands contain much of the molecule's general stretching, twisting and moving movements, and the total range may act as fingerprint for a specific compound. This identification approach has many advantages as an authenticity tool for screening; extremely quick (tests can be performed in 1 to 2 minutes), easy to use and implemented by unskilled personnel (Kelly and Downey, 2005).

Carbendazim and some other fungicides are widely used to control postharvest decay caused by various fungal pathogens in citrus fruit (Fernandez *et al.*, 2001). Mancozeb, a [[1,2-ethylenbis(dithiocarbamate)](2-)] of manganese and zinc blend, is a synthetic pesticide, which has been utilized since 1967 as a fungicide to forestall the development of moulds and to ensure plants and yields against harm caused by parasites. Mancozeb should be splashed on surfaces of leaves and harvests for assurance against moulds. Luckily the harmfulness of Mancozeb is exceptionally low, with LD_{50} estimations of the request for a few $g\ kg^{-1}$ (Armenta *et al.*, 2005).

The determination of a trace amount of carbaryl (organic contaminant) in copper oxychloride necessitate the development and full validation of the method by chromatography. It was determined normally based on HPLC-DAD and confirmation of the identity of the compound with HPLC-PDA-ESI-MS (Karasali *et al.*, 2014) which involves sample preparation, more solvent consumption, time requirement and tedious process.

Gallart-Mateu *et al.*, (2016) analysed the copper oxychloride by diffuse reflectance near-infrared (DR-NIR) spectra and observed a characteristic band in the spectral region between 5340 and 4869 cm^{-1} and states that these bands are related to water absorption in the copper oxychloride structure being overtones and combination of vibrational modes of OH- groups. The formulations containing copper oxychloride are also characterized by a strong band centred at 4050 cm^{-1} possibly due to overtones or combination bands of Cu-Cl fundamental vibrations (Frost *et al.*, 2010).

Thus, the point of this paper is to introduce an environmental agreeable strategy with no sample pre-treatment that can be utilized for the standard assurance in financially detailed fungicides as a genuine option in contrast to dreary, time and reagent expending reference systems. With this background, the commercial fungicide formulations were tested using FTIR for the rapid identification of their active ingredients.

Materials and Methods

Commercial-grade chemicals

Commercially available pesticides were purchased from the local agrochemical store, in which each farmer has access to his or her agricultural chemical needs. Table 1 describes the particulars of the formulations used in the analysis.

Fourier Transform Infrared Spectroscopy (FT-IR)

Fourier Transform Infrared Spectroscopy (FT-IR) is a technique used to achieve an infrared spectrum of solid, liquid or gas absorption, emission, photoconductivity or Raman dispersion. Around the same time, an FTIR spectrometry instrument used was

Nicolet iS10 with OMNIC spectra software. Two screens, such as the Smart-iTR window and Omnic transmission window, were used for sample analysis. A Bio-Rad Excalibur 3000 MX FTIR spectrometer and helium purged MTEC 300 photoacoustic cell were collected here to collect the spectral data. All the spectra were reported at a spectral resolution of 8 cm^{-1} over the 4000-400 cm^{-1} range and co-added with the 1024 scans. The KBr was used as a pelleting medium with powdered formulations and granules, and liquid formulations were fed directly to the iTR slot.

FTIR procedure

Omnic transmission window

The pesticide powder formulations were packed into a thin pellet for FTIR analysis. IR transparent content called KBr has been combined with fungicide formulation at a ratio of 2:1 in a mortar and pestle over 5-10 minutes for the preparation of pesticide pellet samples. The mixture was then converted into pellets by pushing the prepared mixture on a hard disk using a hydraulic press. A complete hydraulic laboratory press producing strength (force) of about 15 tones was used to produce 0.5 to 1 mm thick pellets which were then mounted in a transmission holder and screened.

Handling and comparison of test spectra with database

FTIR spectra of fungicide commercial formulation samples were correlated with the National Institute of Standards and Technology (NIST) database and processed also using Bio-Rad KnowItAll. Software. The NIST offers basic reference data that covers a wide spectrum of logical order including nuclear and sub-atoms, organic and precious stone systems, gases, structural properties,

biotechnology, and optical character recognition, among others. Bio-Rad Labs, Inc. is an on-line spectrum store, with a wide range of spectra, including natural blends, inorganic mixtures and polymers, for sample spectra. KnowItAll provides detailed responses to the concerns of IR, Raman, NIR, NMR, MS, UV-Vis, and chromatography. Throughout tandem with the world's greatest spectral collection, scientists can discern substantially more influential details from their imagination.

