Unleashing the aromatic arsenal: Anti-coronavirus activity of volatile Nigella sativa compounds

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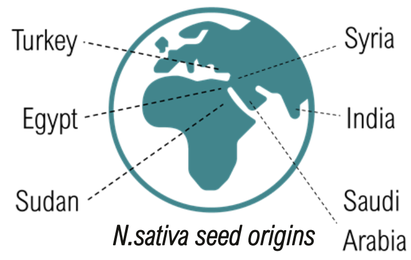
BACKGROUND

In the last 20 years, there have been three instances of coronaviruses jumping from animals to humans, resulting in pandemics[1]. With many more coronaviruses circulating in bats, future emergence into humans is likely. To target future virus emergence and treat infected individuals, the development of novel broad-spectrum antivirals is vital[2].

Nigella sativa (black cumin) is a medicinal plant that contains numerous bioactive compounds, although compositions vary with the source of the plants. *N. sativa* extracts were shown to have antiviral activity against RNA viruses,[3] and thymoquinone (TQ), a major bioactive constituent of *N. sativa* oil, suggested to disrupt binding of SARS-CoV-2 spike protein to its receptor ACE2 in *in silico* studies.[4,5]

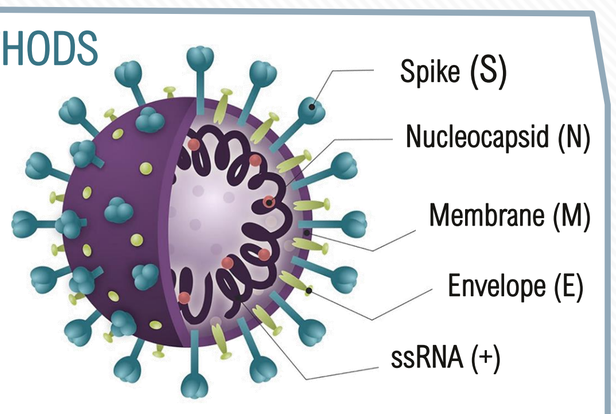


N.sativa seed origins

Here we investigated the antiviral efficacy of the volatile component of *N. sativa* oils from seeds collected globally or commercially-sourced.

METHODS

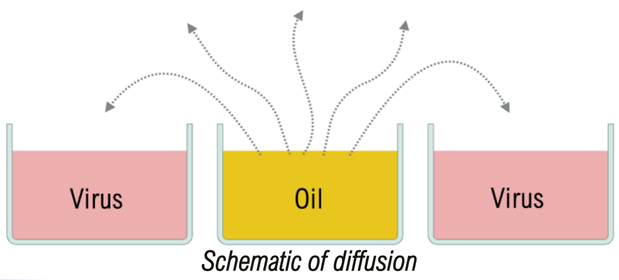
A panel of *N. sativa* oils were screened for antiviral activity against model seasonal human coronaviruses (OC43 and 229E). Pseudoviruses expressing SARS-CoV-1 and SARs-CoV-2 spike proteins were used to assess the impact of oils on viral entry.



Key features of a coronavirus virion

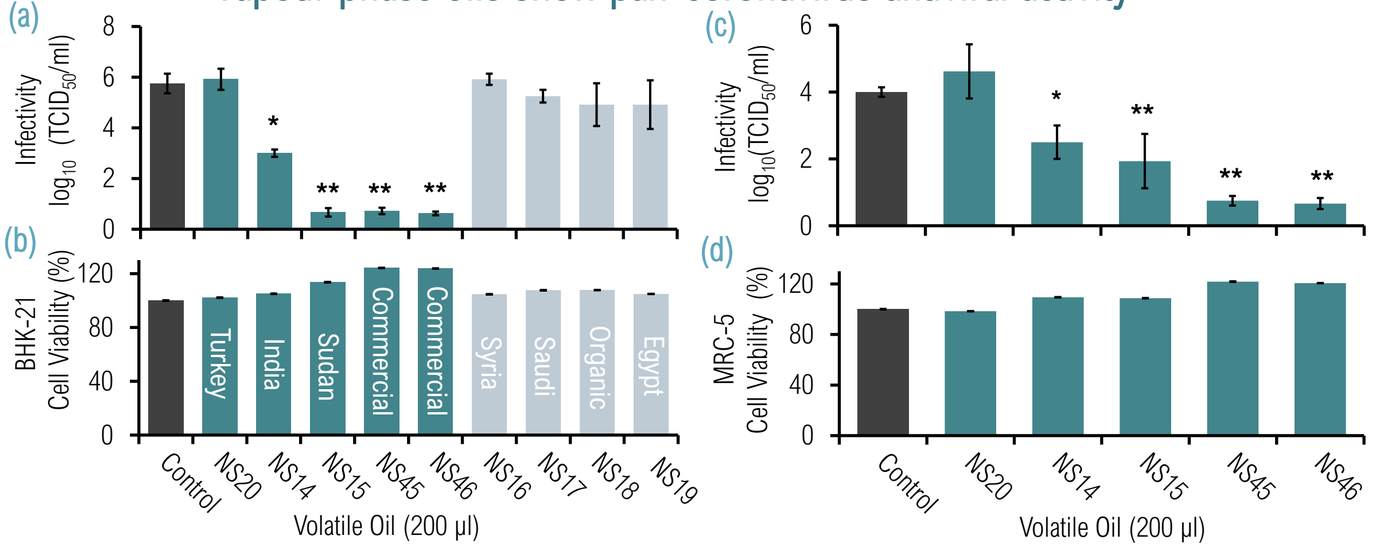
Oil and virus was incubated in adjacent wells of a 96-well plate at 33°C, after which virus solutions were titred.

