

Chemically Characterized Nanoencapsulated Homalomena aromatica Schott. Essential oil as Green Preservative Against Fungal and Aflatoxin B1 Contamination of Stored Spices based on in Vitro and in Situ Efficacy and Favourable Safety Profile on Mice

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1 **Title:** Chemically characterized nanoencapsulated *Homalomena aromatica* Schott. essential oil
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24 **Abstract**

25 Present study deals with the efficacy of nanoencapsulated *Homalomena aromatica* essential oil
26 (HAEO) as a potent green preservative against toxigenic *Aspergillus flavus* strain (AF-LHP-NS
27 7), AFB₁ and free radical mediated deterioration of stored spices. GC-MS analysis revealed
28 linalool (68.51%) as the major component of HAEO. HAEO was encapsulated into chitosan
29 nanomatrix (CS-HAEO-Ne) and characterized through SEM, FTIR and XRD. CS-HAEO-Ne
30 completely inhibited *A. flavus* growth and AFB₁ biosynthesis at 1.25 µL/mL and 1.0 µL/mL,
31 respectively in comparison to unencapsulated HAEO (1.75 µL/mL and 1.25 µL/mL
32 respectively). CS-HAEO-Ne exhibited superior antioxidant efficacy (IC₅₀ (DPPH) = 4.5 µL/mL)
33 over unencapsulated HAEO (IC₅₀ (DPPH) = 15.9 µL/mL). Further, CS-HAEO-Ne caused
34 significant reduction in ergosterol content in treated *A. flavus* and provoked leakage of cellular
35 ions (Ca⁺², Mg⁺² and K⁺) as well as 260 nm and 280 nm absorbing materials. Depletion of
36 methylglyoxal level in treated *A. flavus* cells deals with the novel antiaflatoxicogenic efficacy of
37 CS-HAEO-Ne. CS-HAEO-Ne depicted excellent *in situ* efficacy by inhibiting mold attack and
38 AFB₁ contamination, mineral preservation and acceptable sensorial profile. Moreover, broad
39 safety paradigm (LD₅₀ value = 8006.84 µL/kg) of CS-HAEO-Ne also suggest it as novel green
40 preservative to enhance shelf life of stored spices.

41 **Keywords:** Aflatoxin B₁, Chitosan, *Homalomena aromatica* essential oil, Methylglyoxal,
42 Nanoencapsulation

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46 **Introduction**

47 Spices are aromatic food commodities obtained from different plant parts such as root, seed,
48 leaves, bark, flower, bulb, and fruit that are used all over world in food preparations (Thanushree
49 et al. 2019). Peculiar flavor, colour and aromatic attributes of spices make them highly
50 demanding food ingredient globally. In addition, several spices have been reported for their
51 antimicrobial and antioxidant potential, along with vast therapeutic values such as analgesic,
52 antipyretic, blood purifier, hepatoprotective, carminative, anticancerous, antidiabetic and anti-
53 inflammatory (Gupta 2010; Singh et al. 2020a).

54 Unscientific and inappropriate harvesting, drying and storage techniques as well as warm
55 and humid environmental conditions of storage make spices highly prone towards contamination
56 by mold and their associated mycotoxins. Spices are reported to be second highly mycotoxin
57 contaminated food item after nut products and fruits and vegetables (RASFF 2019). Among wide
58 array of mycotoxins reported from different stored spices such as aflatoxins, citrinin, fumonisins,
59 zearalenone, sterigmatocystin, tenuazonic, alternariol and deoxynivalenol (Pickova et al. 2020),
60 aflatoxins are reported to be the most prevailing spice contaminant (Potorti et al. 2020).
61 Aflatoxins, especially aflatoxin B₁ (AFB₁) contamination in stored spices has become a matter of
62 great concern due to its potential properties of hepatocarcinogen, mutagen, teratogen,
63 nephrotoxic and immunosuppressive agent for which it has been categorized as group 1 human
64 carcinogen by International Agency for Research on Cancer (IARC 2012). Stringent regulations
65 have been imposed by Food and Agricultural Organization (FAO) for the maximum acceptable
66 limit of AFB₁ in spices and set as 5 µg/kg (FAO 2004). Yang et al. (2017) reported that fungal
67 and mycotoxin contamination causes depletion of bioactive components of spices, thereby
68 deteriorating spice quality. Moreover, AFB₁ contamination further provokes oxidative stress

69 resulting to rancidity and degradation of nutritive constituents of food items (Kovesi et al. 2020).
70 Oxidative stress also enhances biosynthesis of methylglyoxal, the AFB₁ inducer molecule.
71 Hence, in order to mitigate biodeterioration of food products caused due to fungal and mycotoxin
72 contamination, chemicals such as butylated derivatives, potassium sorbate, and propyl gallate
73 have been widely used. However, issues of environmental toxicity, resistance development,
74 residual toxicity and carcinogenic effects (Rajkumar et al. 2020a) greatly limit their applicability.
75 On the contrary, plant based green preservatives have been considered as better alternative to
76 synthetic food preservatives based on eco-friendly and broad safety profile. Among various
77 phytochemicals, aromatic plant essential oils (EOs) and their active components have been
78 highly encouraged to be used as novel green preservative due to its considerable antibacterial,
79 antifungal, antimycotoxigenic, insecticidal and antioxidant efficacy. Moreover, several EOs such
80 as *Coriandrum sativum*, *Ocimum basilicum*, *Mentha piperita*, *Matricaria chamomilla*, *Cuminum*
81 *cyminum* and EO bioactives such as limonene, carvone, eugenol, linalool, citral, vanillin, thymol
82 and menthol are also grouped under generally recognized as safe category (GRAS), which
83 strongly recommends botanical formulations as next generation pesticide (Prakash et al. 2018).

84 In spite of tremendous preservative potential, high volatility, less water solubility, intense
85 aroma and easy degradation of active components limit the large scale practical application of
86 EOs in food system (Marques et al. 2019). In order to deal with these challenges,
87 nanoencapsulation of EOs using food grade biopolymer has emerged as a novel and efficient
88 technique. In recent past, chitosan biopolymer has gained prime attention as superior
89 encapsulating agent, based on its biodegradability, non-mammalian toxicity, hydrophilicity,
90 controlled release and emulsion forming property (Wu and Liu 2008). Among different
91 nanoencapsulation techniques utilized, ionic-gelation method is more preferred. This method is

92 comparatively simple, economical and non-toxic. Further, suitability for both hydrophilic and
93 lipophilic component also suggests ionic-gelation method as an efficient strategy to formulate
94 nanoemulsion.

95 *Homalomena aromatica* (Sugandh mantri) is an aromatic perennial herb reported for
96 therapeutic values (Roy et al. 2019). *Homalomena aromatica* EO (HAEO) has been widely used
97 in perfumery and cosmetics and reported to have potent antimicrobial efficacy (Policegoudra et
98 al. 2012). However, the data are unavailable on the antiaflatoxicogenic efficacy of HAEO and
99 exploration of its preservative potential in food system. Moreover, no study has been performed
100 on nanoencapsulation of HAEO in chitosan polymer. Hence, the present study focused on
101 exploration of the efficacy of HAEO loaded chitosan nanoparticle as fungitoxic and antiaflatoxin
102 B₁ candidate to prevent deterioration of spices under storage. Study comprised of biosynthesis of
103 HAEO nanoparticle, its characterization, evaluation of antioxidant, antifungal and
104 antimycotoxigenic efficacy along with probable mode of actions. The study also includes *in vivo*
105 investigation on spice sample, sensorial analysis and assessment of lethal toxic dose in
106 comparison with unencapsulated HAEO in order to strengthen recommendation as promising
107 preservative agent in food sectors with sufficient consumer acceptance.

108 **Methodology**

109 **Chemicals**

110 Chemicals such as low molecular weight chitosan, glacial acetic acid, dichloromethane
111 (DCM), tripolyphosphate (TPP), Tween 20, Tween 80, chloroform (CHCl₃), perchloric acid,
112 nitric acid (HNO₃) methylglyoxal, diaminobenzene (DAB), thiobarbituric acid (TBA),
113 trichloroacetic acid (TCA), potassium hydroxide (KOH), isoamyl alcohol, toluene, sucrose,
114 acetonitrile, hydrochloric acid (HCl), n-heptane, methanol, sucrose, magnesium sulphate

115 (MgSO₄), potassium nitrate (KNO₃), yeast extract, DPPH and sodium chloride (NaCl) were
116 procured from SRL Pvt LTD and Hi-Media Mumbai, India.

117 **Test fungal species**

118 Aflatoxin secreting strain of *Aspergillus flavus* AF-LHP-NS 7 selected during mycobiota
119 analysis of different spices was utilized for further investigations. Storage fungi including
120 *Aspergillus niger*, *A. repens*, *A. luchuensis*, *A. terreus*, *Fusarium oxysporum*, *F. graminearum*,
121 *Penicillium italicum*, *P. chrysogenum*, *Mucor sp.*, *Rhizopus sp.*, *Alternaria alternata*, *Curvularia*
122 *lunata* and *Mycelia sterilia* were used during fungitoxic experiments.

123 **Extraction and phytochemical analysis of HAEO**

124 Rhizomes of *Homalomena aromatica* procured from Varanasi, Uttar Pradesh, India, were
125 transferred to Clevenger's apparatus for 5 hour in order to extract EO.

126 Chemical characterization of HAEO was done through GC-MS analysis. TG-5 MS fused
127 silica capillary column of dimensions 30 m × 0.25 mm × 0.25 μm fitted inside Thermo Scientific
128 1300 GC interfaced with TSQ Duo triple quadruple mass spectrophotometer. Sample was
129 injected to column at 70 °C temperature with programmed increment up to 250 °C. Individual
130 phyto components were identified based on spectral peaks available in NIST, Wiley, and other
131 published literature (Carneiro et al. 2020).

132 **Synthesis of HAEO entrapped in chitosan based emulsion (CS-HAEO-Ne)**

133 CS-HAEO-Ne was prepared using ionic gelation technique (Rajkumar et al. 2020b). 1%
134 v/v glacial acetic acid (GAA) was added to 1.5% chitosan solution prepared in distilled water,
135 and mixed at 27 °C for 24 hour. Different w/v ratio of chitosan and HAEO *i.e.* 1:0, 1:0.2, 1:0.4,
136 1:0.6, 1:0.8 and 1:1 was prepared by mixing different amount of HAEO *i.e.* 0.00, 0.06, 0.12,
137 0.18, 0.24 and 0.30 g to chitosan solution. Further drop wise STPP (4 mg/mL) was added in

138 order to obtain nanoemulsion. Prepared nanoemulsion was lyophilized (Alpha 1-2 LD plus Entry
139 Laboratory Freeze Drier for aqueous samples, John Morris Scientific, Sydney, Australia) at -62
140 °C for 72 hour and kept at 4 °C for further experiments.

141 **Physico-chemical analysis of CS-HAEO-Ne**

142 Structural and morphological analysis of lyophilized CS-Ne and CS-HAEO-Ne was
143 performed through scanning electron microscopy (SEM) (EVO-18 researcher, Zeiss). A 10 fold
144 dilution of 1 mg lyophilized CS-Ne and CS-HAEO-Ne was done followed by 10 min of
145 sonication. A thin film of prepared solution was spreaded over glass slide, gold coated and
146 viewed using SEM. Lyophilized CS-Ne and CS-HAEO-Ne was further used for FTIR analysis in
147 between absorbance 500-4000 cm^{-1} at 4 cm^{-1} . Crystallinity of lyophilized CS-Ne and CS-HAEO-
148 Ne was determined through XRD analysis using diffractometer at 2θ degree between 5-50°, step
149 angle 0.02° min^{-1} with scan rate of 5° min^{-1} .

150 **Estimation of nanoencapsulation efficiency (NEE) and loading capacity (LC)**

151 Content of HAEO in CS-HAEO-Ne was estimated by UV-visible spectrophotometry.
152 CS-HAEO-Ne (0.1 mL) was added in ethyl acetate (3 mL) followed by centrifugation at
153 10,000×g for 15 min. Content of HAEO was determined by taking the optical density of
154 supernatant at absorbance maxima of HAEO *viz.* 265 nm and using calibration graph ($R^2 =$
155 0.998) of HAEO mixed in ethyl acetate. CS-Ne prepared in the same way was treated as control.
156 NEE and LC were calculated through formula

157 Nanoencapsulation efficiency(NEE)

$$158 = \frac{\text{Total amount of loaded HAEO} - \text{amount of HAEO into nanoemulsion}}{\text{Initial amount of HAEO}} \\ 159 \times 100$$

160
$$\text{Loading capacity(LC)} = \frac{\text{Mass of loaded HAEO}}{\text{Mass of loaded nanoemulsion}} \times 100$$

161 ***In vitro* release profile of CS-HAEO-Ne**

162 *In vitro* release profile of CS-HAEO-Ne was calculated following Chaudhari et al. (2020)
163 with slight modification. 500 µL of CS-HAEO-Ne was added to 5 mL phosphate buffer saline
164 (PBS and ethanol 3:2 v/v) under gentle agitation for 0-96 h at 30 °C. 3 mL suspension was
165 removed at specific time interval which was replenished with the equal volume of fresh buffer.
166 Amount of HAEO at each time was calculated by measuring absorbance at 293 nm as well as
167 using standard calibration curve. Release profile of HAEO was calculated using the following
168 formula.

169
$$\text{Cumulative release of HAEO (\%)} = \frac{\text{Cumulative amount of HAEO released at each time interval}}{\text{Initial mass of HAEO loaded in the sample}} \times 100$$

170 **Antifungal and AFB₁ inhibitory efficacy of HAEO and CS-HAEO-Ne**

171 Antifungal activity of HAEO and CS-HAEO-Ne was estimated as minimum inhibitory
172 concentration (MIC). Toxigenic *A. flavus* AFLHP NS-7 strain was treated with different
173 concentration of HAEO (0.25, 0.50, 0.75, 1.0, 1.25, 1.50 and 1.75 µL/mL) and CS-HAEO-Ne
174 (0.25, 0.50, 0.75, 1.0, 1.25 µL/mL) for 10 days. Sample without any treatment worked as control.
175 Minimum concentration of HAEO and CS-HAEO-Ne that completely inhibited growth of
176 AFLHP NS-7 was considered as its MIC.

177 Antiaflatoxic efficacy of HAEO and CS-HAEO-Ne was calculated in terms of
178 minimum aflatoxin inhibitory concentration (MAIC) (Rasooli and Abyaneh 2004). To determine
179 the amount of AFB₁ content, filtered media was extracted with chloroform and developed in
180 TLC plate by using mobile phase toluene, isoamyl alcohol and methanol in 90:32:2 (v/v/v).

181 Absorbance of spots was recorded at 360 nm and AFB₁ content was calculated based on the
182 formula given below.

$$183 \text{ AFB}_1 (\mu\text{g/mL}) = \frac{\text{Absorbance at 360 nm} \times \text{Molecular weight of AFB}_1}{\text{Molar extinction coefficient of AFB}_1 \times \text{Path length}} \times 1000$$

184 Where, molecular mass of AFB₁ is 312 g/mol, molar extinction coefficient is 21800 mol cm⁻¹ and
185 path length is 1 cm.

186 % inhibition of AFB₁ was calculated as

$$187 \% \text{ inhibition} = \frac{\text{AFB}_1 (\text{control}) - \text{AFB}_1 (\text{treatment})}{\text{AFB}_1 (\text{control})} \times 100$$

188 **Antifungal action of HAEO and CS-HAEO-Ne**

189 **Ergosterol quantification**

190 In order to estimate ergosterol content of fungal plasma membrane, *A. flavus* cells were
191 treated with different concentration of HAEO and CS-HAEO-Ne (0.25, 0.50, 0.75, 1.0, 1.25 and
192 0.25, 0.50 and 0.75 μL/mL, respectively) and kept in B.O.D. incubator for 5 days at 27 ± 2 °C.
193 Samples without HAEO and CS-HAEO-Ne worked as controls. *A. flavus* biomass was harvested
194 from each sample and net wet weights of mycelia were measured followed by vortexing in 25%
195 KOH solution. Thereafter, ergosterol from samples was extracted using n-heptane and water 2:5
196 (v/v) and scanned spectrophotometrically between 230-300 nm. Formula given by Tian et al.
197 (2012) was used to quantify ergosterol.

$$198 \% \text{ ergosterol} + \% 24 (28) \text{ dehydroergosterol} = (A_{282} / 290) / \text{Pellet weight}$$

$$199 \% 24 (28) \text{ dehydroergosterol} = (A_{230} / 518) / \text{Pellet weight}$$

$$200 \% \text{ ergosterol} = (\% \text{ ergosterol} + \% 24 (28) \text{ dehydroergosterol}) - \% 24 (28) \text{ dehydroergosterol}$$

201 Where 290 and 518 are the E values determined for crystalline ergosterol and dehydroergosterol,
202 respectively.

203 **Effect on cellular cations (Ca²⁺, K⁺ and Mg²⁺) and 260 and 280 absorbing materials**

204 Seven days grown biomass of *A. flavus* obtained from liquid SMKY media was
205 fumigated HAEO (0.25 to 1.75 $\mu\text{L}/\text{mL}$ and 2 MIC) and CS-HAEO-Ne (0.25 to 1.25 $\mu\text{L}/\text{mL}$ and
206 2 MIC). Efflux of calcium, potassium and magnesium ions was analyzed by atomic absorption
207 spectroscopy (Perkin Elmer AAnalyst 800, USA). For measuring release of 260 and 280 nm
208 absorbing materials, 7 days grown *A. flavus* biomass was treated with different concentration of
209 HAEO and CS-HAEO-Ne *i.e.*, 0.25 to 1.75 $\mu\text{L}/\text{mL}$ and 0.25 to 1.25 $\mu\text{L}/\text{mL}$ as well as 2 MIC
210 doses, respectively for 24 h and absorbance of samples were taken at 260 and 280 nm.