Results and Discussion

Carbendazim

Carbendazim is a member of the benzimidazoles class that is 2-aminobenzimidazole in which the primary amino group is substituted by a methoxycarbonyl group. Its commercial-grade Carbendazim 50% WP was subjected to FTIR analysis and then compared with the spectrabase and bio-rad software (Fig:1) to identify the functional groups. It shows antisymmetric deformation of primary amine NH_3 at $1580\text{-}1640\text{ cm}^{-1}$, symmetric deformation of NH_3 at $1340\text{-}1250\text{ cm}^{-1}$ and nitrile group C-N=C of C=N bond stretching at $1650\text{-}1550\text{ cm}^{-1}$ (Table 2). This is similar to the characteristics of IR spectra published in the PubMed and spectrabase for carbendazim standard. Hence the C=N bond stretching of nitrile group C-N=C in carbendazim at 1630 cm^{-1} wavenumber could be considered for identifying and quantifying the carbendazim active ingredient in commercial fungicide formulation. Similar stretching of C=N bond at 1628 cm^{-1} for carbendazim was reported by Sandhya *et al.*, (2016). The 100 % peak intensity at 1007.14 cm^{-1} and 95.26 peak intensity at 1029.80 cm^{-1} was also observed in the present study which needs to be explored further for characterizing the carbendazim by FTIR.

Mancozeb

Mancozeb (75% WP) is a dithiocarbamate non-systemic fungicide (the chemical formula is $\text{C}_8\text{H}_{12}\text{MnN}_4\text{S}_8\text{Zn}$ which denotes the compound Dithane 945) was analysed in FTIR to identify the characteristic spectra of mancozeb (Fig. 2). The strong intense and medium-weak bonds were observed at 1580 to 1520 cm^{-1} and 1450 to 1350 cm^{-1} which shows the presence of triazine ring and C_3N_3 bond stretching (Table 2). The strong stretching of NH bond at 3400 to 3200 cm^{-1} and deformation of NH bond at 1580 to 1530 cm^{-1} and 750 to 550 cm^{-1} was also observed for Mancozeb. Similar intense band for mancozeb at 1525 and 1510 cm^{-1} was observed by Armenta *et al.*, (2005) correspond to amide II band in its structures. There was a medium-strong stretching of NH bond at 3350 to 3250 cm^{-1} and the similar band was also observed by Armenta *et al.*, (2005) for Mancozeb standard. The mancozeb characteristics spectral range corresponding to amide band was observed between 1505 to 1525 cm^{-1} and area or height measured at this range could be used to identify and quantify the mancozeb content in commercial formulation after validation.

Carbendazim + Mancozeb

Fig. 3 shows the FTIR spectra of Carbendazim and Mancozeb active ingredients in Carbendazim 12% + Mancozeb 63% WP mixed commercial formulation. The overlay spectra (Fig 3) of mancozeb, carbendazim and their mixture shows the difference in the spectra at 2500 to 3500 cm^{-1} which is present in the mancozeb and mixture spectra and absent in carbendazim spectrum. Mixture spectra shows 100% intensity at 1506 cm^{-1} , 1510.54 cm^{-1} , 1512.01 cm^{-1} , 1521.62 cm^{-1} and 1525.06 cm^{-1} which is the characteristic amide band for mancozeb (Table 2). The peak at 3153.66 cm^{-1} and

3293.48 cm^{-1} shows 67.41% and 66.02% intensity and was similar to that observed for carbendazim 50% WP alone. The characteristics C=N band at 1628 cm^{-1} for carbendazim was also observed in the mixed spectra and confirmed that both the active ingredients of mancozeb and carbendazim

could be detected in mixed commercial fungicides formulation without overlapping of bands and spectra. Quantification can be done after thorough validation. Identification of mancozeb in mixed pesticide formulation containing mancozeb plus Cymoxanil was also reported by the Armenta *et al.*, (2005).