To assess cytotoxicity, cells were seeded in wells with oils in adjacent wells, and viability measured by an MTS assay.



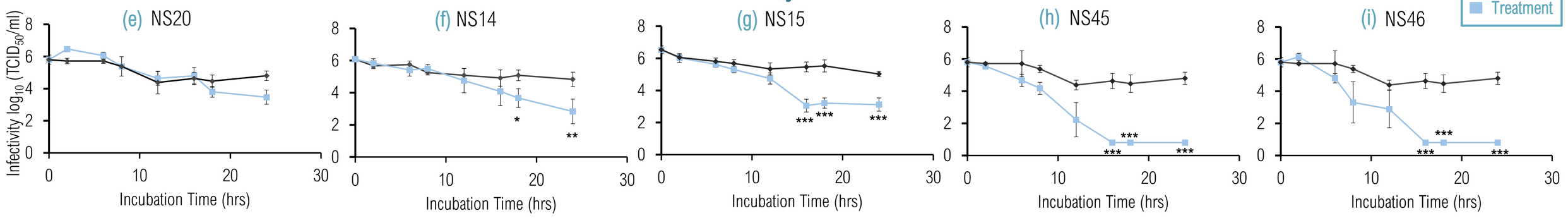
Schematic of diffusion

Vapour phase oils show pan-coronavirus antiviral activity



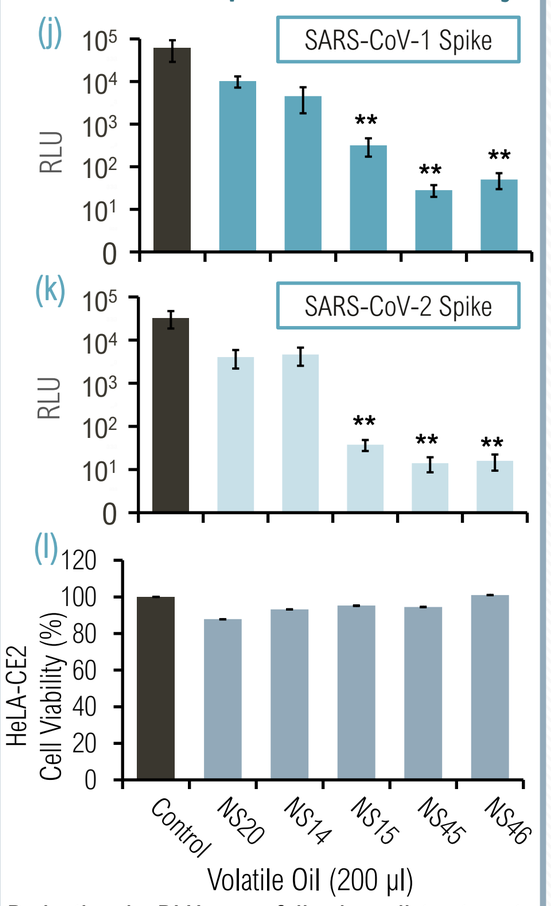
Four *N. sativa* oils showed significant reduction in coronavirus infectivity. A panel of *N. sativa* oils were incubated with OC43 (a) or 229E (b) to test the antiviral activity of the volatile component of the oils. After 24 h, virus titres were quantified on BHK-21 (c) or MRC-5 (d) cells. NS14, NS15, and the commercially-sourced NS45 and NS46 showed the greatest reduction in infectivity compared to the no treatment control. None of the volatiles were cytotoxic to BHK-21 or MRC-5 cells. Mean ± SEM; ** $p \leq 0.01$; * $p \leq 0.05$; LOD 0.5 $\log_{10}$ $TCID_{50}$/mL