211 **HAEO and CS-HAEO-Ne effect on methylglyoxal (MG)**

212 Seven days old *A. flavus* mycelia was treated at different doses *viz.* MIC and 2MIC (1.75
213 and 3.5 $\mu\text{L}/\text{mL}$) of HAEO and MIC and 2MIC (1.25 to 2.5 $\mu\text{L}/\text{mL}$) of CS-HAEO-Ne for 24 h.
214 Samples without HAEO and CS-HAEO-Ne were treated as controls. Estimation of methylglyoxal
215 was done following the methods of Yadav et al. (2005). 300 mg sample from each set was
216 crushed in 3 mL of perchloric acid (0.5 M) and subjected to centrifugation at 4 °C on 10000 x g
217 for 10 min. Supernatant was neutralized (pH = 7) by saturated potassium carbonate solution and
218 centrifuged again at 10000 x g for 10 min (4 °C). Reaction mixture containing 0.5 mL 1,2
219 diaminobenzene (DAB), 0.2 mL HClO_4 (5 M) and 1.3 mL neutralized supernatant was prepared
220 and its optical density was recorded at 341 nm. Total amount of MG was estimated using the
221 standard curve of pure MG (10-100 μM).

222 **Antioxidant potential of HAEO, CS-Ne and CS-HAEO-Ne**

223 Free radical removal potential of HAEO, CS-Ne and CS-HAEO-Ne was calculated using
224 the DPPH assay following slightly modified method of Balasubramani et al. (2017). IC_{50} (50 %
225 radical scavenging potential of HAEO, CS-Ne and CS-HAEO-Ne) was determined using the
226 following formula

227 % Radical scavenging potential = $(A_0 - A_1) / A_0 \times 100$

228 Where A_0 and A_1 are expressing the absorbance of blank and samples (HAEO, CS-Ne
229 and CS-HAEO-Ne) at 517 nm, respectively.

230 **Estimation of phenolics present in HAEO, CS-Ne and CS-HAEO-Ne**

231 Amount of phenolics in HAEO, CS-Ne and CS-HAEO-Ne was calculated following
232 Dzhahfezova et al. (2020) with slight modifications. Reaction mixture containing Folin-
233 Ciocalteu's reagent and sample was allowed to stand for 2 h in dark after addition of 3 mL
234 Na_2CO_3 and optical densities of samples were measured at 760 nm. The result was calculated in
235 terms of μg gallic acid equivalent (GAE)/g.

$$236 \text{ Absorbance (760 nm) = } 0.0012 \times \text{GA } (\mu\text{g}) + 0.024$$

237 **Study on in vivo AFB₁ inhibitory efficacy of HAEO and CS-HAEO-Ne in (*Nigella* 238 *sativa*) food system: High performance liquid chromatography (HPLC) assay**

239 *In situ* efficacy of HAEO and CS-HAEO-Ne were estimated by fumigating black cumin
240 seeds (model spice system; 250 g) in 500 mL air tight plastic containers for storage period of 18
241 months. Spice samples were fumigated with HAEO and CS-HAEO-Ne at their respective MIC
242 concentrations. Control sets were devoid of any treatment.

243 AFB₁ content in stored spice samples were determined through HPLC method following
244 Sheijooni-Fumani et al. (2011). 5 g of grinded spice samples were mixed with methanol and
245 double distilled water (8/10; v/v), centrifuged and supernatant was mixed with 300 μL
246 chloroform and 6 mL water containing 3% KBr. Obtained reaction mixtures were centrifuged
247 again (5000 x g), settled portion was isolated and dried at 85 °C on water bath and further
248 dissolved in 500 μL of HPLC grade methanol for injecting into HPLC column. Amount of AFB₁
249 ($\mu\text{g}/\text{kg}$) was determined at 365 nm from the prepared standard curve of AFB₁ (50-500 ng/50 μL)

250 (Upadhyay et al. 2018). Methanol, acetonitrile and water (17:19:64 v/v/v) was used as mobile
251 phase (1.2 mL/min flow rate) to separate AFB₁ on C18 reverse phase column (4.6 mm × 25 cm ×
252 5 µm) at ambient temperature of 25 °C.

253

254 **Analysis of lipid peroxidation and mineral loss in HAEO and CS-HAEO-Ne treated** 255 **black cumin (*Nigella sativa*) seeds**

256 *In vivo* preservative efficacy of HAEO and CS-HAEO-Ne was tested in terms of lipid
257 peroxidation inhibitory action at their respective MIC and 2 MIC value following Iseri et al.
258 (2013). Lipid peroxidation was measured using thiobarbituric acid reactive substance (TBRAS)
259 assay. In order to execute experiment, 1 g of grinded spice sample was added to 5 mL TBA
260 reagent comprising 0.375 % TBA, 15 % TCA, and 0.2 N HCl. Further, samples were subjected
261 to water bath at 95 °C for 25 min, following centrifugation (10000 x g for 10 min). Thereafter,
262 absorbance of supernatant was recorded at 532 nm and 600 nm and results were expressed as µM
263 equivalent MDA/g FW. Mineral biodeterioration level in HAEO and CS-HAEO-Ne treated spice
264 samples were evaluated through atomic absorption spectrometry (Perkin Elmer AAnalyst 800,
265 USA).

266 **Sensorial analysis of fumigated black cumin (*Nigella sativa*) seeds with HAEO and** 267 **CS-HAEO-Ne**

268 Effect of HAEO and CS-HAEO-Ne fumigation (at MIC concentration) on sensorial
269 characteristics of stored black cumin seeds (18 months duration) was analyzed by a panel
270 comprising of 10 panelists of both genders. 7 point hedonic scale (5 = extremely like, 4 = slightly
271 like, 3 = neither like nor dislike, 2 = slightly dislike, 1 = extremely dislike) was used to estimate
272 taste, colour and odor of stored spice samples.

273 **Safety profile assessment of HAEO and CS-HAEO-Ne**

274 Safety profile of HAEO and CS-HAEO-Ne was evaluated in terms of toxicity assay on
275 male mice using oral administration and represented in terms of LD₅₀ value (Singh et al. 2020b).
276 Different amount of HAEO and CS-HAEO-Ne mixed with stock solution (Tween 20 and
277 deionized distilled water, 1:1) were administered orally to each mice group (10 mice). Mice
278 administered with stock and CS-Ne was considered as control. LD₅₀ value was based on the
279 number of mice dead within 24 h of study period and calculated through probit analysis.

280 **Statistical analysis**

281 The experiments were carried out in triple sets and the data represented as mean (n = 3) ±
282 standard error (SE). Further it was analyzed by one way ANOVA followed by Tukey's B
283 multiple comparison test at significant (P < 0.05) differences. SPSS and Sigma plot program
284 were used for data analysis and creating graphs.

285 **Result and discussion**

286 **Extraction and GC-MS of HAEO**

287 HAEO was extracted from rhizome of the plant and per cent yield was found to be 8.6
288 mL/kg. Phytochemical analysis of HAEO through GC-MS analysis revealed 26 compounds
289 comprising 91.71% of EO. Linalool was found to be the major component contributing 68.51%
290 of total bioactive components. The outcomes of the present study are in line with the previous
291 study of Policegoudra et al. (2012) describing linalool (62.5%) as the major bioactive
292 component. Per cent occurrence and retention time of different compounds are presented in
293 Table 1. Bioefficacy of EOs are based on their bioactive components whose composition and
294 amount may vary with variation in geological and environmental conditions, harvesting stage
295 and oil extraction procedure, ultimately affecting the biological activity of EOs (Dhifi et al.

296 2016). Thus, analysis of EO bioactive composition is a crucial step before its detailed bioactivity
297 evaluation.

298 **Preparation of HAEO loaded chitosan nanoemulsion (CS-HAEO-Ne)**

299 CS-HAEO-Ne was prepared using tripolyphosphae (TPP) as cross linking agent
300 following ionic gelation technique. Interaction between protonated -NH₂ group of chitosan and
301 negative charged ions of TTP leads to formation of biocompatible nanoparticles. CS-HAEO-Ne
302 was formed following the two step strategy *i.e.* droplet formation and solidification.

303 Encapsulation enhance stability of volatile aromatic substances such as EOs and their
304 bioactive components against environmental factors *i.e.* light, chemical, oxygen, pressure and
305 heat mediated degradation (Delshadi et al. 2020). Ionotropic gelation method is well known for
306 encapsulating bioactive principles due to its non toxic, organic solvent free, appropriate and
307 easily controllable properties (Esmaeili and Asgari 2015). Chitosan, obtained by deacetylation of
308 chitin, was selected as coating matrix as it an efficient, non toxic, biodegradable and film/ gel
309 forming polymer matrix. Effective entrapment of HAEO was determined through preparing
310 different chitosan and HAEO ratios *viz.* 1:0, 1:0.2, 1:0.4, 1:0.6, 1:0.8 and 1:1.

311 **Physico-chemical characterization of CS-HAEO-Ne**

312 **Scanning electron microscopic (SEM) analysis**

313 SEM analysis was done to analyze the morphological features of CS-Ne and CS-HAEO-
314 Ne which depicted spherical structure and smooth surface of the prepared nanoparticles. Size of
315 CS-Ne and CS-HAEO-Ne was found in range of 37-81.4 nm and 48.3-94.6 nm, respectively
316 (Fig. 1A, 1B). Increment in size of CS-Ne after encapsulation of HAEO could be to the result of
317 swelling of chitosan matrix by entrapped HAEO (Kumar et al. 2019). This finding on enhanced

318 particle size of EO loaded nanoparticles in comparison to unencapsulated chitosan nanoparticle
319 is supported by previous reports of Hosseini et al. (2013).

320 **Fourier transform infrared (FTIR) spectroscopic analysis**

321 FTIR analysis of pure chitosan powder, CS-Ne, HAEO and CS-HAEO-Ne showing
322 chemical interaction between chitosan and HAEO are presented in Fig. 2. Chitosan indicated
323 specified peaks at 3445 cm^{-1} for -OH and -NH stretching, 2916 cm^{-1} for symmetric or asymmetric
324 -CH stretching and at 1066 cm^{-1} for C-O-C linkage. In addition 1645 cm^{-1} , 1581 cm^{-1} and 1316
325 cm^{-1} peaks represented presence of amide I, amide II and amide III group (Branca et al. 2016).
326 Prominent peak at 1375 cm^{-1} in chitosan represent -C-N- bond stretching. Presence of new peaks
327 in CS-Ne at 1556 cm^{-1} (N-H bending) and 888 cm^{-1} (P-O stretching) specified the electrostatic
328 bonding between amide group of CS and phosphate moiety of TPP. Furthermore, in HAEO
329 several peaks at/in between $1644\text{-}1447\text{cm}^{-1}$ (for phenyl ring), 1713 cm^{-1} for presence of ether
330 group, 3541 cm^{-1} (O-H stretching), 2966 cm^{-1} (-CH stretching) and 739 cm^{-1} (aromatic C-H
331 bending) appeared. Most of the aforementioned peaks were retained in the spectra of CS-HAEO-
332 Ne. Shifting of peak from 2966 cm^{-1} in spectra of HAEO to 2922cm^{-1} in spectra of CS-HAEO-Ne
333 also denotes successful encapsulation of HAEO inside chitosan polymer.

334 **X-ray diffraction (XRD) analysis**

335 Crystallographic pattern of chitosan powder, CS-Ne and CS-HAEO-Ne has been shown
336 in Fig. 3. Diffraction spectrum of chitosan powder represented peaks at 2θ value 10.2° and 19.8° ,
337 denoting the characteristic peak of chitosan viz. 10° and 20° and increased crystallinity (Su et al.
338 2020). However, the diffractogram of CS-Ne and CS-HAEO-Ne depicted flattening and
339 broadening of characteristic peaks suggesting destruction of chitosan crystallinity as a result of
340 successful TPP cross linking with chitosan and loading of HAEO into the polymer matrix.

341 **Estimation of per cent nanoencapsulation efficiency (NEE) and loading capacity**
342 **(LC) of CS-HAEO-Ne**

343 NEE and LC of HAEO inside chitosan nanomatrix was determined through UV-visible
344 spectrophotometric analysis which revealed that NEE and LC of CS-HAEO-Ne ranged between
345 22.0-83.41 % and 0.15-2.32 % (Table 2). NEE showed dose dependent increment up to the ratio
346 1:1.08 (CS:HAEO), representing good entrapment of HAEO inside chitosan nanomatrix.
347 However, decline in NEE was recorded at the ratio 1:1 (CS: HAEO) depicting insufficiency of
348 chitosan matrix to entrap further more HAEO. The result is in accordance with the previous
349 reports of Feyzioglu and Tornuk (2016).

350 ***In vitro* release of CS-HAEO-Ne**

351 The *in vitro* cumulative release mechanism of HAEO from CS-HAEO-Ne was measured
352 at room temperature for 1:0.8 ratio of chitosan to HAEO. The release of EO comprised of two
353 steps *i.e.* initial rapid release and then constant release as shown in Fig. 4. This result is
354 supported by the previous study of Li et al. (2018) describing controlled release of curcumin
355 encapsulated in chitosan. Initial fast release phase could be a response of unencapsulated EO that
356 is adsorbed on the surface of polymeric matrix or due to diffusion of EO from higher
357 concentration till attainment of equilibrium. After 6 h, release was recorded to be 51.36 %
358 followed by 26.90 % and 3.27 % release in between (10-24) and (24-96) h respectively.
359 Maximum release of EO observed was 81.55 % after 96 h, showing that 1.87 % HAEO out of
360 83.41 % was still entrapped inside the polymeric matrix. The result suggested that encapsulation
361 maintained the stability of volatile compounds through its control release.

362 ***In vitro* antifungal and antiaflatoxicogenic efficacy of HAEO and CS-HAEO-Ne**

363 Minimum inhibitory concentration (MIC) and AFB₁ inhibitory concentration of HAEO
364 was 1.75 µL/mL and 1.25 µL/mL, respectively. However, CS-HAEO-Ne showed enhanced the
365 bioefficacy against *A. flavus* and its MIC and MAIC value declined to 1.25 µL/mL and 1.0
366 µL/mL, respectively (Table 3). In addition, both HAEO and CS-HAEO-Ne significantly
367 suppressed the growth of other storage molds (*A. niger*, *A. repens*, *A. luchuensis*, *A. terreus*,
368 *Fusarium oxysporum*, *F. graminearum*, *Penicillium italicum*, *Mucor sp.*, *Rhizopus sp.*, *P.*
369 *chrysogenum* *Alternaria alternata*, *Curvularia lunata* and *Mycelia sterilia*) at their respective
370 MIC values viz., 1.75 µL/mL and 1.25 µL/mL (Fig. 5). Potent fungitoxic profile of HAEO and
371 its nanoformulation also favored for their application as botanical preservative for stored
372 products. The boosted bio-efficacy of CS-HAEO-Ne in comparison to HAEO could be the result
373 of controlled release and improved stability of constituent volatiles, enhanced water solubility,
374 wider surface area and fastened adsorption through cell wall and membrane (Hasheminejad et al.
375 2019). The antifungal efficacy of HAEO is more efficacious than other reported EOs such as
376 *Pelargonium roseum* EO (3.8 µL/mL), *Thymus vulgaris* EO (2.3 µL/mL) and *Cymbopogon*
377 *nardus* EO (6.4 µL/mL) (Zabka et al. 2009) and other synthetic preservatives such as propionic
378 acid and sodium sulphite having MIC value ranged from 2 to > 83 µL/mL against *P. verrucosum*
379 and two strain of *A. westerdijkiae* (Schlosser and Prange 2018). The boosted potency of
380 encapsulated HAEO could also be due to additive action of chitosan and HAEO. Cationic charge
381 of chitosan has been reported to interact with anionic charges of oxygenated lactone ring causing
382 enhanced antiaflatoxigenic efficacy (Cortes-Higareda et al. 2019). Hence, CS-HAEO-Ne could
383 be highly preferred green preservative over other synthetic preservatives with potent toxicity.

384 **Antifungal mode of action of HAEO and CS-HAEO Ne**

385 **Effect on ergosterol content and leakage of vital cellular ions**

386 Ergosterol is the unique sterol associated with fungal plasma membrane, responsible to maintain
387 proper functioning of membrane by controlling membrane permeability and integrity. Ergosterol
388 content in treated *A. flavus* cells declined in dose dependent manner with increasing doses of
389 0.25, 0.50, 0.75, 1.0 and 1.25 $\mu\text{L}/\text{mL}$. HAEO concentrations showed 33.96 %, 40.78 %, 46.81 %, 61.91 %
390 and 82.31 % decline of ergosterol content, respectively. However, CS-HAEO-Ne
391 inhibited ergosterol content to 11.02 %, 33.26 % and 96.88% at just 0.25, 0.50 and 0.75 $\mu\text{L}/\text{mL}$
392 respectively (Fig. 6). Obtained results are supported by the investigations of Khan et al. (2010).
393 Antifungal drugs such as azoles are reported to inhibit ergosterol biosynthesis through targeting
394 cytochrome 450 lanosterol 14 α -demethylase enzyme, the ERG11 gene product involved in
395 ergosterol pathway (Lupetti et al. 2002). Thus, HAEO and CS-HAEO-Ne mediated depletion in
396 ergosterol content could also be based on downregulation of lanosterol 14 α demethylase enzyme
397 functioning, involved in crucial step of ergosterol biosynthesis *i.e.* 14 α demethylation. Decline of
398 ergosterol content would make membrane porous leading to loss of vital ions *viz.* Ca⁺², Mg⁺² and
399 K⁺ as well as 260 nm and 280 nm absorbing material (Table 4), responsible for vital metabolic
400 activities of cell. Therefore, present study concluded fungal plasma membrane as one of the
401 prime targets for antifungal action of HAEO and CS-HAEO-Ne as the cause of cell death
402 through altering vital cellular mechanisms of fungus.