Table.1 Details of fungicide formulations selected for the study

Active ingredients	Formulations	Molecular Formula	IUPAC Name
Carbendazim	50% WP	$\text{C}_9\text{H}_9\text{N}_3\text{O}_2$	methyl N-(1H-benzimidazol-2-yl)carbamate
Carbendazim + Mancozeb	12% + 64% WP	-	-
Copperoxychloride	50% WG	$\text{Cl}_2\text{Cu}_4\text{H}_{12}\text{O}_6$	Copper chloro hypochlorite
Mancozeb	75% WP	$\text{C}_8\text{H}_{12}\text{MnN}_4\text{S}_8\text{Zn}$	zinc;manganese(2+);N-[2-(sulfidocarbothioylamino)ethyl]carbamodithioate
Sulphur	80% WDG	-	-

Table.2 Fungicides active ingredients determination in commercial formulations using FTIR Spectrometry by KBr pelleting method

Pesticides	Measurement mode	Sample throughput (hr^{-1})	Waste generation	Characteristics band & vibrations	Wavenumber (cm^{-1})
Carbendazim	KBr disks	5-6	10-20 mg	C=N bond stretching of nitrile group C-N=C	1630
Mancozeb	KBr disks	5-6	10-20 mg	NH bond stretching	1505 to 1525
Carbendazim + Mancozeb	KBr disks	5-6	10-20 mg	C=N bond & NH bond	1505-1630
Copper oxychloride	KBr disks	5-6	10-20 mg	O-H bond stretching halogen group C-Cl bond stretching	3300-3150 & 800-700
Sulphur	KBr disks	5-6	10-20 mg	S=O bond stretching	1090-990 & 870-810

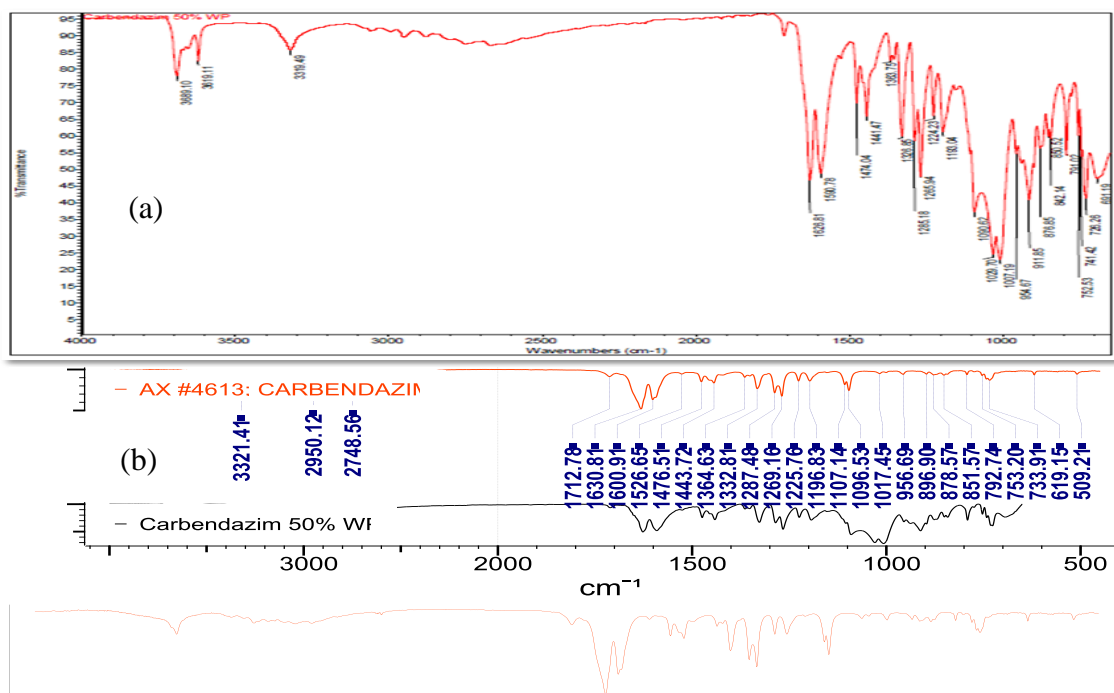


Fig.1 Infrared spectra of commercial carbendazim (a) and its processed spectra (b) by Bio-Rad software

