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RESEARCH ARTICLE

Synthesis and Biological Activity of Eugenol-Copper Complex

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Abstract: Metal chelate of bioactive metal ion Cu(II) with eugenol was chelated for improving the ligand's efficiency and the complex synthesized was characterized through FT-IR, TG-DTA, AAS and elemental analysis. The composition of eugenol-copper in complex was found to be 1:1 through Job's method. *In vitro* antimicrobial test of eugenol, eugenol-copper complex and $CuSO_4.5H_2O$ was conducted against phytopathogenic fungi *Pyricularia grisea* Sacc., *Helminthosporium oryzae* Breda de Haan and *Curvularia lunata* (Wakker) Boedijin through conidial germination test that revealed enhanced fungi-toxicity of the complex compared to either eugenol or $CuSO_4.5H_2O$ alone especially against the former pathogen.

Keywords: Eugenol-copper complex, FT-IR, TG/DTA, Elemental analysis, Job's method, Fungitoxic activity

Introduction

Eugenol is member of phenylpropanoid class of chemical compound and main constituent of essential oil obtained from *Pimenta racemosa* (bay leaves), *Cinnamomum verum* (cinnamon leaf), *Syzygium aromaticum* (clove) and *Ocimum* species[1-3]. The first biological activity of eugenol, antibacterial, activity was reported by H.A. Bartels[4]. It possesses antifungal, bactericidal, insecticidal, larvicidal, nematicidal[5-8]. It also shows antidepressant[9], antioxidant, anti-inflammatory activities coupled with anti-animal toxin with analgesic properties for human antiseptic in traditional medical practices[10-12]. It finds wide application in perfumeries, flavourings and in medicines. Eugenol affects the peripheral aspects of cardiovascular system. The heart is not the principle site of action since eugenol has little effect on the electrical activity and only slightly reduces the contractile force unless a little fatal dose is used[13]. It is used in combination with zinc oxide as a surgical dressing, pulp capping cavity liner, temporary cement, in mouth washes and endodontic therapy, in study of mucous secretions and in gastric cytology[14,15]. It is important that in several studies, this natural compound was reported to be non- genotoxic and non-carcinogenic[3,12]. In the present study we synthesized complex of eugenol with copper to enhance the antifungal activity of the former against phytopathogenic fungi *viz*., *Pyricularia*

grisea Sacc., *Helminthosporium oryzae* Breda de Haan and *Curvularia lunata* (Wakker) Boedijin responsible to cause blast, brown spot and grain discoloration diseases in rice affecting yield.

Experimental

All the chemicals used were of A.R. grade and the solvents were of G.R. grade.

Synthesis of complex

Eugenol and $CuSO_4.5H_2O$ (in form of solution in methanol) in ratio 5:1 were mixed together and kept for 3-4 days at room temperature. The blue precipitate was obtained that was filtered and washed with diethyl ether and dried in desiccator. After recrystallization, the powder obtained was scrapped out from the beaker and kept in clean glass vials.

Spectral and elemental analysis of the complex

The complex was characterized by FT-IR by Perkin Elmer spectrum RX1, thermogravimetric analysis (TG), differential thermal analysis (DTA) were done through Perkin Elmer STA 6000 and C H N analysis by Elementar Vario EL III and Cu analysis through AAS by ECIL 4139 .

Composition of the complex

The composition of the complex was determined by Job's method using Cecil CE 7400 at 780 nm. The concentration of eugenol and $CuSO_4.5H_2O$ was $3x10^{-4}$ M.