403 Methylglyoxal (MG), an endogeneous product of metabolic pathways such as polyol
404 pathway, glycolytic pathway and amino acetone metabolism is reported to be highly reactive and
405 strong glycaling agent (Antognelli et al. 2013). MG is also reported to induce cytotoxic effects
406 through inducing apoptosis *via* enhancing reactive oxygen species production or through
407 accumulation of MG mediated advanced glycation end products. Moreover, MG has been also
408 reported to have inductive role in AFB₁ production. Chen et al. (2004) reported upregulation of

409 the major regulatory gene *aflR* and other AFB₁ biosynthetic gene *nor1* by MG. In present
410 experiment MG content in control was found to be 232.4 $\mu\text{M/g}$ FW, while it decreased in HAEO
411 treated cells in dose dependent manner. Further, CS-HAEO-Ne depicted maximum suppression
412 of MG formation in treated cells at relatively low concentration in comparison to unencapsulated
413 HAEO (Fig. 7). Obtained outcome is in accordance with the findings of Chaudhari et al. (2020).
414 Considerable difference between bioactivity of unencapsulated and encapsulated HAEO might
415 be due to enhanced bioavailability along with targeted and slow release caused by encapsulation.
416 Based on the above findings, we hypothesize that inhibition of MG formation might have a
417 significant role in antiaflatoxic activity of HAEO or CS-HAEO-Ne.

418 **Antioxidant efficacy**

419 DPPH based free radical scavenging assay basically relies on the principle of antioxidant
420 mediated quenching of single electron form DPPH radical and subsequently decolorization of
421 purple colour of DPPH solution. Encapsulation of EOs along with boosting its bioefficacy also
422 enhances its antioxidant potency through protecting bioactive components of EOs from
423 environmental degradation caused due to light and temperature. In the present experiment CS-
424 HAEO-Ne depicted enhanced antioxidant potency over HAEO. IC₅₀ value for HAEO and CS-
425 HAEO-Ne were recorded as 15.98 $\mu\text{L/mL}$ and 4.57 $\mu\text{L/mL}$, respectively, describing
426 enhancement of antioxidant potency through nanoencapsulation (Fig. 8). In addition, chitosan
427 was found to be deprived of promising antioxidant efficacy. Siva et al. (2020) also reported
428 enhanced antioxidant efficacy of isoeugenol encapsulated inside methyl β -cyclodextrin in
429 comparison to its free form. In addition, Cetin Babaoglu et al. (2017) suggested that boosted free
430 radical scavenging potency of hydropropyl beta cyclodextrin (HP β CD) loaded clove EO was
431 either due to its enhanced water solubility or preservation of phenolic compounds under Clove-

432 HP β CD complex from oxidative degradation. IC₅₀ value of CS-HAEO-Ne is lower in
433 comparison to that recorded for other synthetic antioxidants as ascorbic acid and BHT (Ricci et
434 al. 2005), suggesting encapsulated HAEO as future sustainable green food preservative.

435 Total phenolic content of chitosan, HAEO and CS-HAEO-Ne was found as 0.18 (μ g
436 gallic acid equivalent/g HAEO), 3.37 and 5.91 μ g gallic acid equivalent respectively. The present
437 result showed improved total phenolic content of HAEO after encapsulation which is in
438 accordance with the previous study of Attallah et al. (2020). Such enhancement could result due
439 to enhanced surface to volume ratio of nanomeric particle size of EO. In addition, ameliorated
440 water solubility of EO phenolic content as well as its improved protection against evaporation
441 loss by environmental gradient also contributes towards enhanced total phenolics.

442 ***In situ* antifungal and antiaflatoxic potential of HAEO and CS-HAEO Ne on** 443 **black cumin seeds (model food system)**

444 Based on *in vitro* investigations, HAEO and CS-HAEO-Ne was found to be efficient
445 antifungal and antiaflatoxic agent. However, in order to recommend large scale
446 commercialization, it is mandatory to analyze *in situ* efficacy. HPLC analysis of 18 months
447 stored black cumin seed sample manifested potent AFB₁ inhibitory efficacy of HAEO and CS-
448 HAEO-Ne. HPLC result depicted 208.37 μ g/Kg concentration of AFB₁ in control samples.
449 While both HAEO and CS-HAEO-Ne completely inhibited AFB₁ biosynthesis at their respective
450 MIC concentrations. AFB₁ concentration at MIC of both HAEO and CS-HAEO-Ne was found to
451 be 7.39 μ g/Kg and 7.34 μ g/Kg (Fig. 9). Highly efficacious *in situ* antifungal and
452 antiaflatoxic potency of non-encapsulated as well as encapsulated HAEO is based on its
453 diverse *in vitro* antifungal mode of actions such as disruption of membrane permeability through

454 depleting ergosterol content, leakage of vital cellular components and ions as well as through
455 inhibiting biosynthesis of aflatoxin inducer molecule (methylglyoxal).

456 **Estimation of lipid peroxidation inhibitory efficacy and mineral preservation**
457 **potency of HAEO and CS-HAEO-Ne fumigated *Nigella sativa* seeds**

458 Reactive oxygen species (ROS) like superoxide radical and peroxide radicals due to its highly
459 reactive nature interact with biomolecules such as nucleic acids, proteins and polyunsaturated
460 fatty acids (PUFAs). Reaction between ROS and PUFA ultimately leads to generation of
461 malondialdehyde (MDA), F₂-isoprostanes and 4-hydrox-2-nonenal (HNE), a biomarker molecule
462 of oxidative stress or lipid peroxidation (Tsikas 2017). The MDA, a significant biomarker of
463 lipid peroxidation, generate pink colour MDA-thiobarbituric acid complex which is measured via
464 TBARS assay in order to quantify lipid peroxidation in a sample. In control sets for HAEO and
465 CS-HAEO Ne MDA content was noted as 351.21 and 334.39 $\mu\text{M/g}$ FW. However, MDA
466 content in HAEO treated samples was declined and found to be 224.51 and 129.03 $\mu\text{M/g}$ FW at
467 its MIC and 2 MIC value, respectively. Conversely, samples fumigated with encapsulated HAEO
468 revealed presence of only 176.8 and 105.8 $\mu\text{M/g}$ FW MDA content at relatively low
469 concentration (Fig. 10). Outcome of the result is corroborated with the previous report of Hu et
470 al. (2015) suggesting enhanced preservative potential of cinnamon essential oil loaded chitosan
471 nanoparticle in order to prevent lipid peroxidation and maintain sensory quality of stored meat
472 based on synergism between antioxidant potency of HAEO and chitosan. Moreover, earlier
473 reported resistance quality of chitosan coating towards oxygen permeability and of chitosan
474 amine group with malondialdehyde (Sathivel et al. 2007) might also be one of the major causes
475 related with boosting of preservative potential of nanoencapsulated EO. Furthermore, entrapped
476 HAEO inside chitosan matrix have been protected against environmental degradation, have

477 sustainable release profile and potent free radical scavenging property might be a promising
478 reason owing to its shelf life enhancer efficacy. Mineral content of stored food substances are
479 also lost due to action of storage fungi. Fagbohun and Ogundahunsi (2019) reported diminished
480 mineral content in stored *Citrullus lanatus* seeds, as the minerals are metabolized and utilized by
481 the storage fungi for their growth and physiological activity. Black cumin seed is reported to
482 have vast medicinal history and it is huge repository of nutritional substances and minerals such
483 as iron, sodium, potassium, phosphorus, calcium, manganese, zinc, magnesium, and copper. Our
484 experimental analysis revealed that fumigation of HAEO as well as CS-NHAEO-Ne has
485 capability to preserve nutritional property of black cumin via protecting its mineral content loss.
486 Table 5 presents mineral content in control, HAEO and CS-NHAEO-Ne fumigated black cumin
487 seed at its MIC and 2 MIC concentrations. Hence, the present investigation recommends CS-
488 NHAEO to be used as a sustainable green shelf life enhancer food additive substance.

489 **Sensorial profile of black cumin (*Nigella sativa*) seeds fumigated with HAEO and** 490 **CS-HAEO-Ne**

491 Sensorial properties of food products are important perspective with respect to consumer's
492 acceptance. Therefore, in order to recommend HAEO loaded nanoformulation as commercial
493 green food preservative, it is a very crucial to evaluate sensorial attributes of fumigated samples
494 for its wide consumer acceptance in global market. Considering this, three different sets of 18
495 months stored samples *i.e.* stored black cumin seed without fumigation, black cumin seed
496 fumigated with HAEO at MIC concentration *i.e.* 1.75 $\mu\text{L/ml}$ and black cumin seed fumigated
497 with CS-HAEO-Ne at its MIC concentration *i.e.* 1.25 $\mu\text{L/ml}$ sensorial aspects was assessed.
498 Obtained result is presented in Fig. 11. The obtained sensorial score for odor, taste, texture and
499 colour was lower for control set, in comparison to fumigated samples. However, in between

500 HAEO and CS-HAEO-Ne treated samples CS-HAEO-Ne is having better score suggesting food
501 grade coating as an efficient strategy to prevent undesirable sensorial effect of HAEO on food
502 products. Entrapment of HAEO inside polymeric nanomatrix masked the intense aroma of
503 essential oil and caused its controlled release as well as lower concentration of EO is required;
504 besides, above mentioned potent radical scavenging action, prevention of lipid peroxidation and
505 efficient antifungal characteristics of encapsulated HAEO also contributed to its food items
506 sensorial properties preservation quality. Pabast et al. (2018) reported nanoencapsulated *Satureja*
507 *khuzestanica* EO as better substance over free EO to extend shelf life of lamb meat with
508 improved sensorial quality.

509 **Safety assessment of HAEO and CS-HAEO-Ne: Acute oral toxicity test on male** 510 **mice**

511 In acute oral toxicity assay, LD₅₀ value for HAEO was determined as 11334.6 µL/kg body
512 weight, while it is 8006.84 µL/kg for CS-HAEO-Ne. Controls containing Tween 20 and chitosan
513 nanoemulsion are non toxic to the mice. The outcome is in accordance with the previous report
514 of Ribeiro et al. (2014), indicating that encapsulated *Eucalyptus citriodora* EO have enhanced
515 acute oral toxicity in comparison to free EO. LD₅₀ value of HAEO was higher as compared to
516 some previous studies focused on plant products like *Nepeta cataria* EO *i.e.* 2710 mg/kg BW
517 (Zhu et al. 2009), *Artemisia annua* *i.e.* 790 mg/kg (Perazzo et al. 2003), thymol and carvacrol
518 bioactive component *i.e.* 980 mg/kg and 810 mg/kg (Bahuguna et al. 2020) recommending
519 nanoformulated HAEO as safer next generation green preservative.

520 **Conclusion**

521 The findings of present study recommend utilization of nanoformulated essential oils as
522 efficacious antifungal agent. Encapsulated HAEO exhibited improved antifungal,

523 antiaflatoxigenic as well as free radical scavenging activity over the unencapsulated HAEO.
524 Noticeable destruction of ergosterol level, efflux of important cellular ions and inhibition of
525 methylglyoxal biosynthesis suggested possible mechanisms underlying antifungal and AFB₁
526 suppression potencies of encapsulated HAEO. Moreover, CS-HAEO Ne was also found to have
527 significant *in vivo* AFB₁ inhibitory potency as well as protective role against lipid peroxidation
528 and mineral loss in stored *Nigella sativa* seeds without compromising its organoleptic attributes.
529 Thus, the above findings provide an exciting future opportunity for food industries to prefer
530 HAEO nanoformulation as a natural and safe alternative of synthetic chemicals due to its potent
531 preservative potential and safety profile.

532 **Author contribution**

533 Shikha Tiwari: Conceptualization, writing-original review draft, funding acquisition; Neha
534 Upadhyay: Review and editing; formal analysis; Bijendra Kumar Singh: Experimental analysis;
535 Vipin Kumar Singh: Review and editing, data curation; Nawal Kishore Dubey: writing-review
536 and editing, supervision. All authors have reviewed and approved the final manuscript.

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540 **Data availability**

541 All data analysed during this study are included in this manuscript.

542 **Declarations**

543 **Ethics approval**

544 The animal based experiment was performed according to the ethical standards of the institution.

545 **Consent to participate**

546 All authors participated in this work.

547 **Consent for publication**

548 All authors agree to publish this article in the Environmental Science and Pollution Research.

549 **Conflict of interest**

550 Authors declare that they have no competing interests.

551 **References**

552 Antognelli C, Mezzasoma L, Fettucciari K, Talesa VN (2013) A novel mechanism of
553 methylglyoxal cytotoxicity in prostate cancer cells. *Int J Biochem Cell Biol* 45:836-844.

554 Attallah OA, Shetta A, Elshishiny F, Mamdouh W (2020) Essential oil loaded pectin/chitosan
555 nanoparticles preparation and optimization *via* Box–Behnken design against MCF-7
556 breast cancer cell lines. *RSC Advances* 10:8703-8708.

557 Bahuguna A, Ramalingam S, Arumugam A, Natarajan D, Kim M (2020) Molecular and *in silico*
558 evidences explain the anti-inflammatory effect of *Trachyspermum ammi* essential oil in
559 lipopolysaccharide induced macrophages. *Process Biochem* 96:138-145.

560 Balasubramani S, Rajendhiran T, Moola AK, Diana RKB (2017) Development of nanoemulsion
561 from *Vitex negundo* L. essential oil and their efficacy of antioxidant, antimicrobial and
562 larvicidal activities (*Aedes aegypti* L.). *Environ Sci Pollut Res* 24:15125-15133.

563 Branca, C., D'Angelo, G., Crupi, C., Khouzami, K., Rifici, S., Ruello, G., & Wanderlingh, U.
564 (2016). Role of the OH and NH vibrational groups in polysaccharide-nanocomposite
565 interactions: A FTIR-ATR study on chitosan and chitosan/clay films. *Polymer* 99:614-
566 622.

567 Carneiro JNP, da Cruz RP, Campina FF, do Socorro Costa M, Dos Santos ATL, Sales DL,
568 Bezerra CF, da Silva LE, de Araujo JP, do Amaral W, Rebelo RA, Begnini IM, de Lima
569 LF, Coutinho DM, Morais-Braga MFB (2020) GC/MS analysis and antimicrobial activity
570 of the *Piper mikanianum* (Kunth) Steud. essential oil. Food Chem Toxicol 135:1-8.

571 Cetin Babaoglu H, Bayrak A, Ozdemir N, Ozgun N (2017) Encapsulation of clove essential oil
572 in hydroxypropyl beta-cyclodextrin for characterization, controlled release, and
573 antioxidant activity. J Food Process Preserv 41:1-8.

574 Chaudhari AK, Singh VK, Das S, Prasad J, Dwivedy AK, Dubey NK (2020) Improvement of *in*
575 *vitro* and *in situ* antifungal, AFB₁ inhibitory and antioxidant activity of *Origanum*
576 *majorana* L. essential oil through nanoemulsion and recommending as novel food
577 preservative. Food Chem Toxicol 143:1-12.

578 Chen ZY, Brown RL, Damann KE, Cleveland TE (2004) Identification of a maize kernel stress-
579 related protein and its effect on aflatoxin accumulation. Phytopathology 94:938-945.

580 Cortes-Higareda M, de Lorena Ramos-Garcia M, Correa-Pacheco ZN, Del Rio-Garcia JC,
581 Bautista-Banos S (2019) Nanostructured chitosan/propolis formulations: characterization
582 and effect on the growth of *Aspergillus flavus* and production of aflatoxins. Heliyon 5:1-
583 7.

584 Delshadi R, Bahrami A, Tafti AG, Barba FJ, Williams LL (2020) Micro and nano-encapsulation
585 of vegetable and essential oils to develop functional food products with improved
586 nutritional profiles. Trends Food Sci Technol 104:72-83.

587 Dhifi W, Bellili S, Jazi S, Bahloul N, Mnif W (2016) Essential oils' chemical characterization
588 and investigation of some biological activities: A critical review. *Medicines* 3:1-16.

589 Dzhhanfezova T, Barba-Espin G, Muller R, Joernsgaard B, Hegelund JN, Madsen B, Larsen DH,
590 Vega MM, Toldam-Andersen TB (2020) Anthocyanin profile, antioxidant activity and
591 total phenolic content of a strawberry (*Fragaria × ananassa* Duch) genetic resource
592 collection. *Food Biosci* 36:1-7.

593 Esmaeili A, Asgari A (2015) *In vitro* release and biological activities of *Carum copticum*
594 essential oil (CEO) loaded chitosan nanoparticles. *Int J Biol Macromol* 81:283-290.

595 Fagbohun E, Ogundahunsi AS (2019) Effects of storage on nutritional, mineral composition and
596 mycoflora of stored sundried *Citrullus lanatus* Thunberg (Melon) seeds. *Int J Biochem*
597 *Res Rev* 28:1-7.

598 Feyzioglu GC, Tornuk F (2016) Development of chitosan nanoparticles loaded with summer
599 savory (*Satureja hortensis* L.) essential oil for antimicrobial and antioxidant delivery
600 applications. *LWT- Food Sci Technol* 70:104-110.

601 Food and Agriculture organization (FAO, 2004). Regulations for mycotoxins in food and feed in
602 2003. FAO, Food and Nutrition, paper 81. FAO, Rome, Italy.

603 Gupta M (2010) Pharmacological properties and traditional therapeutic uses of important Indian
604 spices: A review. *Int J Food Prop* 13:1092-1116.

605 Hasheminejad N, Khodaiyan F, Safari M (2019) Improving the antifungal activity of clove
606 essential oil encapsulated by chitosan nanoparticles. *Food Chem* 275:113-122.