Kinetics of antiviral activity varies between oil sources



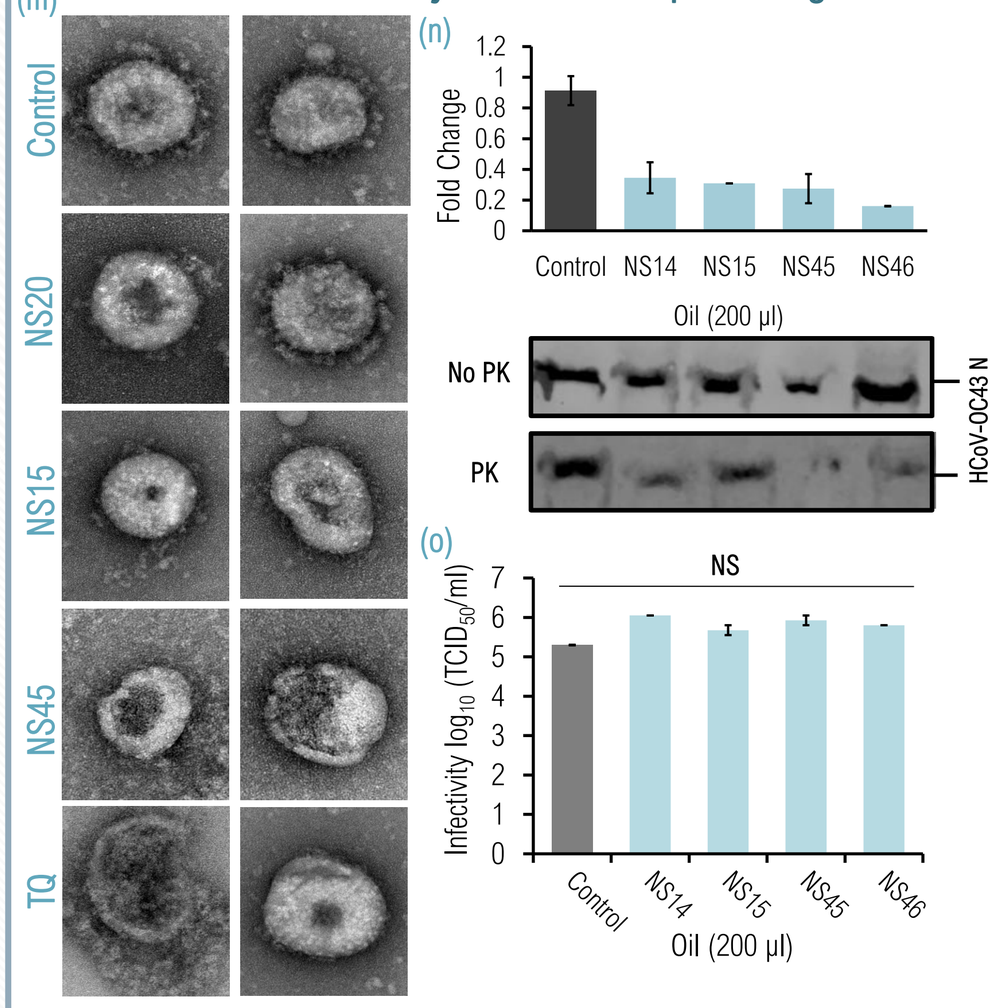
Commercially sourced oils show the greatest antiviral activity against HCoV-OC43 over 24 hours. The four antiviral oils (f-i) were incubated with 8 $\log_{10}$ $TCID_{50}$/mL OC43 for 24 hrs and virus infectivity was assessed at various timepoints during this treatment. NS45 and NS46 show a reduction in infectivity as early as 6 hrs while the antiviral efficacy of NS14 and NS15 occurs later at 16-18 hrs incubation. NS20 (e), used as a negative control did not show any antiviral activity over 24 hrs incubation. Mean ± SEM; *** $p \leq 0.001$;** $p \leq 0.01$; * $p \leq 0.05$; LOD 0.5 $\log_{10}$ $TCID_{50}$/mL