Antifungal activity of the complex

Complex, eugenol and $CuSO_4.5H_2O$ were tested against fungal pathogens *viz*. *P. grisea*, *H.oryzae* and *C.lunata* under *in vitro* conditions through conidial germination test as per method given by Nene and Thapliyal[16] at 100 ppm, 10 ppm, 1 ppm, 0.1 ppm, 0.01 ppm. Conidial suspension of 7 day old pure culture of the test pathogens with 30-35 conidia per microscopic field (1.26 mm^2) were placed separately on each glass slide having treatment and incubated in moist chamber at 24 ^{0}C for 24 h. Observations on conidial germination (%) and the patterns of fungitoxicity were recorded using phase contrast microscope, Olympus BX51 at 10X magnification after 24 h of incubation. Appropriate controls were maintained keeping three replications in each case and the experiment was repeated thrice. Data on germination was transformed to angular value and statistically analyzed. No conidial germination was accorded the numerical value 2% for the purpose of statistical analysis that was done using Cropstat 7.2 developed by International Rice Research Institute (IRRI).

Results and Discussion

Spectral and elemental analysis

FT-IR analysis that revealed that peak at 3514 cm^{-1} in eugenol disappeared due to coordination of OH group in metal in case of complex. Peak at 3367 cm^{-1} appeared due to coordinated water. Peak at 1619 cm^{-1} in C=C in aromatic ring remains as such. 1092 cm^{-1}, 1159 cm^{-1} and 1198 cm^{-1} is due to C-O-C stretching (symmetric and asymmetric). New band at 603 cm^{-1}, 585 cm^{-1}, 517 cm^{-1} is due to Cu-O bonding (Figure 1) which is not found in FT-IR spectra of eugenol. These are characteristic peaks of metal-oxygen bonding. Hasanvand *et al*.,[17] and Volanti *et al*.,[18] reported peaks at 580 cm^{-1}, 610 cm^{-1} and 520 cm^{-1} for Cu-O bonding.

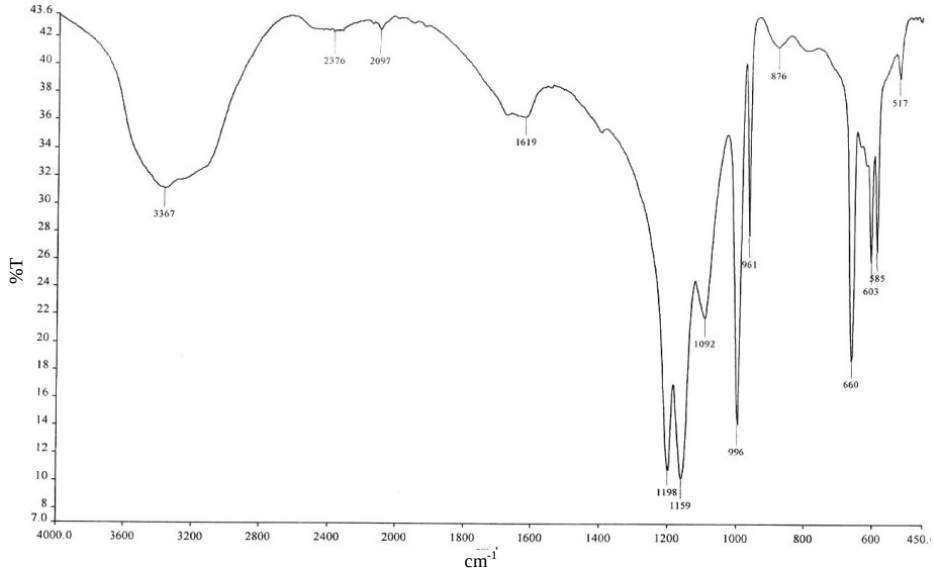

Figure 1. FT-IR spectrum of complex

Thermal analysis methods are widely used for thermodynamic investigation of the complex formed. TG curve of the complex is shown in Figure 2. According to TG data of complex from temperature 35.76 ºC to 735.5 ºC, decomposition of the complex showed following steps. The first weight loss from 40-47 ºC was about 3% corresponding to release of absorbed water molecule.