607 Hosseini SF, Zandi M, Rezaei M, Farahmandghavi F (2013) Two-step method for encapsulation
608 of oregano essential oil in chitosan nanoparticles: preparation, characterization and *in*
609 *vitro* release study. *Carbohydr Polym* 95:50-56.

610 Hu J, Wang X, Xiao Z, Bi W (2015) Effect of chitosan nanoparticles loaded with cinnamon
611 essential oil on the quality of chilled pork. *LWT-Food Sci Technol* 63:519-526.

612 International agency for research on cancer (IARC) (2012) Chemical agents and related
613 occupations. IARC monographs on the evaluation of carcinogenic risks to humans,
614 Volume 100F.

615 Iseri OD, Korpe DA, Sahin FI, Haberal M (2013) Hydrogen peroxide pretreatment of roots
616 enhanced oxidative stress response of tomato under cold stress. *Acta Physiol*
617 *Plant* 35:1905-1913.

618 Khan A, Ahmad A, Akhtar F, Yousuf S, Xess I, Khan LA, Manzoor N (2010) *Ocimum sanctum*
619 essential oil and its active principles exert their antifungal activity by disrupting
620 ergosterol biosynthesis and membrane integrity. *Res Microbiol* 161:816-823.

621 Kovesi B, Cserhati M, Erdelyi M, Zandoki E, Mezes M, Balogh K (2020) Lack of dose-and
622 time-dependent effects of aflatoxin b₁ on gene expression and enzymes associated with
623 lipid peroxidation and the glutathione redox system in chicken. *Toxins* 12:1-11.

624 Kumar A, Kujur A, Singh PP, Prakash B (2019) Nanoencapsulated plant-based bioactive
625 formulation against food-borne molds and aflatoxin B1 contamination: Preparation,
626 characterization and stability evaluation in the food system. *Food Chem* 287:139-150.

627 Li MF, Chen L, Xu MZ, Zhang JL, Wang Q, Zeng QZ, Wei XC, Yuan Y (2018) The formation
628 of zein-chitosan complex coacervated particles: Relationship to encapsulation and
629 controlled release properties. *Int J Biol Macromol* 116:1232-1239.

630 Lupetti A, Danesi R, Campa M, Del Tacca M, Kelly S (2002) Molecular basis of resistance to
631 azole antifungals. *Trends Mol Med* 8:76-81.

632 Marques CS, Carvalho SG, Bertoli LD, Villanova JCO, Pinheiro PF, dos Santos DCM, Yoshida
633 MI, de Freitas JCC, Cipriano DF, Bernardes PC (2019) β -Cyclodextrin inclusion
634 complexes with essential oils: Obtention, characterization, antimicrobial activity and
635 potential application for food preservative sachets. *Food Res Int* 119:499-509.

636 Pabast M, Shariatifar N, Beikzadeh S, Jahed G (2018) Effects of chitosan coatings incorporating
637 with free or nano-encapsulated Satureja plant essential oil on quality characteristics of
638 lamb meat. *Food Control* 91:185-192.

639 Perazzo FF, Carvalho JCT, Carvalho JE, Rehder VLG (2003) Central properties of the essential
640 oil and the crude ethanol extract from aerial parts of *Artemisia annua* L. *Pharmacol*
641 *Res* 48:497-502.

642 Pickova D, Ostry V, Malir J, Toman J, Malir F (2020) A Review on Mycotoxins and Microfungi
643 in Spices in the Light of the Last Five Years. *Toxins* 12:1-33.

644 Policegoudra RS, Goswami S, Aradhya SM, Chatterjee S, Datta S, Sivaswamy R, Chattopadhyay
645 P, Singh L (2012) Bioactive constituents of *Homalomena aromatica* essential oil and its
646 antifungal activity against dermatophytes and yeasts. *J Mycol Méd* 22: 83-87.

647 Potorti AG, Tropea A, Lo Turco V, Pellizzeri V, Belfita A, Dugo G, Di Bella G (2020)
648 Mycotoxins in spices and culinary herbs from Italy and Tunisia. *Nat Prod Res* 34:167-
649 171.

650 Prakash B, Kujur A, Yadav A, Kumar A, Singh PP, Dubey, N. K. (2018) Nanoencapsulation: An
651 efficient technology to boost the antimicrobial potential of plant essential oils in food
652 system. *Food Control* 89:1-11.

653 Rajkumar V, Gunasekaran C, Dharmaraj J, Chinnaraj P, Paul CA, Kanithachristy I (2020b)
654 Structural characterization of chitosan nanoparticle loaded with *Piper nigrum* essential oil
655 for biological efficacy against the stored grain pest control. *Pestic Biochem Phys* 166:1-9.

656 Rajkumar V, Gunasekaran C, Paul CA, & Dharmaraj J (2020a) Development of encapsulated
657 peppermint essential oil in chitosan nanoparticles: characterization and biological
658 efficacy against stored-grain pest control. *Pestic Biochem Phys* 170:1-9.

659 RASFF (2019) Rapid alert system for food and feed. Annual report-
660 2019.https://ec.europa.eu/food/safety/rasff/index_en.htm

661 Rasooli I, Abyaneh MR (2004) Inhibitory effects of Thyme oils on growth and aflatoxin
662 production by *Aspergillus parasiticus*. *Food Control* 15:479-483.

663 Ribeiro JC, Ribeiro WLC, Camurça-Vasconcelos ALF, Macedo ITF, Santos JML, Paula HCB,
664 Filho JVA, Magalhaes RD, Bevilaqua CML (2014) Efficacy of free and
665 nanoencapsulated *Eucalyptus citriodora* essential oils on sheep gastrointestinal
666 nematodes and toxicity for mice. *Vet Parasitol* 204:243-248.

667 Ricci D, Fraternali D, Giamperi L, Bucchini A, Epifano F, Burini G, Curini M (2005) Chemical
668 composition, antimicrobial and antioxidant activity of the essential oil of *Teucrium*
669 *marum* (Lamiaceae). J Ethnopharmacol 98:195-200.

670 Roy SJ, Baruah PS, Lahkar L, Gurung L, Saikia D, Tanti B (2019) Phytochemical analysis and
671 antioxidant activities of *Homalomena aromatic* Schott. J Pharmacogn Phytochem 8:1379-
672 1385.

673 Sathivel S, Liu Q, Huang J, Prinyawiwatkul W (2007) The influence of chitosan glazing on the
674 quality of skinless pink salmon (*Oncorhynchus gorbuscha*) fillets during frozen
675 storage. Journal Food Eng 83:366-373.

676 Schlosser I, Prange A (2018) Antifungal activity of selected natural preservatives against the
677 foodborne molds *Penicillium verrucosum* and *Aspergillus westerdijkiae*. FEMS
678 microbiology letters 365: fny125.

679 Sheijooni-Fumani N, Hassan J, Yousefi SR (2011) Determination of aflatoxin B₁ in cereals by
680 homogeneous liquid–liquid extraction coupled to high performance liquid
681 chromatography-fluorescence detection. J Sep Sci 34:1333-1337.

682 Singh A, Chaudhari AK, Das S, Singh VK, Dwivedy AK, Shivalingam RK, Dubey NK (2020b)
683 Assessment of preservative potential of *Bunium persicum* (Boiss) essential oil against
684 fungal and aflatoxin contamination of stored masticatories and improvement in efficacy
685 through encapsulation into chitosan nanomatrix. Environ Sci Pollut Res 27:27635-27650.

686 Singh N, Rao AS, Nandal A, Kumar S, Yadav SS, Ganaie SA, Narasimhan B (2020a)
687 Phytochemical and pharmacological review of *Cinnamomum verum* J. Presl-a versatile
688 spice used in food and nutrition. Food Chem 338:1-24.

689 Siva S, Li C, Cui H, Meenatchi V, Lin L (2020) Encapsulation of essential oil components with
690 methyl- β -cyclodextrin using ultrasonication: Solubility, characterization, DPPH and
691 antibacterial assay. Ultrason Sonochem 64:1-12.

692 Su H, Huang C, Liu Y, Kong S, Wang J, Huang H, Zhang B (2020) Preparation and
693 characterization of *Cinnamomum* essential oil-chitosan nanocomposites: Physical,
694 Structural, and Antioxidant Activities. Processes 8:1-13.

695 Thanushree MP, Sailendri D, Yoha KS, Moses JA, Anandharamakrishnan C (2019) Mycotoxin
696 contamination in food: An exposition on spices. Trends Food Sci Technol 93:69-80.

697 Tian J, Huang B, Luo X, Zeng H, Ban X, He J, Wang Y (2012) The control of *Aspergillus flavus*
698 with *Cinnamomum jensenianum* Hand.-Mazz essential oil and its potential use as a food
699 preservative. Food Chem 130:520-527.

700 Tsikas D (2017) Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and
701 relatives in biological samples: Analytical and biological challenges. Anal Biochem
702 524:13-30.

703 Upadhyay N, Singh VK, Dwivedy AK, Das S, Chaudhari AK, Dubey NK (2018) *Cistus*
704 *ladanifer* L. essential oil as a plant based preservative against molds infesting oil seeds,
705 aflatoxin B₁ secretion, oxidative deterioration and methylglyoxal biosynthesis. LWT-
706 Food Sci Technol 92:395-403.

707 Wu L, Liu M (2008) Preparation and properties of chitosan-coated NPK compound fertilizer
708 with controlled-release and water-retention. *Carbohydr Polym* 72:240-247.

709 Yadav SK, Singla-Pareek SL, Reddy MK, Sopory SK (2005) Transgenic tobacco plants
710 overexpressing glyoxalase enzymes resist an increase in methylglyoxal and maintain
711 higher reduced glutathione levels under salinity stress. *Federation of European*
712 *Biochemical Societies (FEBS) Letters* 579:6265-6271.

713 Yang Z, Wang H, Ying G, Yang M, Nian Y, Liu J, Kong W (2017) Relationship of mycotoxins
714 accumulation and bioactive components variation in ginger after fungal
715 inoculation. *Front Pharmacol* 8:331.

716 Zabka M, Pavela R, Slezakova L (2009) Antifungal effect of *Pimenta dioica* essential oil against
717 dangerous pathogenic and toxinogenic fungi. *Ind Crops Prod* 30:250-253.

718 Zhu JJ, Zeng XP, Berkebile D, Du HJ, Tong Y, Qian K (2009) Efficacy and safety of catnip
719 (*Nepeta cataria*) as a novel filth fly repellent. *Med Vet Entomol* 23:209-216.

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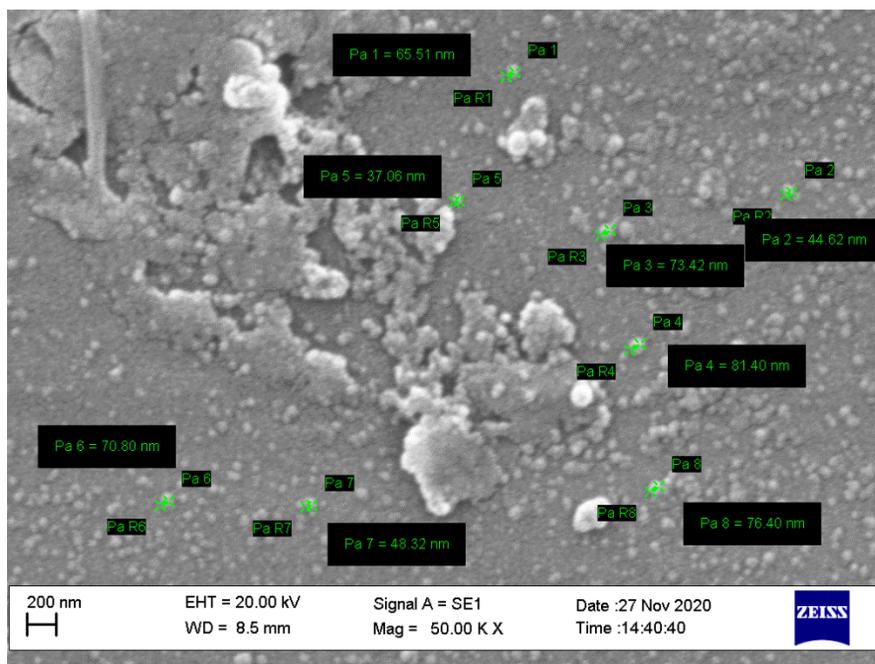
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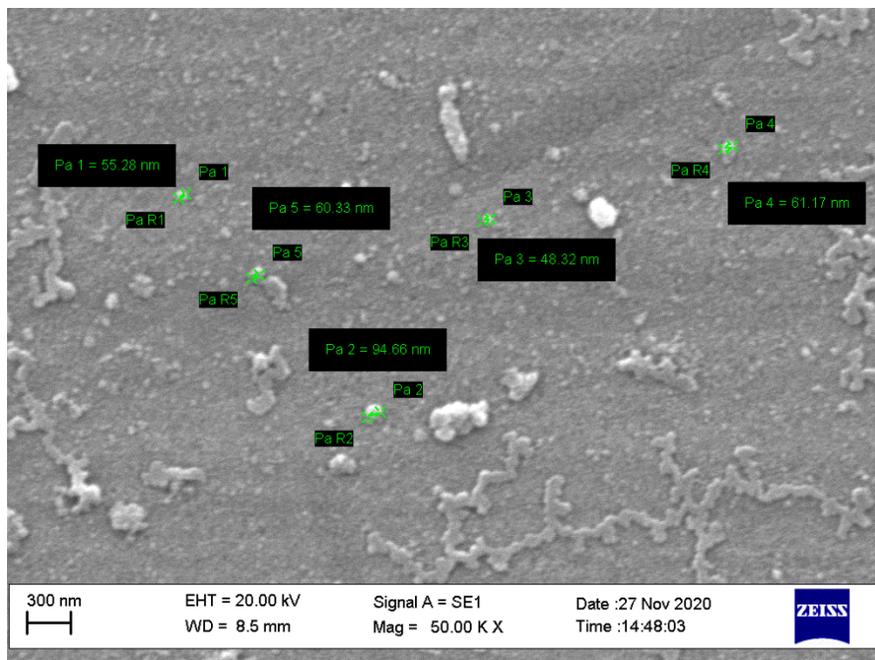
735 **Figures**

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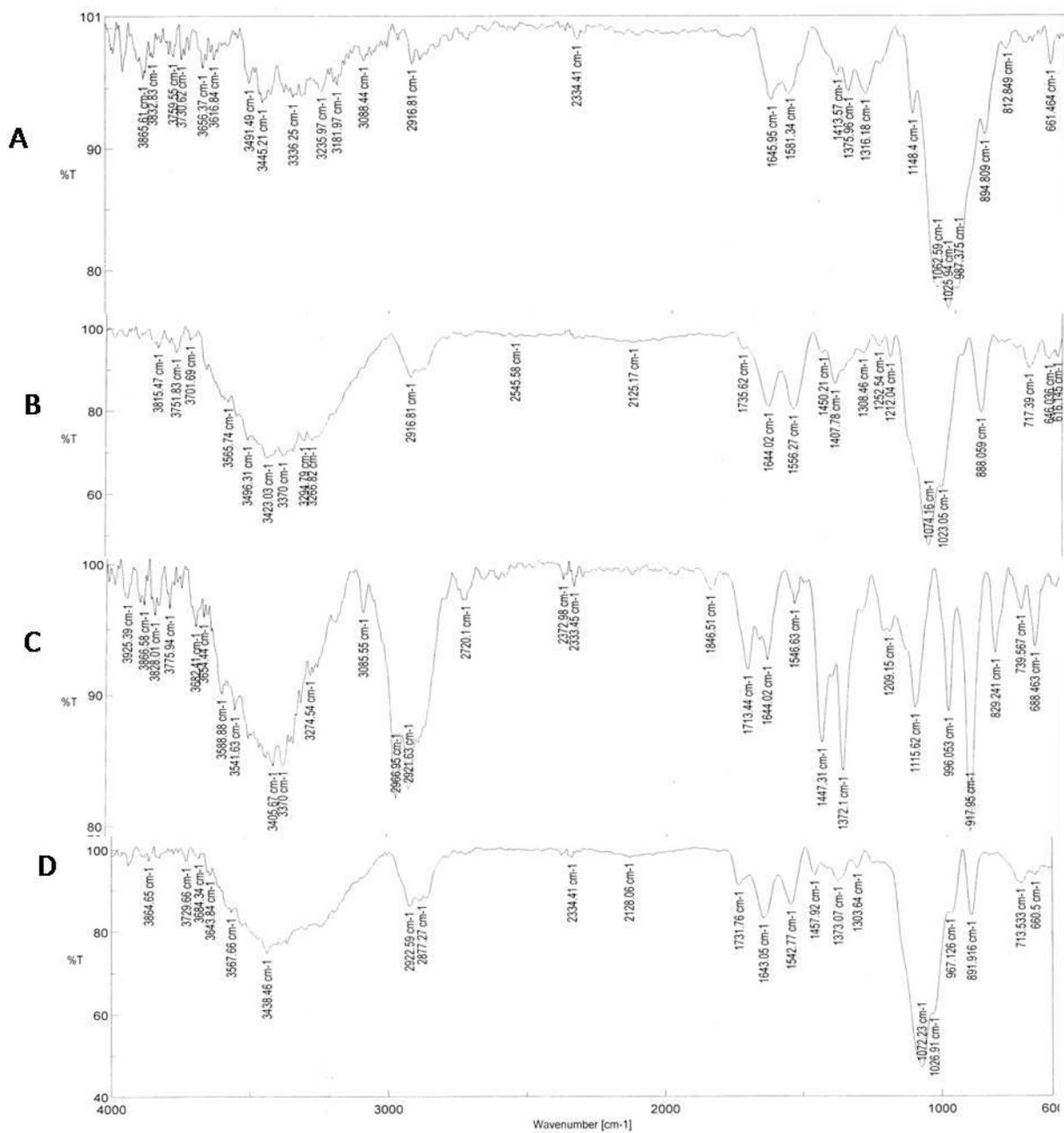
738 (A)