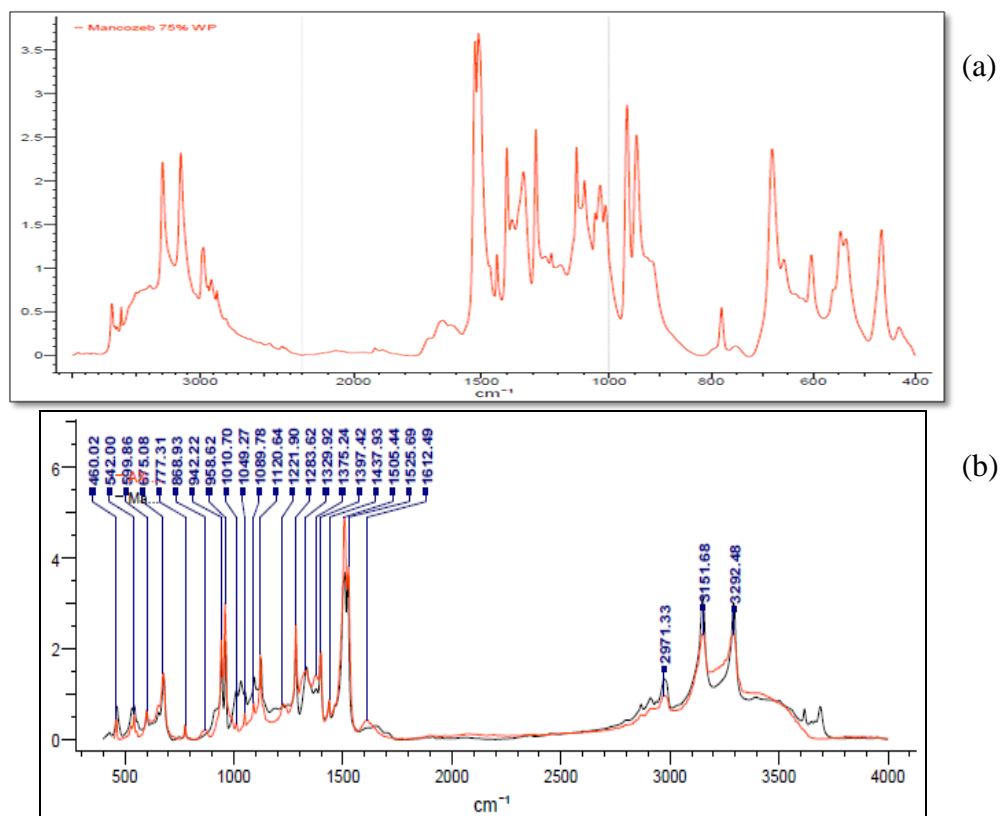


Fig.2 Infrared analysed spectra of commercial Mancozeb (a) and its processed spectra (b) by Bio-Rad software

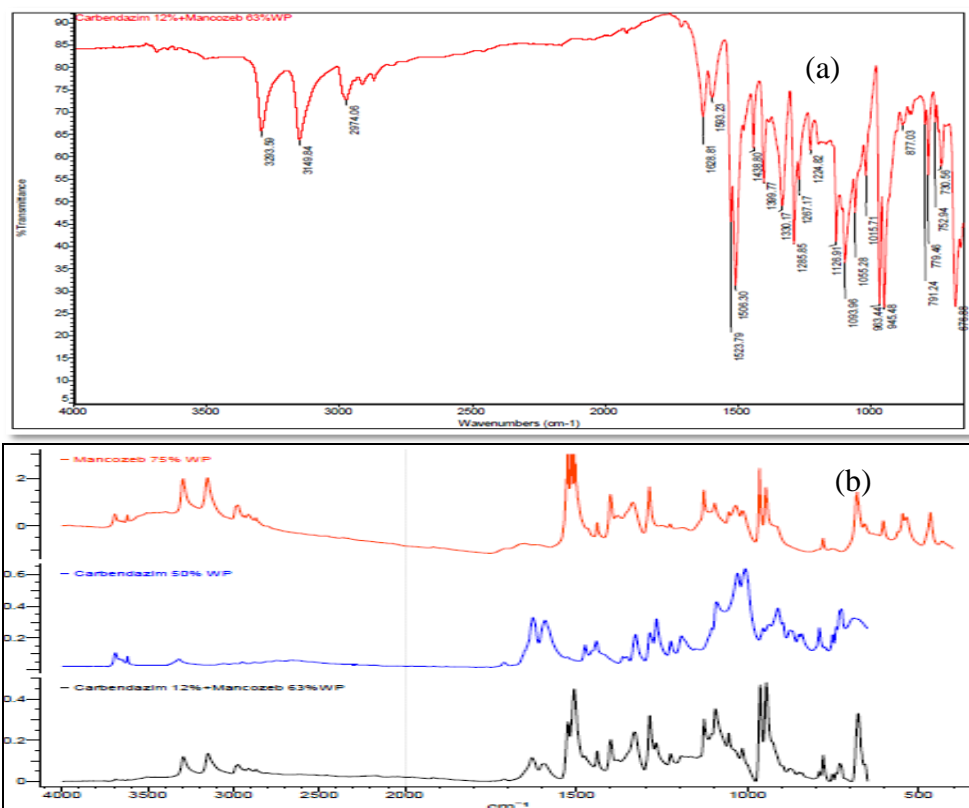


Fig.3 Infrared analysed spectra of commercial (a) and Bio-Rad software processed spectrum (b) of Carbendazim+Mancozeb