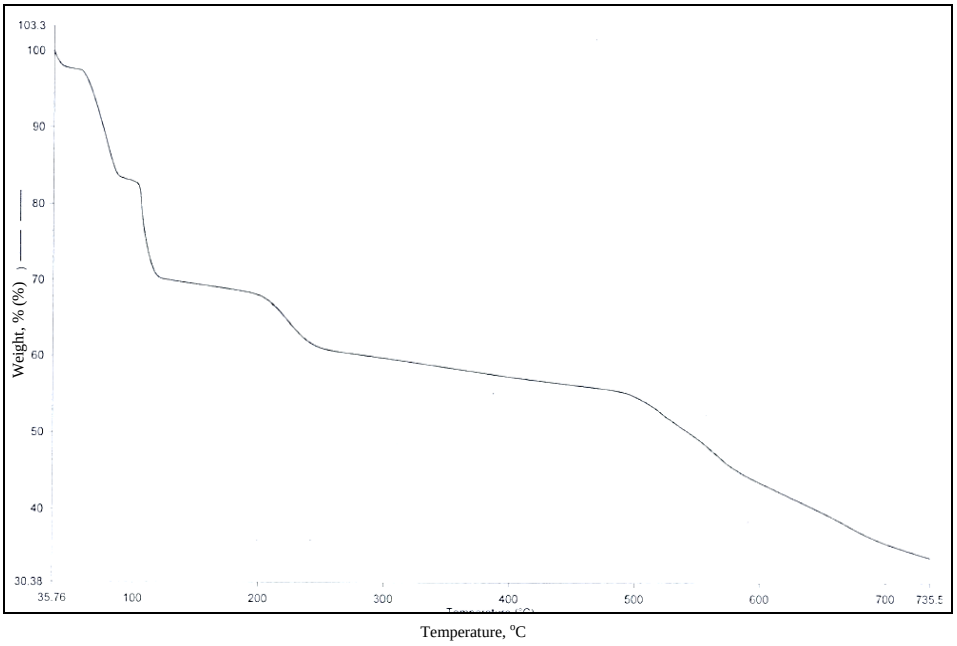

Figure 2.TG spectrum of complex

The second weight loss from 50 ºC-98 ºC was 12% corresponding to the release of two coordinated water molecules, with the maximum rate of weight loss occurring at 75.16 ºC. Third weight loss of 12% from 100 ºC to 110 ºC indicated the release of two more coordinated water molecule from the complex. Maximum decomposition rate of water molecules was recorded at 109.19 ºC (Figure 2 and 3). The next weight loss of 13% occurred from 110 ºC to 225 ºC corresponds to release of allylic group from the complex. The maximum decomposition rate of allylic group was recorded at 222.63 ºC. The weight loss from 230 ºC to 500 ºC was 3% - 4% corresponds to the decomposition of complex forming gases *viz*. CO, CO_2. Beyond 500 ºC, weight loss was about 26% and 7% corresponding to release of benzene ring and methoxy group. Also two peaks 521 ºC and 563 ºC in DTA suggests the decomposition of benzene and methoxy group. Above 700 ºC, a black residue remains corresponding to CuO. The enthalpy data reveals that the peak at 225 ºC shows exothermic reaction and that at 109 ºC shows endothermic reaction (Figure 4).