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740 (B)

741 **Fig. 1** SEM image of (A) CS Ne and (B) CS-HAEO-Ne



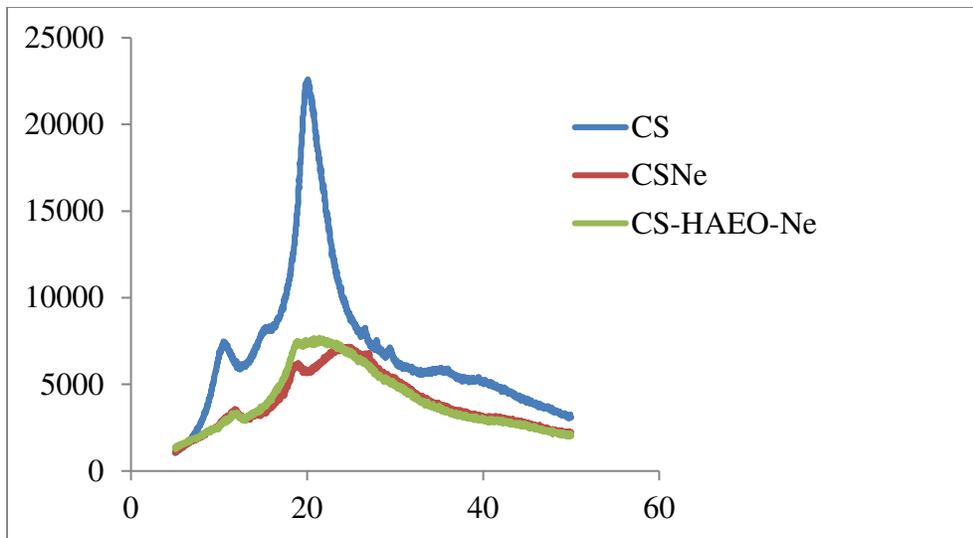
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744 **Fig. 2** FTIR spectra of (A) CS, (B) CS Ne, (C) EO and (D) CS-HAEO-Ne

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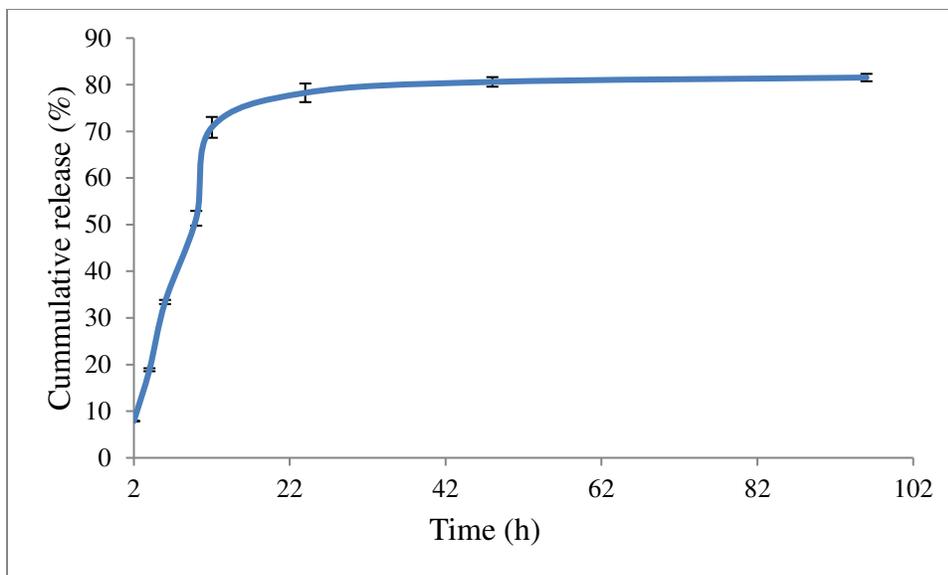
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749 **Fig. 3** XRD spectra of CS, CS Ne and CS-HAEO-Ne

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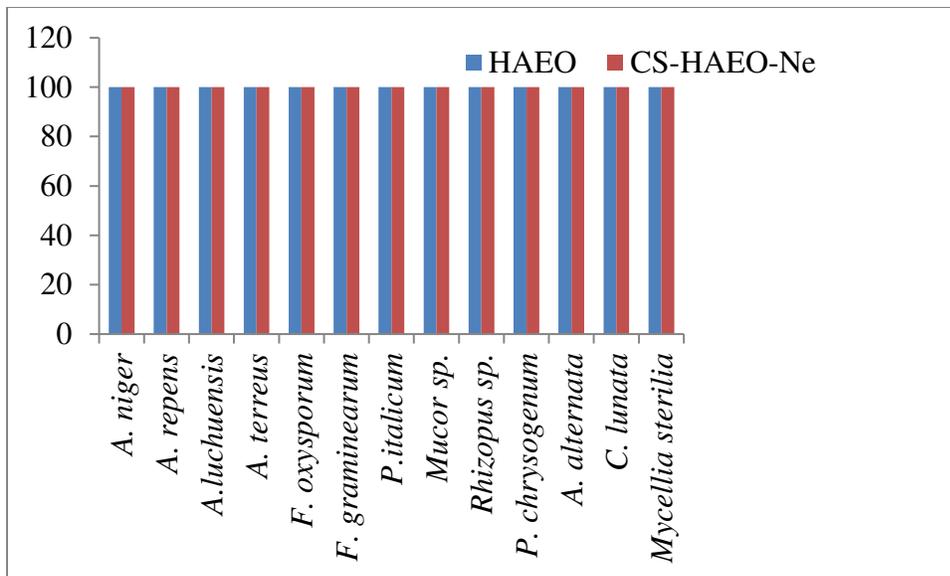


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752 **Fig. 4** *In vitro* release profile of CS-HAEO-Ne

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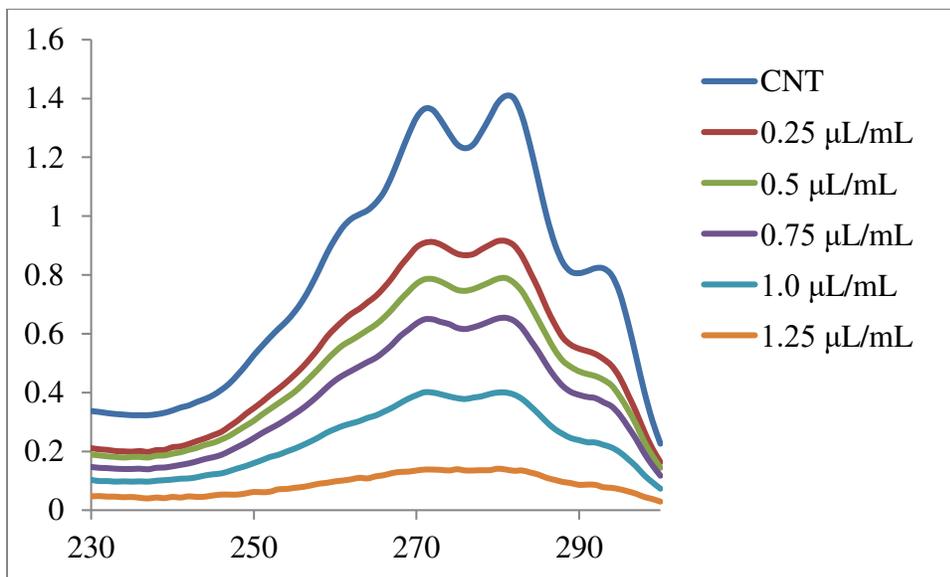
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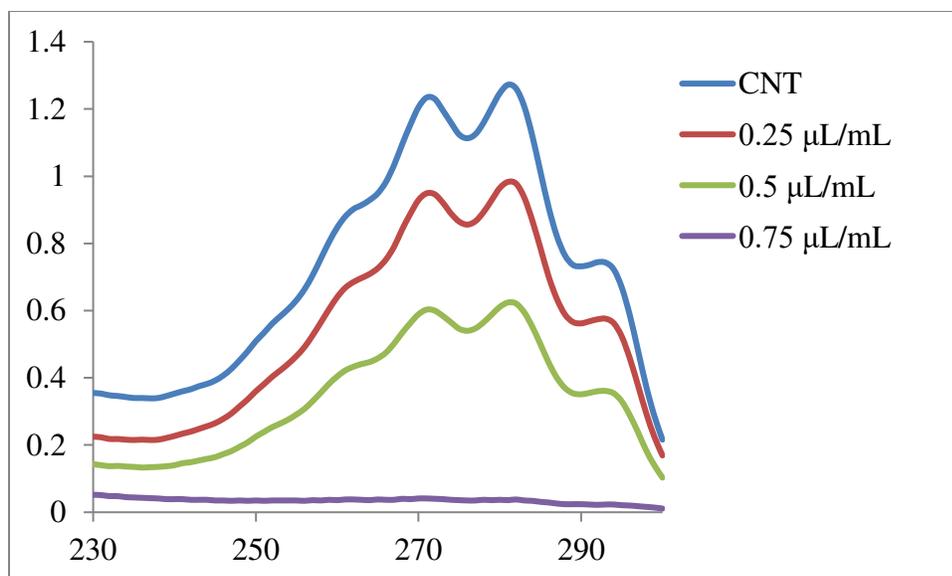
756 **Fig. 5** Fungitoxic spectrum of HAEO and CS-HAEO-Ne at their respective MIC concentration

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759 (A)

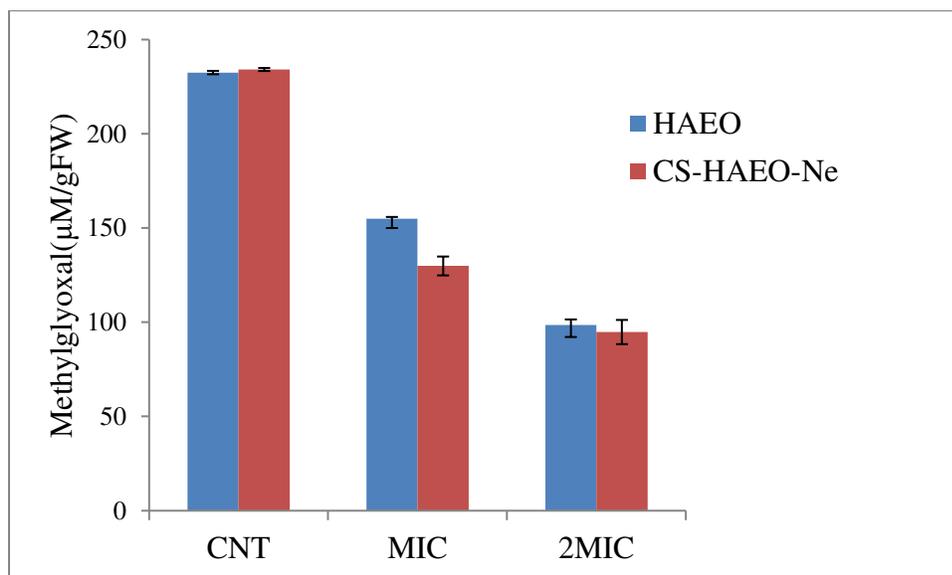


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761 (B)

762 **Fig. 6** Ergosterol inhibition at different concentration of (A) HAEO and (B) CS-HAEO-Ne

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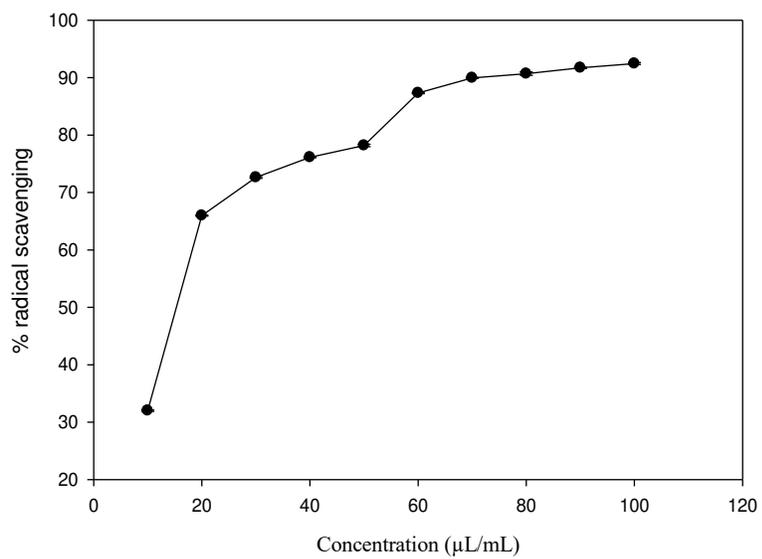


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766 **Fig. 7** Effect of (A) HAEO and (B) CS-HAEO-Ne on methylglyoxal of AF-LHP NS 7

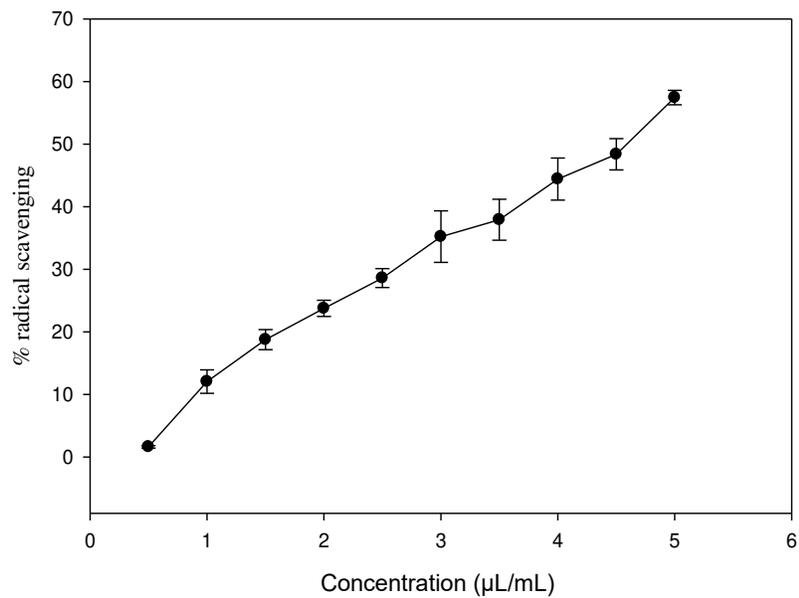
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769 (A)

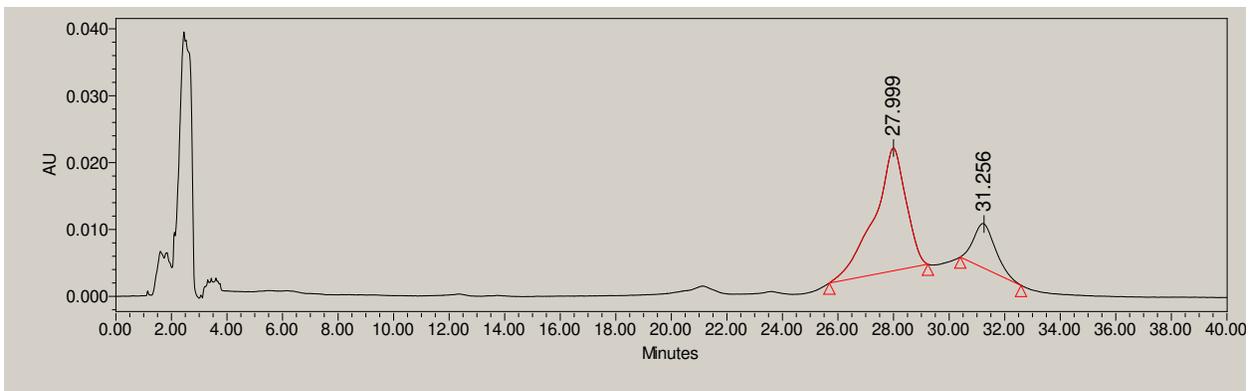
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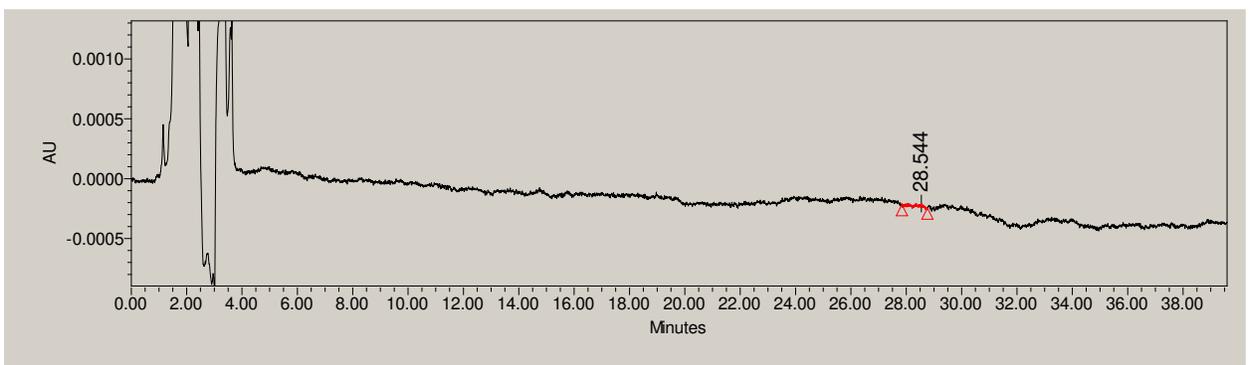
772 (B)

773 **Fig. 8** DPPH free radical scavenging activity of (A) HAEO and (B) CS-HAEO-Ne