Antiviral oils prevent viral entry



Reduction in RLU seen following oil treatment of pseudoviruses. SARS-CoV-1 and SARS-CoV-2 spike expressing psuedotyped lentiviruses were employed to assess the impact of the oils on spike mediated entry. Pseudoviruses contain luciferase reporter to indicate entry into cells, measured as relative light units (RLU). The oils significantly reduce infection of cells by both SARS-1 (j) and SARS-2 (k) spike pseudoviruses. Reduction in RLU is due to pseudovirus inhibition not due to oils causing toxicity to cells (l). Mean ± SEM; ** $p \leq 0.01$; * $p \leq 0.05$.

Antiviral oils may cause envelope damage



Viral structure may be affected by the volatile oils causing reduced infectivity. TEM images of OC43 virions show structural changes to the virion following treatment with volatile oils or TQ compared to the control (m). To assess envelope integrity nucleocapsid protection assays were performed where virus was incubated alongside oils and then subjected to proteinase-K (PK) treatment. Envelope damage allows PK can enter the virion and degrade the nucleoprotGemma Coein, however an intact envelope reduces if the PK degradation. Treated oils show a reduction in levels of nucleoprotein indicating envelope damage (n). Murine Norovirus (MNV-1), a non-enveloped virus, was treated with bioactive oils and no reduction in infection was seen (o). Activity is potentially reduced due to mechanism of action of oils on the envelope of coronavirus . Mean ± SEM

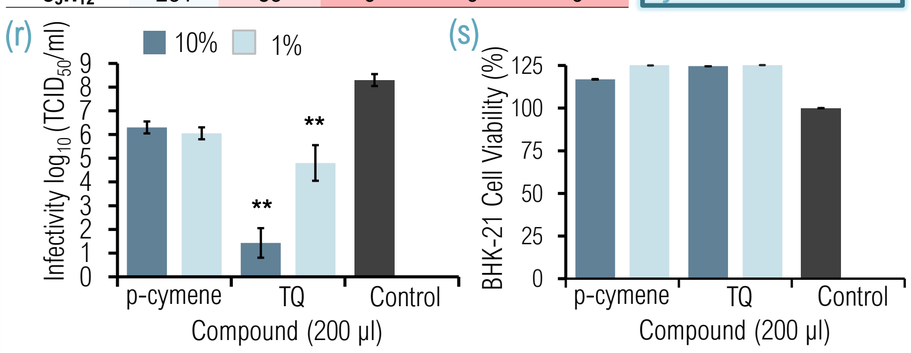
TQ and p-cymene reduce viral infectivity

(p)

Concentration (PPM)	NS20	NS14	NS15	NS45	NS46
TQ	0	0	100	1478	4
p-Cymene	208	476	2679	2269	627
$C_{10}H_{16}$	65	114	289	158	107
$C_{11}H_{20}O$	0	0	317	497	44
$C_{15}H_{24}$	0	0	49	76	15
$C_{11}H_{20}O$	0	0	89	101	17
C_5H_{12}	251	35	0	0	0

(q)

Concentration (PPM)	1%	10%
TQ	1168	15684
p-cymene	6597	34060



Individual bioactive components in the volatile fraction of oils were determined using GC-MS. Composition of the oils vary based on source location (p). Concentrations of diffused compounds during 24 hr treatment were determined using GC-MS (q). Virus treated with diffused compounds at varying concentrations show TQ has significant antiviral activity at low concentrations (r) which are non-toxic to cells (s).
Mean ± SEM; ** $p \leq 0.01$; * $p \leq 0.05$; LOD 0.5 $\log_{10}$ $TCID_{50}$/mL

CONCLUSIONS AND FUTURE

- Volatiles from *N. sativa* oils show antiviral activity against OC43 and 229E.
- TEM, pseudoviruses, nucleocapsid protection assays and non-enveloped norovirus suggest that *N.sativa* oils impact the viral envelope. Further experiments are being carried out to investigate this.
- We identified TQ amongst other bioactive compounds in the volatile fraction of the oils, with a broadly concentration-dependent correlation with their antiviral efficacies.
- Bioactive compounds identified by GC-MS are being tested for their antiviral activities, individually and in combination to determine any synergistic effects.

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