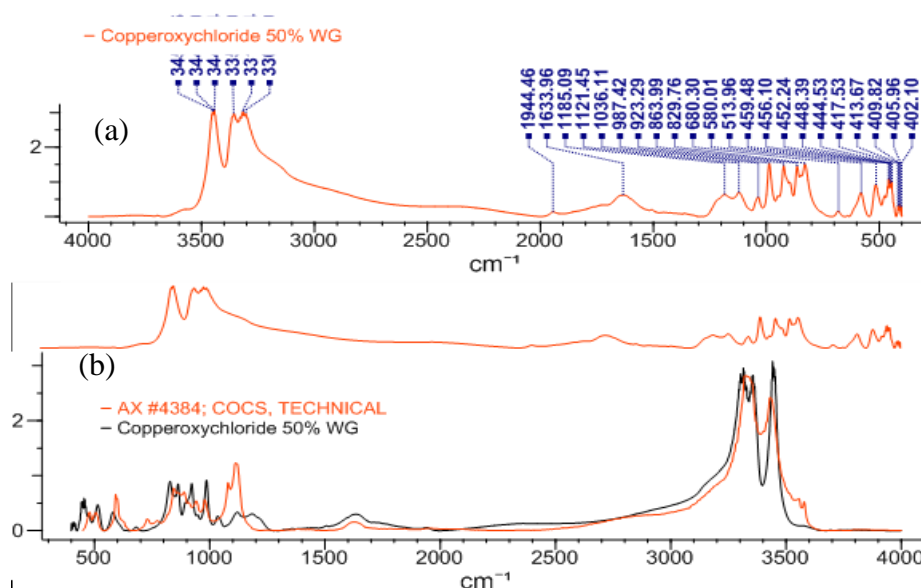


Fig.4 FIIR analysed spectra (a) of commercial copper oxychloride processed by Bio-Rad software (b)

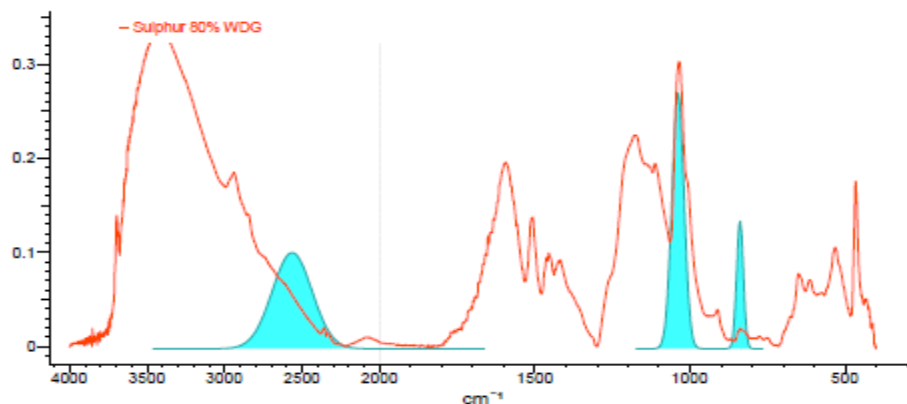


Fig.5 FTIR analysed spectra of commercial sulphur fungicide (shaded peaks shows the functional groups at wavenumbers processed using Bio-Rad's software)

Copper oxychloride

The FTIR spectrum of commercial copper oxychloride 50% WG was examined with the bio-rad's KnowItAll software (Fig:4). When analyzing the copper oxychloride spectrum of commercial-grade, the O-H bond stretching from the range of 3300-3150 cm^{-1} and a halogen group C-Cl bond stretching from the range of 800-700 cm^{-1} was observed (Fig 4 and Table 2). These are the characteristic functional group in copper oxychloride. Overlaying of the FTIR spectrum of copper oxychloride with Bio-Rad's spectrum gave 100% match and confirms that this method could be used to identify and quantify the active ingredient in commercial grades.