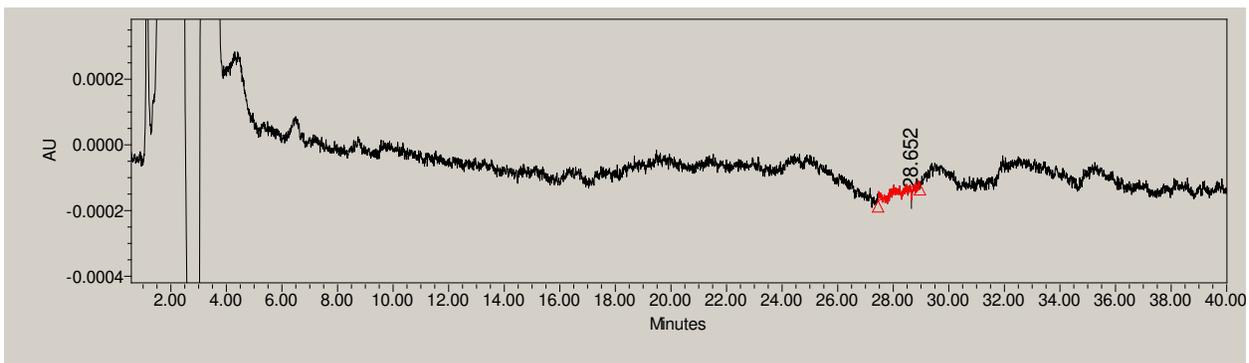
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775 (A)



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777 (B)



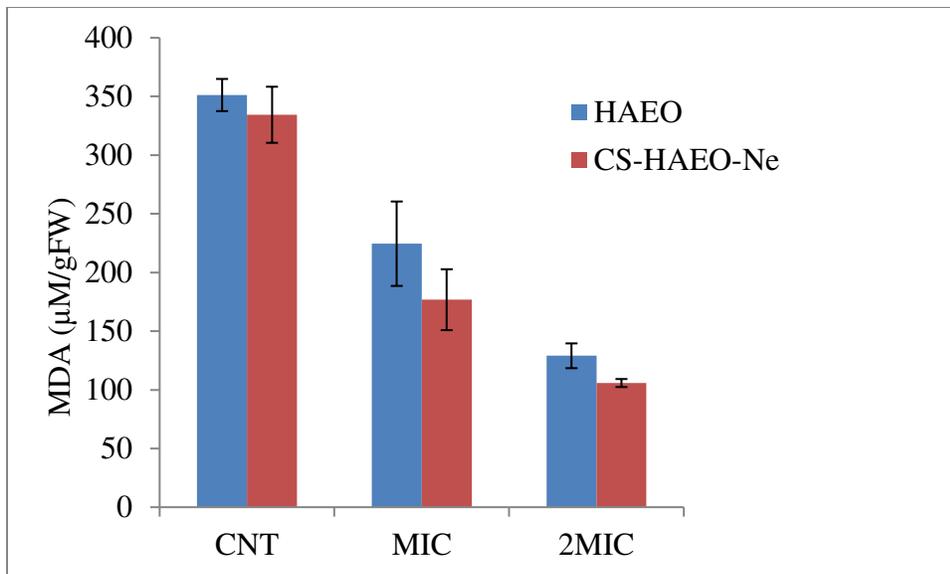
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779 (C)

780 **Fig. 9** *In vivo* antiaflatoxicogenic efficacy of HAEO and CS-HAEO-Ne; (A) CNT, (B) HAEO and
 781 (C) CS-HAEO-Ne

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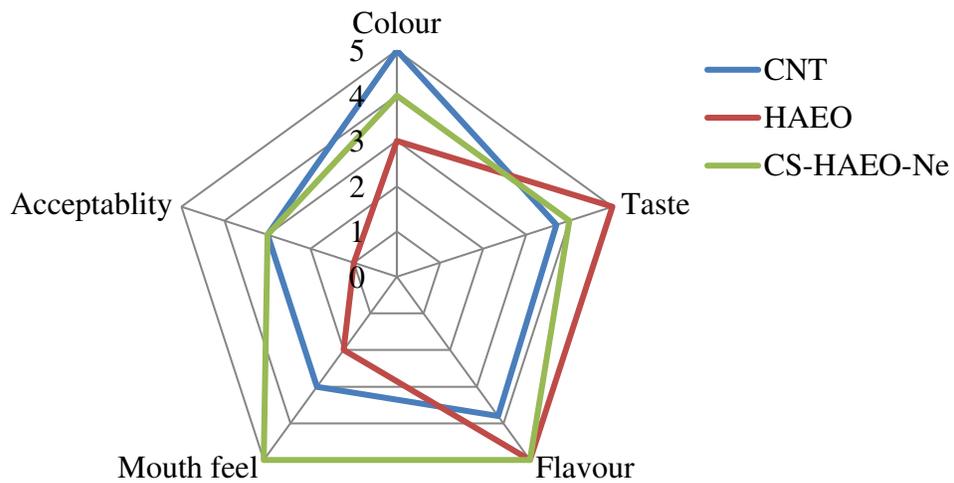
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785 **Fig. 10** Effect of HAEO and CS-HAEO-Ne fumigation on lipid peroxidation of stored black
 786 cumin (*Nigella sativa*) seeds

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788

789 **Fig. 11** Sensorial profile of HAEO and CS-HAEO-Ne fumigated black cumin (*Nigella sativa*)

790 seed

791

792

793

794 **Table 1** Chemical Profile of *Homalomena aromatica* essential oil (HAEO)

S.NO.	Bioactive Components	Retention time	Percentage
1	Terpinolene	4.91	0.23
2	β-pinene	5.25	0.28
3	α-pinene	5.75	3.16
4	α-terpinene	5.89	0.45
5	o-cymene	6.13	1.04
6	D-limonene	6.21	1.21
7	β-ocimene	6.72	0.09
8	γ-terpinene	7.05	1.1
9	Linalool oxide	7.57	0.33
12	Linalool	8.44	68.51
13	Hotrienol	8.62	0.2
14	Terpinen-4-ol	11.06	8.26
15	m-cymen-8-ol	11.48	0.28
16	Terpineol	11.61	2.24
17	cis-geraniol	13.14	0.35
18	Geraniol	14.16	0.81
19	Espatulenol	26.49	0.57
20	Globulol	27.35	0.14
21	Tau-cadinol acetate	28.76	0.35
22	α-cadinol	28.8	0.64
23	Caryophyllene oxide	30.46	1.47
		Total	91.71

795 **Note:** Compounds in bold are major components

796

797 **Table 2** % nanoencapsulation efficiency and % loading capacity of HAEO inside chitosan
798 polymeric matrix

Chitosan: HAEO (w/v)	NEE %	LC %
1:0.0	0.00±0.00 ^a	0.00±0.00 ^a
1:0.2	22.01±0.25 ^b	0.15±0.00 ^b
1:0.4	24.82±0.37 ^c	0.35±0.01 ^c
1:0.6	55.68±0.25 ^d	1.16±0.01 ^d
1:0.8	83.42±0.38^e	2.32±0.01^e
1:1	60.07±0.05 ^f	2.08±0.00 ^f

799 NEE= nanoencapsulation efficiency, LC= Loading capacity

800 Values are mean (n=3) ± standard error

801 Significance difference between the means (p < 0.05, ANOVA test)

802

803

804

805 **Table 3** Effect of HAEO and CS-HAEO-Ne on mycelial dry weight (MDW) and AFB₁
 806 production by AF-LHP-NS 7

Concentration ($\mu\text{L/mL}$)	HAEO			CS-HAEO-Ne		
	MDW (g)	AFB ₁ ($\mu\text{g/mL}$)	% inhibition	MDW (g)	AFB ₁ ($\mu\text{g/mL}$)	% inhibition
CNT	0.69 \pm 0.03 ^a	3.94 \pm 0.05 ^a	0	0.67 \pm 0.01 ^a	3.76 \pm 0.05 ^a	0
0.25	0.63 \pm 0.01 ^a	3.76 \pm 0.07 ^b	4.74	0.55 \pm 0.00 ^b	3.39 \pm 0.05 ^b	9.92
0.50	0.53 \pm 0.00 ^a	2.74 \pm 0.04 ^c	30.47	0.38 \pm 0.01 ^c	2.42 \pm 0.07 ^c	35.54
0.75	0.47 \pm 0.00 ^b	2.38 \pm 0.06 ^d	39.78	0.29 \pm 0.01 ^d	1.53 \pm 0.05 ^d	59.29
1.0**	0.22 \pm 0.07 ^c	0.34 \pm 0.02 ^e	92.57	0.13 \pm 0.01 ^e	0.00 \pm 0.00 ^e	100
1.25*	0.00 \pm 0.00 ^{cd}	0.00 \pm 0.00 ^f	100	0.00 \pm 0.00 ^f	-	-
1.5	0.00 \pm 0.00 ^d	0.00 \pm 0.00	-	-	-	-
1.75	0.00 \pm 0.00 ^e	0.00 \pm 0.00	-	-	-	-

807 AFB₁ = aflatoxin B₁

808 Values are mean (n = 3) \pm standard error

809 Significance difference between the means (p<0.05, ANOVA test)

810 CNT = Control

811

812

813

814 **Table 4** Effect of HAEO and CS-HAEO-Ne on vital cellular constituent's release of AF-LHP-NS 7

Conc. ($\mu\text{L/mL}$)	Leakage of vital cellular constituents									
	Ca^{+2} (mg/L)		Mg^{+2} (mg/L)		K^{+} (mg/L)		260 nm absorbing material		280 nm absorbing material	
	HAEO	CS-HAEO-Ne	HAEO	CS-HAEO-Ne	HAEO	CS-HAEO-Ne	HAEO	CS-HAEO-Ne	HAEO	CS-HAEO-Ne
CNT	6.57 \pm 0.73 ^a	2.83 \pm 0.95 ^a	4.70 \pm 2.14 ^a	3.98 \pm 0.46 ^a	3.48 \pm 0.67 ^a	11.90 \pm 1.75 ^a	0.69 \pm 0.21 ^a	0.10 \pm 0.00 ^a	0.37 \pm 0.28 ^a	0.12 \pm 0.01 ^a
0.25	9.35 \pm 0.85 ^a	8.92 \pm 0.62 ^b	6.48 \pm 1.32 ^{ab}	8.20 \pm 1.41 ^{bc}	30.22 \pm 2.05 ^b	42.10 \pm 2.39 ^{ab}	0.087 \pm 0.00 ^a	0.13 \pm 0.00 ^a	0.10 \pm 0.00 ^a	0.16 \pm 0.00 ^a
0.50	6.55 \pm 2.59 ^{ab}	10.72 \pm 1.35 ^{bc}	7.85 \pm 0.88 ^{abc}	8.55 \pm 1.39 ^{bc}	40.1 \pm 4.95 ^{bc}	81.50 \pm 11.57 ^{abc}	0.15 \pm 0.00 ^a	0.34 \pm 0.01 ^b	0.13 \pm 0.00 ^a	0.32 \pm 0.01 ^b
0.75	10.50 \pm 0.90 ^{ab}	12.43 \pm 1.01 ^{bc}	9.18 \pm 0.43 ^{abc}	9.40 \pm 1.20 ^c	51.48 \pm 5.69 ^{bcd}	91 \pm 11.03 ^{bc}	0.16 \pm 0.00 ^{ab}	0.46 \pm 0.01 ^c	0.23 \pm 0.01 ^b	0.57 \pm 0.01 ^c
1.0	12.13 \pm 1.42 ^{ab}	14.15 \pm 0.635 ^c	10.42 \pm 0.76 ^{abcd}	10.15 \pm 0.78 ^c	56.38 \pm 5.21 ^{cd}	107 \pm 15.17 ^{bc}	0.25 \pm 0.03 ^{ab}	0.54 \pm 0.01 ^d	0.34 \pm 0.01 ^c	0.77 \pm 0.01 ^d
1.25*	13.38 \pm 1.09 ^{ab}	18.72 \pm 0.32 ^d	12.52 \pm 1.21 ^{bcd}	13.05 \pm 0.73 ^c	59.17 \pm 4.23 ^{cd}	118 \pm 22.31 ^c	0.31 \pm 0.01 ^{ab}	0.65 \pm 0.01 ^e	0.37 \pm 0.01 ^c	0.87 \pm 0.03 ^d
1.5	16.07 \pm 2.14 ^{ab}	-	13.10 \pm 1.49 ^{cd}	-	67.33 \pm 6.50 ^{de}	-	0.32 \pm 0.02 ^{ab}	-	0.47 \pm 0.01 ^d	-
1.75**	20.35 \pm 4.41 ^b	-	15.67 \pm 1.45 ^d	-	72.33 \pm 8.64 ^{de}	-	0.54 \pm 0.01 ^{bc}	-	0.58 \pm 0.00 ^e	-
2MIC	37.20 \pm 5.59 ^c	38.08 \pm 1.77 ^e	23.78 \pm 1.48 ^e	25.92 \pm 1.44 ^d	85.97 \pm 5.07 ^e	154 \pm 9.83 ^d	0.68 \pm 0.02 ^c	0.94 \pm 0.04 ^f	0.64 \pm 0.02 ^f	1.36 \pm 0.09 ^e

815 * =Minimum inhibitory concentration for CS-HAEO-Ne, ** =Minimum inhibitory concentration for HAEO, - = not measured

816 Values are mean(n=3) \pm standard error

817 Significance difference between the means (p < 0.05, ANOVA test)

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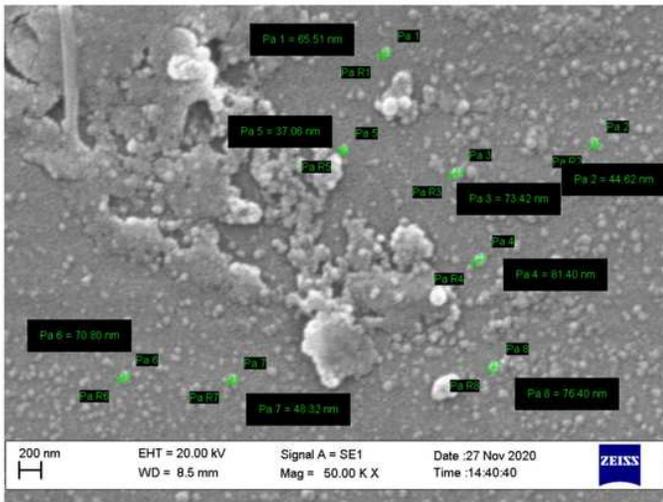
819 **Table 5** Effect of HAEO and CS-HAEO-Ne on *in situ* mineral content of black cumin seeds

Samples	Mineral contents (mg/L)					
	Potassium (K)	Calcium (Ca)	Magnesium (Mg)	Iron (Fe)	Zinc (Zn)	Manganese (Mn)
HAEO CNT	55.77±11.03 ^a	63.17±5.70 ^a	4.55±0.52 ^a	1.87±0.26 ^a	1.55±0.13 ^a	0.88±0.61 ^a
HAEO MIC	79.33±7.37 ^{ab}	180.17±9.94 ^a	20.18±0.77 ^a	12.88±1.08 ^a	6.28±0.28 ^a	2.1±1.15 ^a
CS-HAEO-Ne CNT	40.33±0.81 ^{bc}	77±1.76 ^b	5±0.36 ^b	2.35±0.92 ^b	2.9±0.65 ^b	0.45±0.17 ^b
CS-HAEO-Ne MIC	95±3.75 ^c	194.5±13.60 ^b	22.8±0.82 ^c	14.56±0.57 ^b	5.82±0.16 ^b	2.2±1.21 ^b

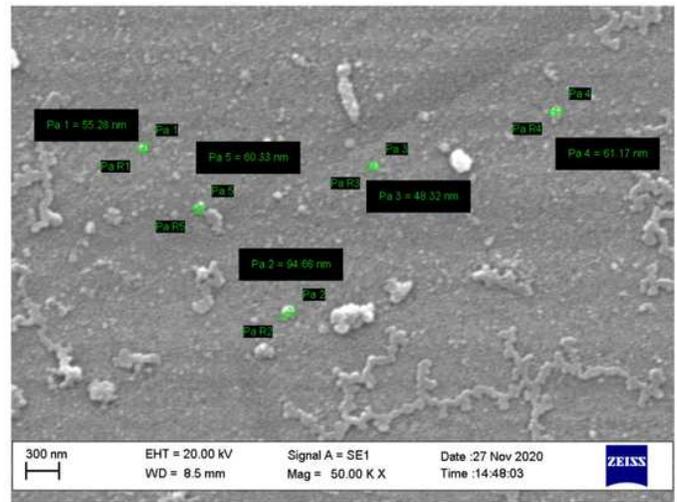
820 Values are mean (n=3) ± standard error

821 Significance difference between the means (p < 0.05, ANOVA test)

Figures



(A)



(B)