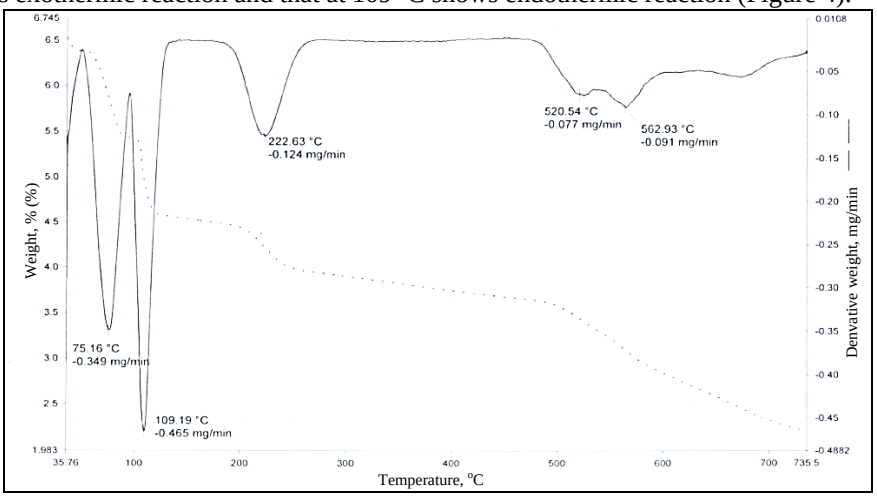

Figure 3. DTA of the complex

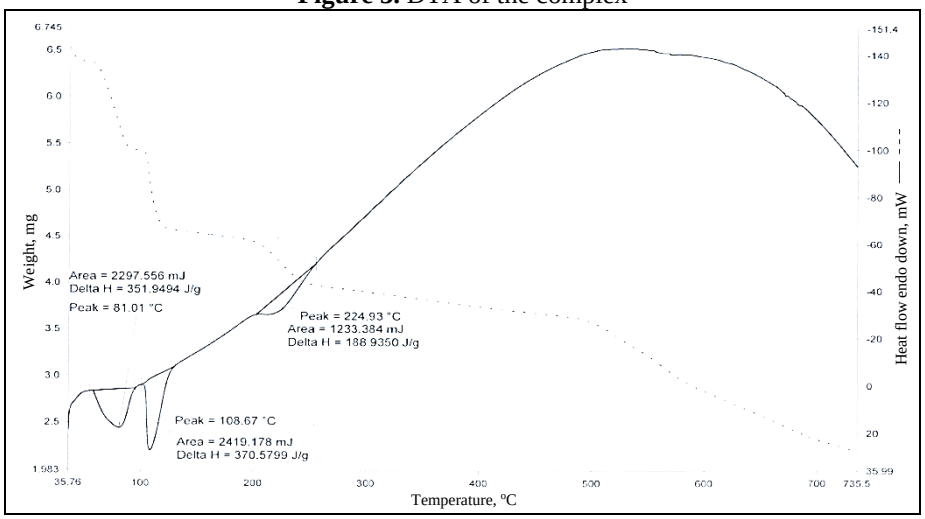

Figure 4. Enthalpy data of complex

Elemental analysis *viz*. C, H, O including Cu done through AAS shows that the percentage of carbon (40.17%), hydrogen (6.30%), oxygen (32.21%) and copper ($2.3x10^5$ ppm *i.e.* 21.31%) were corresponding to the theoretical values of the percentage of these available in the complex (Table 1). Hence C, H, O and Cu percentage data is in agreement with the proposed structure.

Table 1. Percentage of elements in the eugenol-copper complex

Element	% / ppm present
Carbon	40.17 (40.247)*
Hydrogen	6.30 (6.37)*
Oxygen	32.21 (32.193)*
Copper	$2.3x10^5$ ppm =21.31 %
	($2.1x10^5$ ppm = 21.19%)*

**represents theoretical values*

Composition of complex

Through Job's method, the composition of eugenol and copper in complex, was found to be1:1. Based on the above discussion following is the proposed structure:

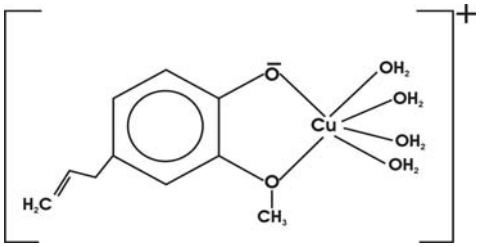

Figure 5. Structure of eugenol-copper complex

Fungitoxic activity of complex

According to Muruganandam *et al.*[19], lipophilic activity of complex increases that extends the residential period on the susceptible host. As result of which the protection period on the host surface increases due to delayed decomposition of complex chelate and in turn efficiency of active ingredient increases. It is known that the existence of metal ions bonded to biologically active compounds may enhance their activities. Singh and Dhakarey[20] also reported that metal complexes with Schiff bases derived from 2-thienyl glyoxal exhibited increased fungitoxic activity than the free ligands.