Sulphur

Sulphur 80% WDG is broad-spectrum contact and protective fungicide and miticide. The commercial-grade sulphur 80% WDG spectrum is examined with FTIR and processed using the bio-rad's KnowItAll software (Fig:5). It shows S=O bond stretching at 1090-990 cm^{-1} and S-O bond stretching at 870-810 cm^{-1} (Table 2). The S=O bond stretching at 1090-990 cm^{-1} could be used to identify and quantify the S active ingredients in pesticides formulation after validation. The peak intensity was 100% at

3445 cm^{-1} and 81.96% at 3695.10 cm^{-1} which needs further processing for characterization.

In conclusion, the proposed spectrometric strategies are quick and environmentally friendly alternatives to the great chromatograph methods for the quality control of active ingredients in commercial fungicide formulations.

This technique could be used to identify the undiluted fungicides active ingredients for quality control purpose and check the adulteration or contamination of the formulations even during transit. It helps to make assurance of fungicide formulations quality with minimum time, sample preparation and solvent. The technique can also be extended to quantify the active ingredients in commercial grades and even residues in farm produce after due validation.

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References

- Armenta, S., S. Garrigues, and Miguel De La Guardia. (2005). "Solid Sampling Fourier Transform Infrared Determination of Mancozeb in Pesticide Formulations." *Talanta* 65 (4): 971–79. <https://doi.org/10.1016/j.talanta.2004.08.029>.
- Esler, M. B., Griffith, D. W., S. R. Wilson and L. P. Steele (2000). Precision trace gas analysis by FT-IR spectroscopy. 1. Simultaneous analysis of CO₂, CH₄, N₂O, and CO in air. *Analytical Chemistry*, 72, 206-215.
- Fernandez, M., Rodriguez, R., Y. Pico and J. Manes, (2001). Liquid chromatographic-mass spectrometric determination of post-harvest fungicides in citrus fruits. *Journal of Chromatography A*, 912(2), 301–310. [https://doi.org/10.1016/S0021-9673\(01\)00576-3](https://doi.org/10.1016/S0021-9673(01)00576-3)
- Frost RL, Reddy BJ, Keeffe EC. (2010). Structure of selected basic copper (II) sulphate mineral-based upon spectroscopy-implications for hydrogen bonding. *J Mol Struct.* 977(1-3):90.
- Gallart-Mateu, D., S. Armenta and M. de la Guardia. (2016). Green near-infrared determination of copper and mancozeb in pesticide formulations. *Anal Bioanal Chem.*, 408:1259–1268.
- Karasali, H., Kasiotis, K. M., K. Machera, and A. Ambrus, (2014). Case study to illustrate an approach for detecting contamination and impurities in pesticide formulations. *Journal of Agricultural and Food Chemistry*, 62(47), 11347–11352. <https://doi.org/10.1021/jf504729g>
- Kelly, J. F. D., and G. Downey, (2005). Detection of sugar adulterants in apple juice using Fourier transform infrared spectroscopy and chemometrics. *Journal of Agricultural and Food Chemistry*, 53(9), 3281–3286. <https://doi.org/10.1021/jf048000w>
- Post, E., Rahner, S., H. Möhler and A. Rager (1995). Study of recyclable polymer automobile undercoatings containing PVC using TG/FTIR. *Thermochimica acta*, 263, 1-6.
- Salari, A., and R. E. Young (1998). Application of attenuated total reflectance FTIR spectroscopy to the analysis of mixtures of pharmaceutical polymorphs. *International Journal of Pharmaceutics*, 163(1–2), 157–166. [https://doi.org/10.1016/S0378-5173\(97\)00378-5](https://doi.org/10.1016/S0378-5173(97)00378-5)
- Sandhya, Kumar, S., Kumar, D. and Dilbaghi, N. (2016). Preparation, characterization, and bio-efficacy evaluation of controlled release carbendazim-loaded polymeric nanoparticles. *Environmental Science and Pollution Research*, 24(1), 926–937.
- Sharma, K. K., S. Gupta, and S. K. Handa, (1997). Fourier transform infrared spectroscopic determination of cypermethrin and deltamethrin in emulsifiable concentrate formulations. *Talanta*, 44(11), 2075–2079. [https://doi.org/10.1016/S0039-9140\(97\)00056-8](https://doi.org/10.1016/S0039-9140(97)00056-8)
- Van de Voort, F., J. Sedman, V. Yaylayan, C. S. Laurent and C. Mucciardi (2004). Quantitative determination of moisture in lubricants by Fourier transform infrared spectroscopy. *Applied spectroscopy*, 58, 193-198.

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