Figure 1

SEM image of (A) CS Ne and (B) CS-HAEO-Ne

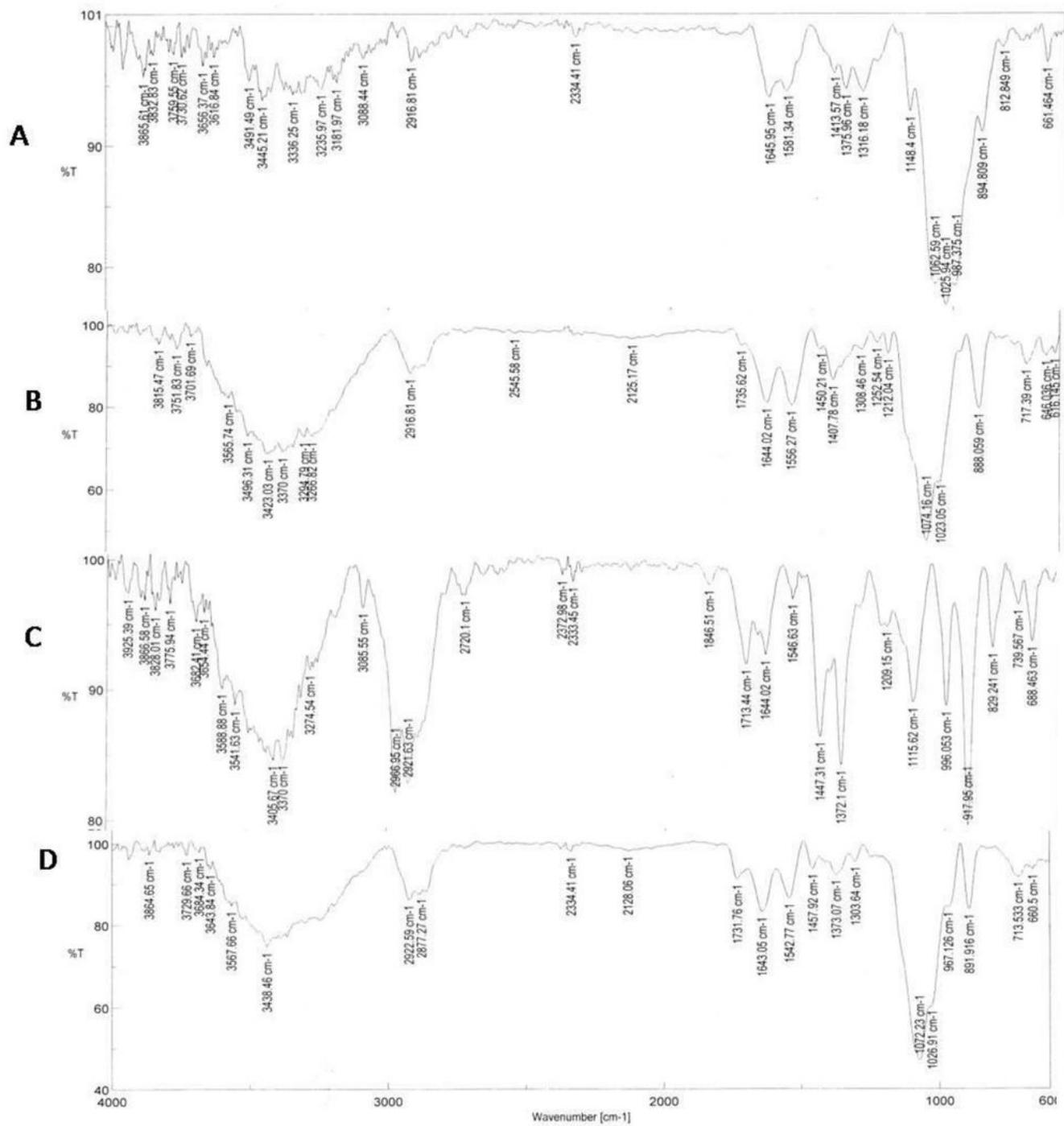


Figure 2

FTIR spectra of (A) CS, (B) CS Ne, (C) EO and (D) CS-HAEO-Ne

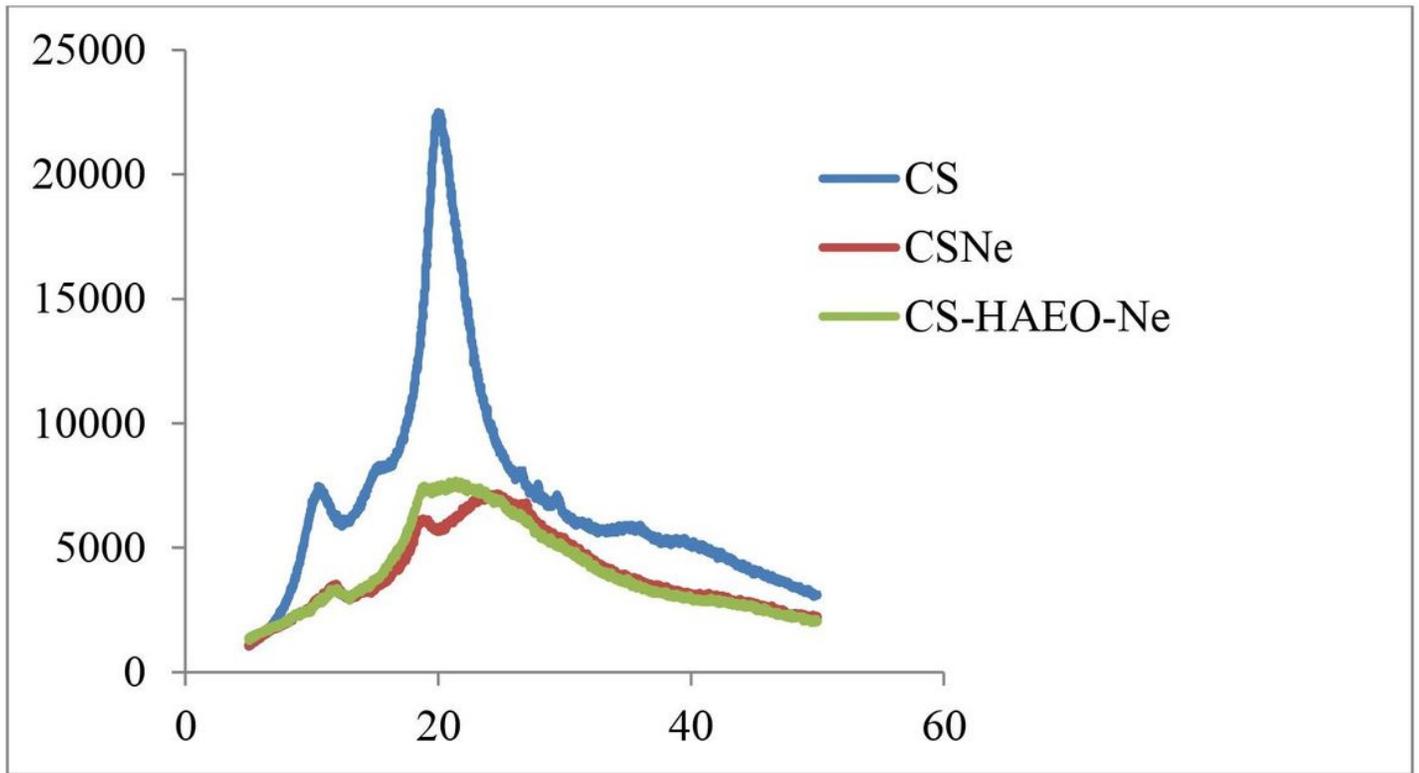


Figure 3

XRD spectra of CS, CS Ne and CS-HAEO-Ne

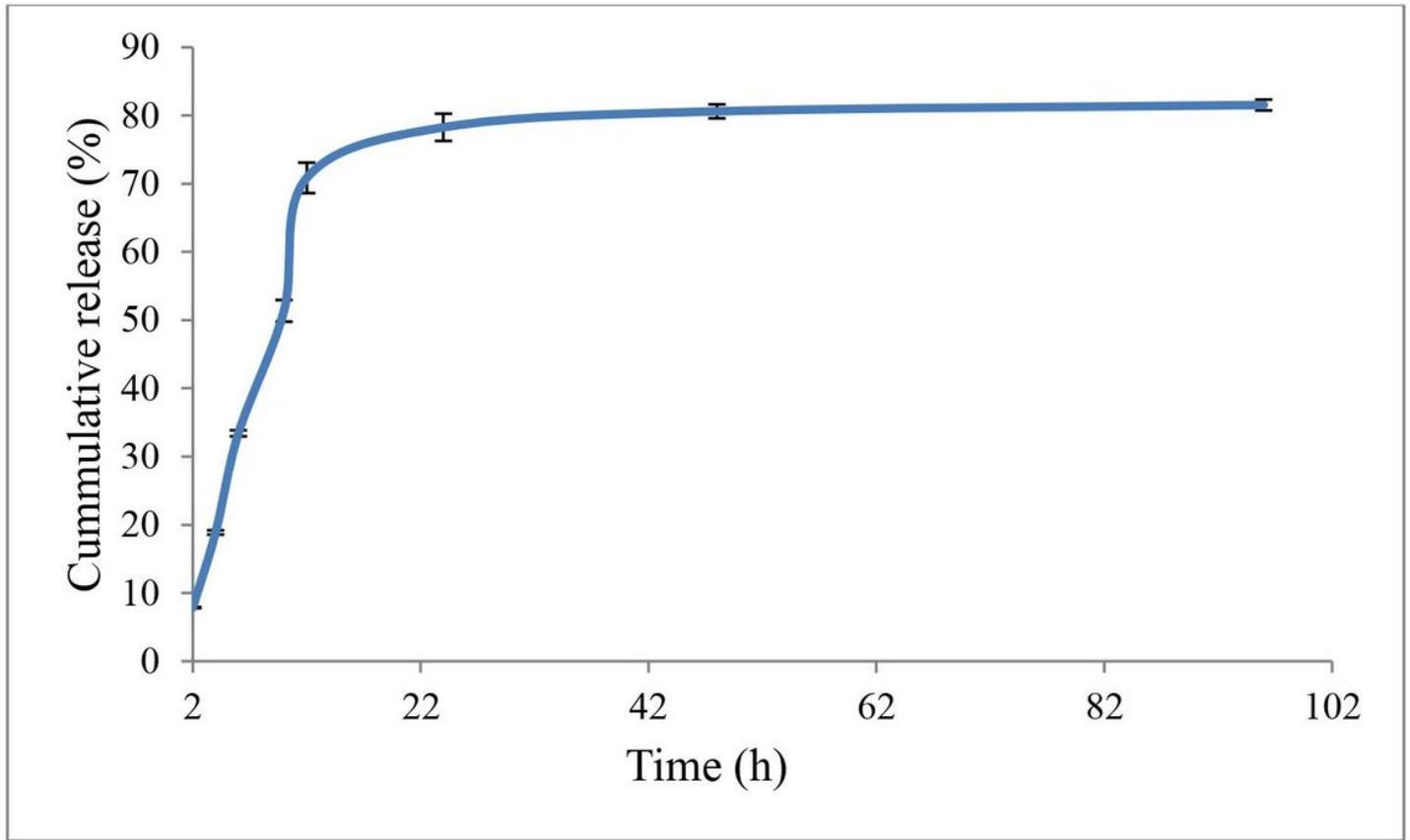


Figure 4

In vitro release profile of CS-HAEO-Ne

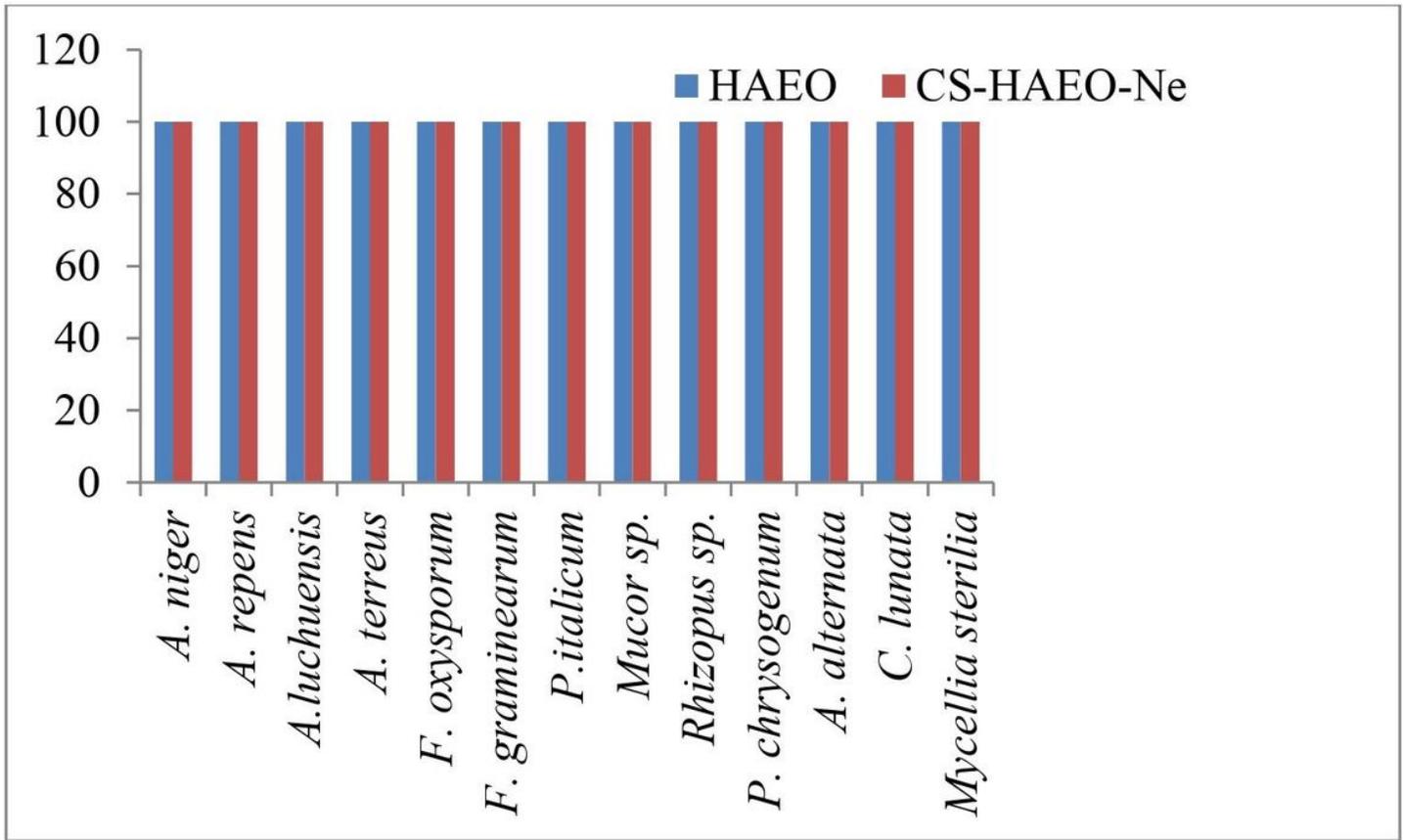
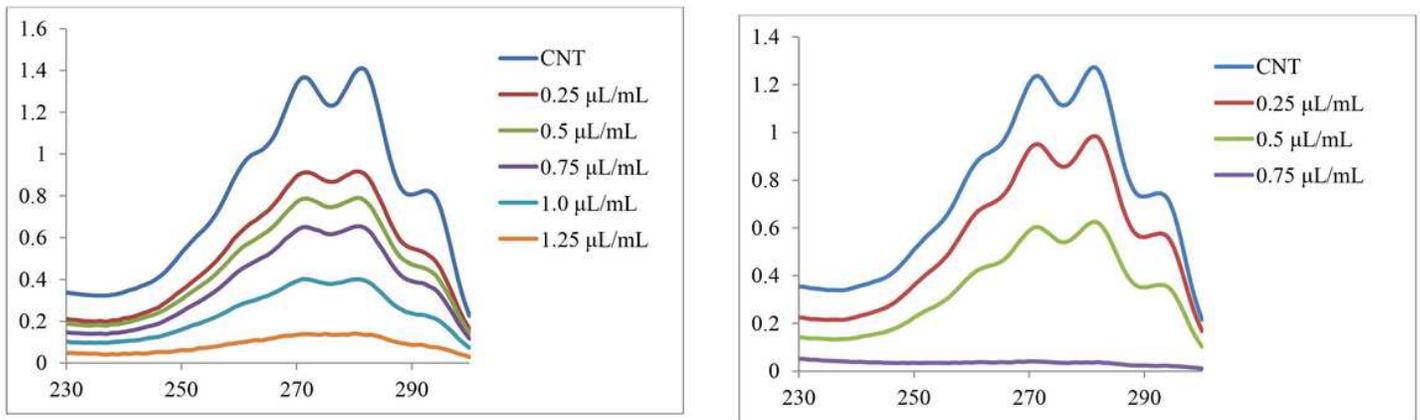


Figure 5

Fungitoxic spectrum of HAEO and CS-HAEO-Ne at their respective MIC concentration



(A)

(B)