Eugenol-copper complex exhibited complete inhibition (2% ± 1.52) of conidial germination at 1 ppm concentration and significant reduction of germination at 0.1 ppm compared to eugenol (98% ± 1.52), $CuSO_4.5H_2O$ (98 % ± 1.52) at same concentration in *P. grisea*. In case of *H. oryzae* and *C. lunata*, complex exhibited significant reduction (85% ± 2.31 in *H. oryzae*, 82% ±1.16 in *C. lunata*) of conidial germination compared to control (98% ± 2.31, 1.16) at 10 ppm concentration. Thus the complex was found to be more effective against *P. grisea* compared to latter two pathogens (Table 2). Varying degree of fungitoxic patterns *viz.* granulated cytoplasm, septal dissolution, granulated, thin and longer germ tube compared to that in control were observed at different concentrations.

Table 2. Fungitoxicity of complex against conidial germination of phytopathogenic fungi of rice crop

Concentration ppm	Pathogen								
	P. grisea			*H. oryzae*			*C. lunata*		
	Complex	Eugenol	$CuSO_4.5H_2O$	Complex	Eugenol	$CuSO_4.5H_2O$	Complex	Eugenol	$CuSO_4.5H_2O$
100	2^{1} (8.13)	$45^{1,2,6}$(42.13)	$10^{1,7,10}$(18.44)	15^{1} (22.75)	$89^{1,7}$ (70.63)	85^{7} (67.21)	4^{1} (11.54)	50^{5} (45.00)	80^{7} (63.44)
10	2^{1} (8.13)	$95^{1,7}$ (77.08)	$80^{1,7}$(63.44)	85^{3} (67.21)	98^{10} (81.87)	95^{3} (77.08)	82^{3} (64.90)	98 (81.87)	90^{3} (71.56)
1	2^{1} (8.13)	98 (81.87)	90^{10} (71.56)	$98^{7,10}$ (81.87)	98 (81.87)	$98^{3,7}$ (81.87)	$98^{7,10}$ (81.87)	98 (81.87)	$98^{7,10}$ (81.87)
0.1	$50^{1,7}$ (45.00)	98 (81.87)	98 (81.87)	98^{10} (81.87)	98 (81.87)	98 (81.87)	98^{10} (81.87)	98 (81.87)	98 (81.87)
0.01	98 (81.83)	98 (81.87)	98 (81.87)	98 (81.87)	98 (81.87)	98 (81.87)	98 (81.87)	98 (81.87)	98 (81.87)
Control	98 (81.83)	98 (81.87)	98 (81.87)	98 (81.87)	98 (81.87)	98 (81.87)	98 (81.87)	98 (81.87)	98 (81.87)

C.D at P=0.05.1.52 for *P. grisea*, 2.31 for *H. oryzae*, 1.16 for *C. lunata*. Data in parentheses represents angular values.
Complete inhibition of conidial germination is represented by 2%. [1]granulated conidial cytoplasm, [3]excessive branched germ tube, [5]thick germ tube, [7]granulated germ tube, [10]thin germ tube.

Conclusion

New eugenol-copper complex was synthesized and characterized through FT-IR, TG/DTA, Elemental, AAS and Job's method. The FT-IR spectrum reveals the presence of Cu-O bond at 603 cm^{-1}, 517 cm^{-1} and 585 cm^{-1}.TG/ DTA corresponds to loss of four water molecule, allylic group and methoxy group. Elemental analysis and AAS done for Cu indicated the percentage of C, H, O and Cu corresponding to those of the calculated values. The composition of eugenol and copper in the complex was found to be 1:1. All these data were in conformity with the proposed structure of the complex. Fungitoxic activity of the complex was found to be enhanced compared to both eugenol and $CuSO_4.5H_2O$ tested alone especially against *P. grisea*, the causal agent of blast, which is globally, one of the most destructive diseases of rice crop. The other two pathogens, *H. oryzae* and *C.lunata* were also successfully inhibited by the complex compared to eugenol alone.

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