Figure 6

Ergosterol inhibition at different concentration of (A) HAEO and (B) CS-HAEO-Ne

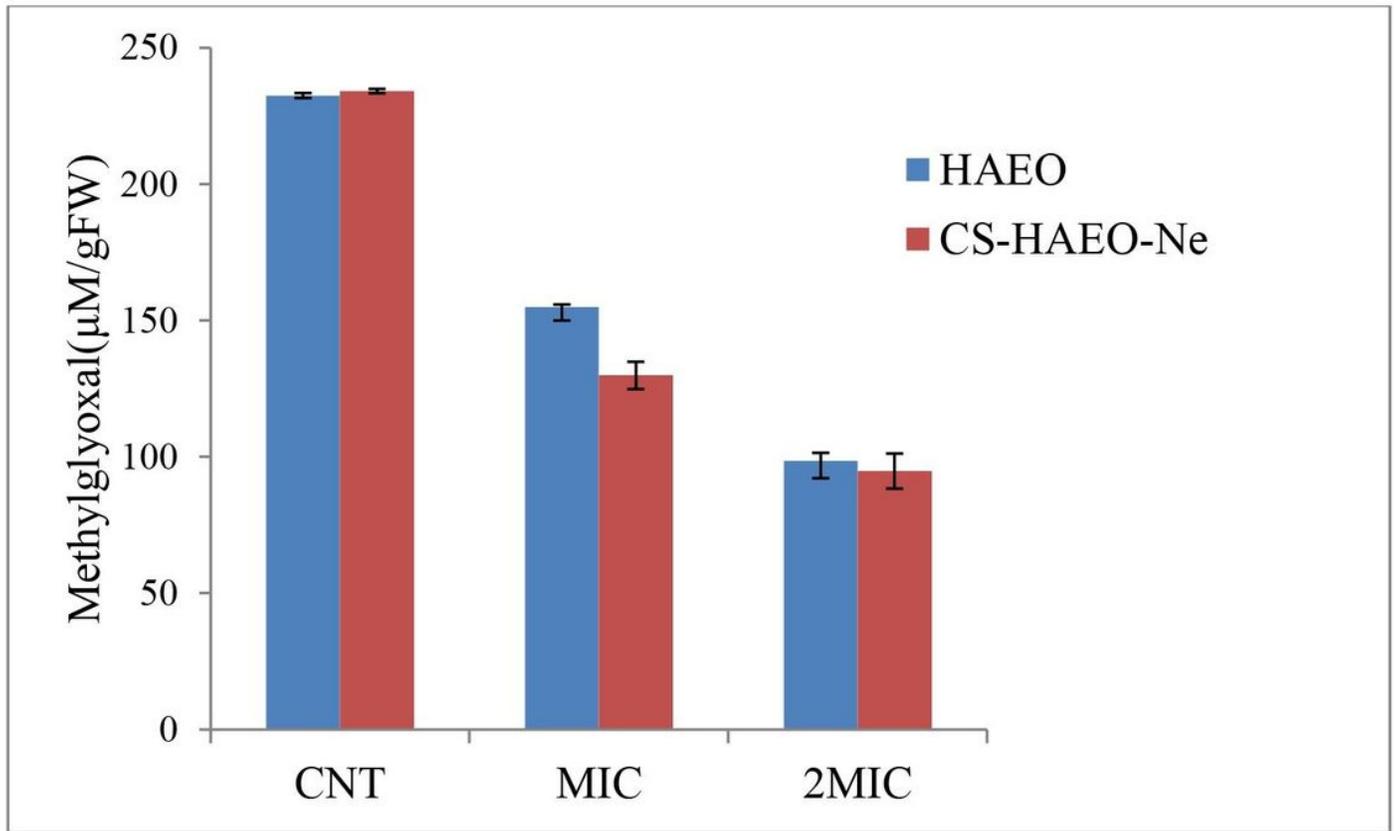
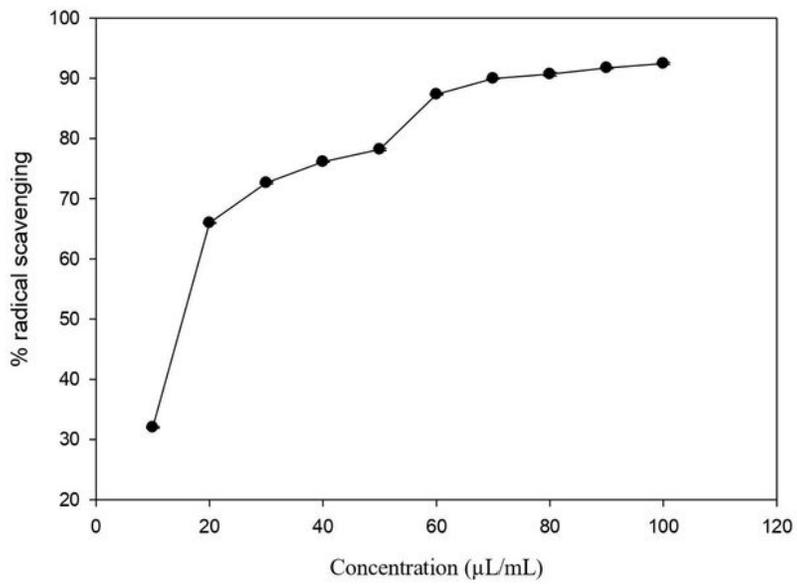
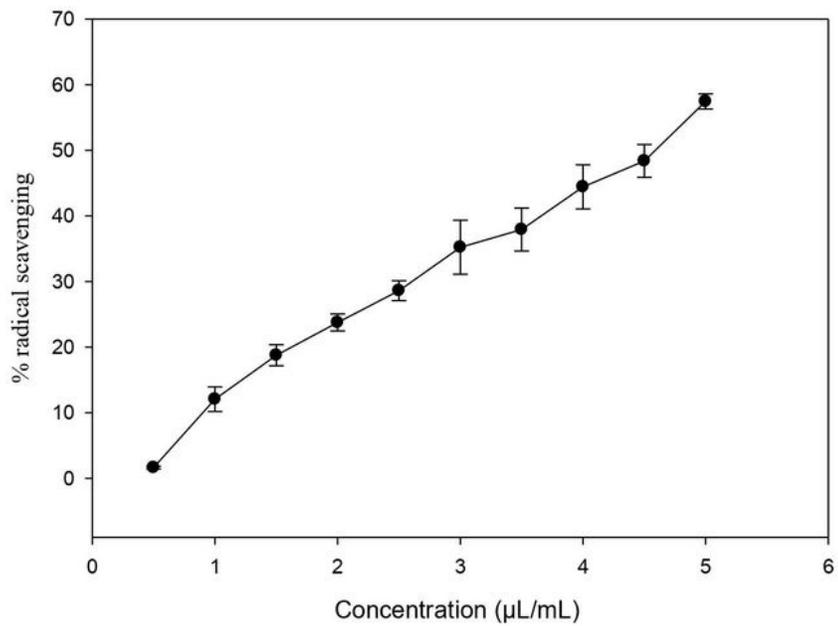


Figure 7

Effect of (A) HAEO and (B) CS-HAEO-Ne on methylglyoxal of AF-LHP NS 7



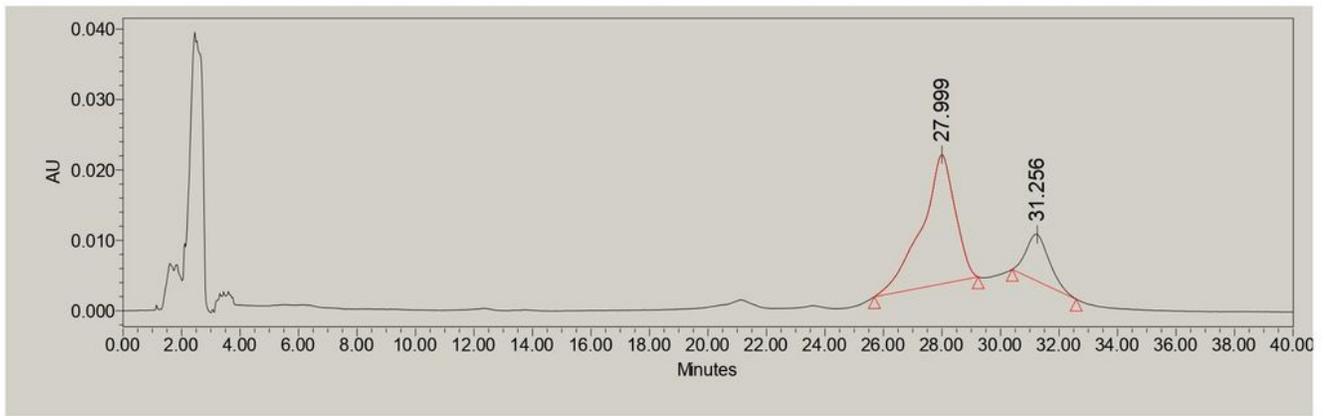
(A)



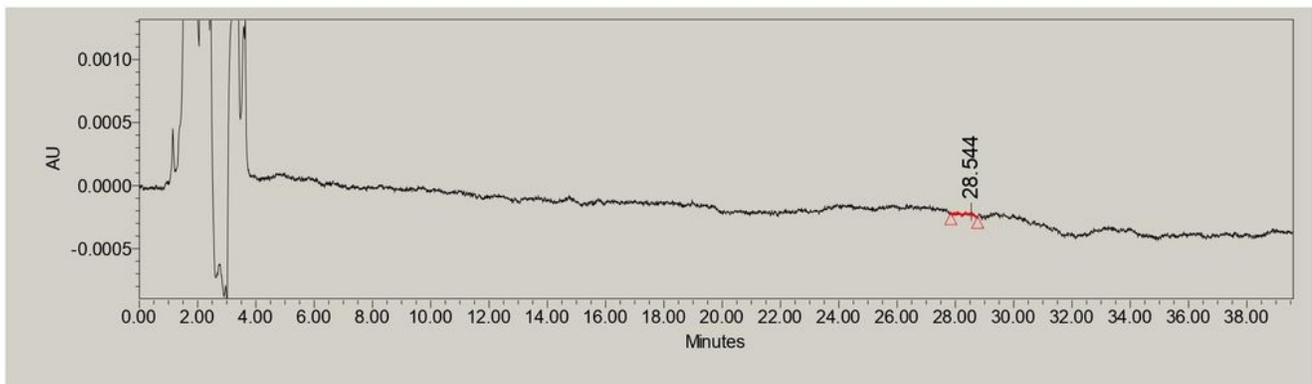
(B)

Figure 8

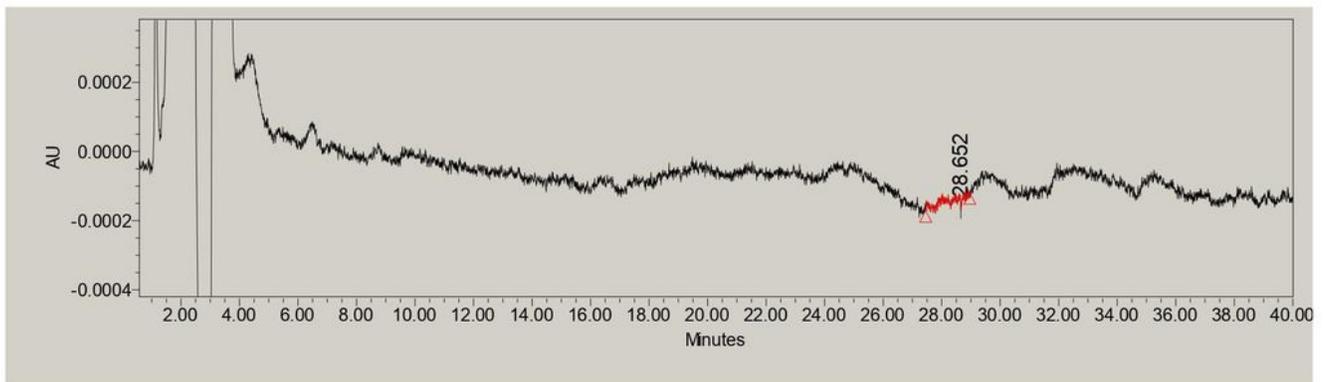
DPPH free radical scavenging activity of (A) HAEO and (B) CS-HAEO-Ne



(A)



(B)



(C)

Figure 9

In vivo antiaflatoxigenic efficacy of HAEO and CS-HAEO-Ne; (A) CNT, (B) HAEO and (C) CS-HAEO-Ne

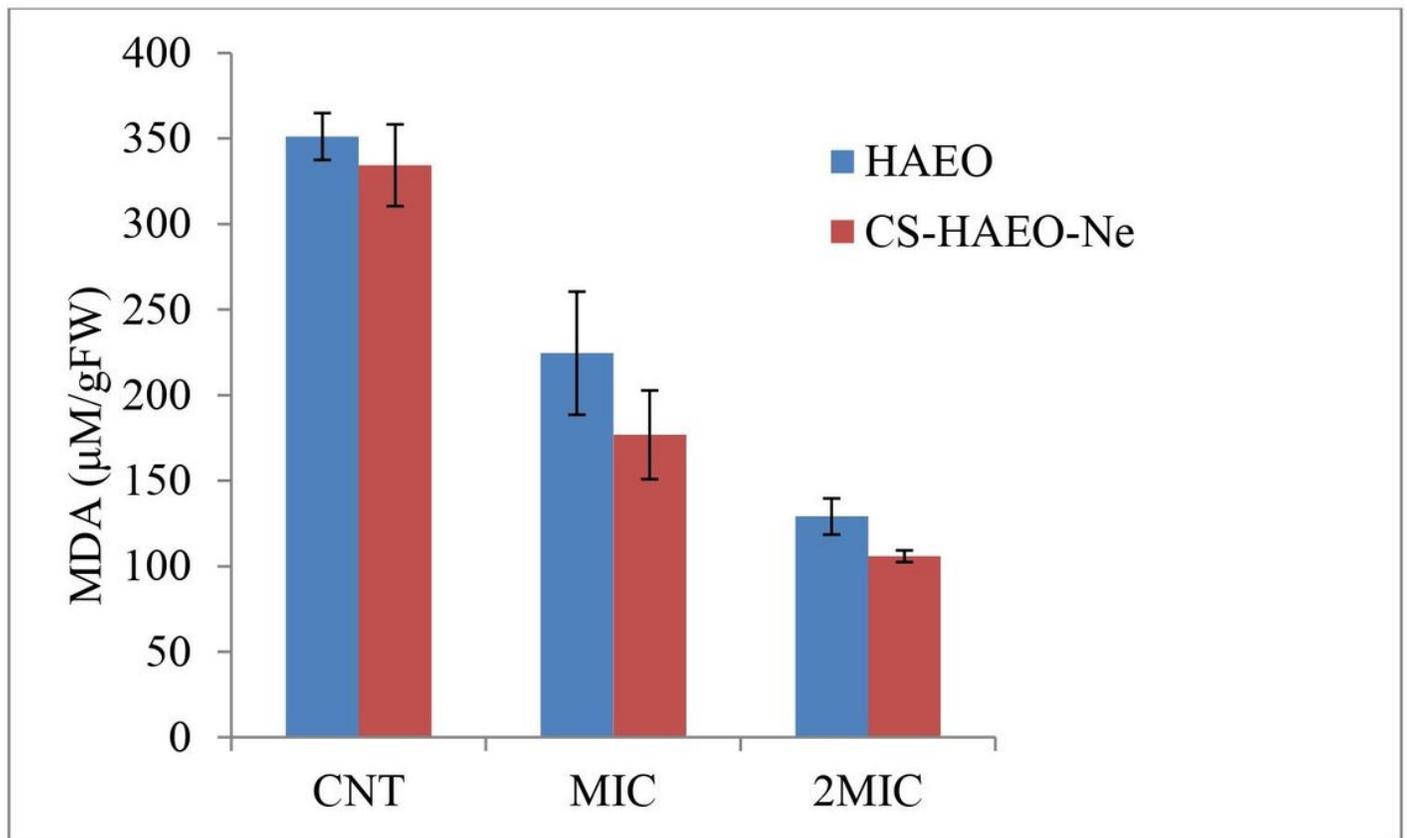


Figure 10

Effect of HAEO and CS-HAEO-Ne fumigation on lipid peroxidation of stored black cumin (*Nigella sativa*) seeds

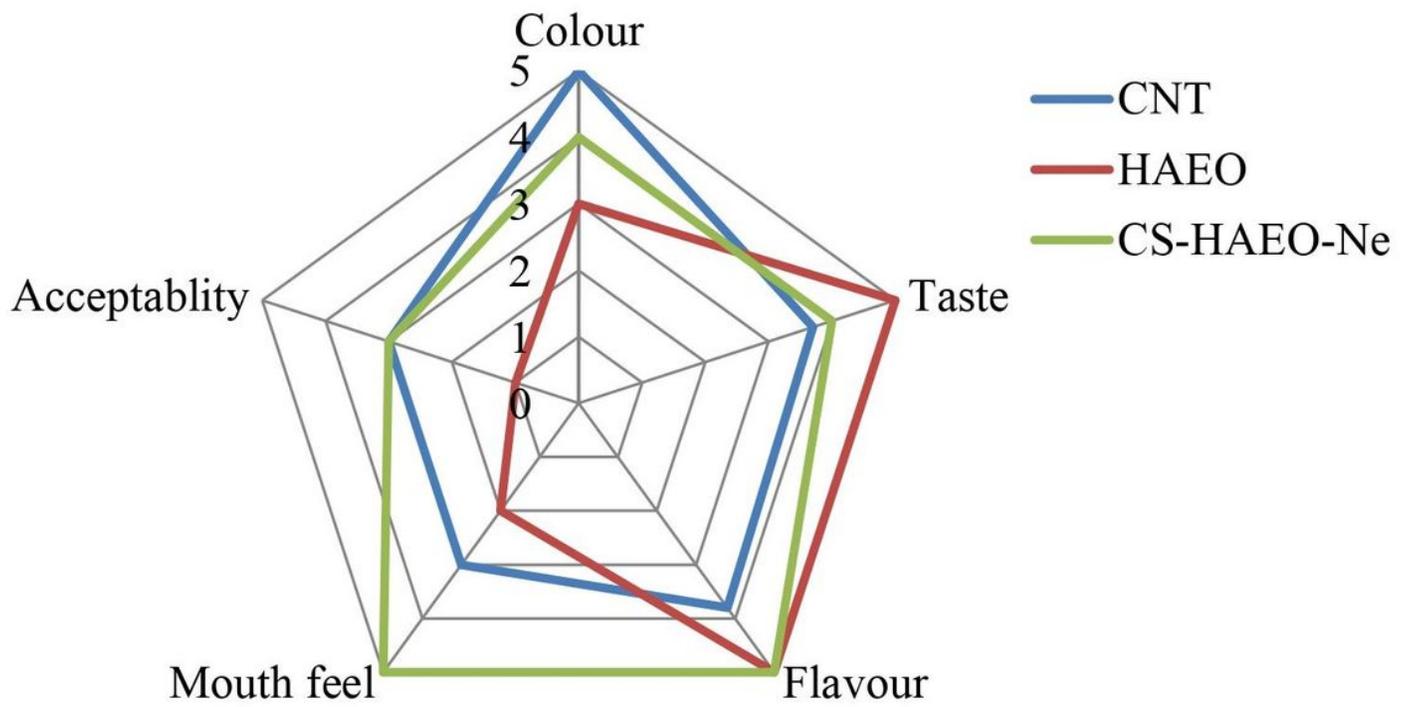


Figure 11

Sensorial profile of HAEO and CS-HAEO-Ne fumigated black cumin (*Nigella sativa*) seed