Are the fatty acids responsible for the higher effect of oil and alcoholic extract of *Nigella sativa* over its aqueous extract on *Trichomonas vaginalis* trophozoites?

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Abstract Trichomoniasis, the disease caused by the flagellate protozoan *Trichomonas vaginalis* is the sexually transmitted infection with the largest annual incidence. Metronidazole is the drug of choice recommended for the treatment of human trichomoniasis but it can lead to drug resistance and many other adverse effects. So, it is necessary for new alternatives for the treatment of this infection. Medicinal plants or herbs could be good alternative regimens to be inexpensive, effective and safe to use. In the present study, the therapeutic potential of *Nigella sativa* aqueous and alcoholic extracts as well as seeds oil was examined. Different concentrations of these plant preparations were incubated in vitro with cultivated *T. vaginalis* trophozoites and its effect on growth was compared with metronidazole under the same conditions. Both the alcoholic extract and oil proved to be valuable agents as efficient as metronidazole in treating *T. vaginalis* infection. The remarkable effect of *N. sativa* oil may be attributed to the fact that the active principles extracted from *N. sativa* seeds are mostly from its essential oil (omega 3, 6, 9 as well as 7 fatty acids). However, further experimental and

clinical investigations are needed to evaluate and standardize the doses of these natural products to be safe and efficient.

Keywords *Trichomonas vaginalis* · Metronidazole · *Nigella sativa* oil · *Nigella sativa* aqueous and alcoholic extracts · Fatty acids

Introduction

Trichomoniasis, the disease caused by the flagellate protozoan *Trichomonas vaginalis* is the sexually transmitted infection with the largest annual incidence, exceeding 276 million new cases annually (WHO 2008).

Trichomoniasis was accounted to about half of all the curable sexually transmitted diseases worldwide (Hook 1999; Cates 1999). In USA, annual incidence of *T. vaginalis* reached 5 million. The general annual adult infection was 180–200 million and being higher than that of gonorrhea, syphilis, and chlamydia infections all together (Schwebke and Burgess 2004). In many Arab countries, trichomoniasis was reported including Jordan (Morsy and EL-Dasouki 1979), Iraq (Mahdi et al. 2001), Egypt (Negm and El-Haleem 2004), Saudi Arabia (Alzanbagi et al. 2005), Libya (Kassem and Majoud 2006) and Tunisia (Zribi et al. 2008).

T. vaginalis colonizes the female and male urogenital tract, and symptoms can vary widely from asymptomatic infections to vaginitis, urethritis, prostatitis (Gardner et al. 1986), low birth weight, preterm delivery, premature rupture of membranes and infertility (Cotch et al. 1997).

Trichomoniasis is of worldwide importance especially because in recent years, it has been implicated in amplifying human immunodeficiency virus transmission. In

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addition, *T. vaginalis* acts as a potential catalyst in the acquisition of secondary infections including human papilloma virus, the organism responsible for pathogenesis of cervical cancer (Rughooputh and Greenwell 2005).

Metronidazole and tinidazole are two drugs of choice recommended for the treatment of human trichomoniasis (Fernando et al. 2007). Metronidazole can lead to drug resistance and potential risks of mutagenesis and carcinogenicity (WHO 2001). In addition, its side effects such as headache, dry mouth, glossitis, and urticaria caused by lenity treatment or high doses have been described (Klebanoff et al. 2001). Tinidazole (Fasigyn), a second metronidazole generation has shown to be an effective therapy in metronidazole-resistant *T. vaginalis*, with several advantages over metronidazole including greater in vitro potency against both sensitive and resistant strains of *T. vaginalis*, a more prolonged duration and improved patient tolerability (Wendel and Workowski 2007). Cross-resistance among metronidazole doses occurred, and thus metronidazole resistant strain was treated with tinidazole but rapid development of tinidazole resistant *T. vaginalis* due to the similarities of metabolic pathway of both has occurred (Lewis et al. 1997). More serious side effects are rare but include eosinophilia, leukopenia, palpitation, confusion, and some central nervous system effects (Harris et al. 2000; Gardner and Hill 2001; Swygard et al. 2004; Upcroft et al. 2006).

Nigella sativa L. (family Ranunculaceae), commonly known as black seed or black cumin, is an annual plant growing in Mediterranean countries and it is one of the native plants that are widely distributed in Egypt. It has been traditionally used in the Indian subcontinent, Arabian countries and Europe for culinary and medicinal purposes as a natural remedy for a number of illnesses and conditions that include asthma, hypertension, diabetes, inflammation, cough, bronchitis, headache, eczema, fever, dizziness and influenza (Harzallah et al. 2012). Recently, many biological activities of *N. sativa* L. seeds have been reported, including: antioxidant, anti-inflammatory, anti-cancer, antimicrobial, antifungal and antiparasitic activities (Abu El Ezz 2005; Haloci et al. 2012). Aqueous and alcoholic extracts, as well as essential oil of *N. sativa* were proved to have many therapeutic effects. In this respect, *N. sativa* alcoholic extract was found to be as effective as metronidazole in the cure of giardiasis (Bishara and Masoud 1992). In addition, aqueous extract has demonstrated inhibitory effect against candidiasis (Khan et al. 2003) and a potential therapeutic effect against *Blastocystis hominis* (El Wakil 2007) and *T. vaginalis* (Tonkal 2009).

New antiprotozoal drugs with high effectiveness, low toxicity and free from side effects are urgently required. Medicinal plants used in the treatment of these diseases can be an alternative resource of novel antiprotozoal drugs

(Freitas et al. 2006). Considering the need for new alternatives for trichomoniasis treatment, the therapeutic potential of *Nigella sativa* aqueous and alcoholic extracts as well as seeds oil were examined.

Materials and methods

Parasites and culture

Trichomonas vaginalis was isolated from vaginal washouts of female patients attending the outpatient clinic, Gynecology and Obstetrics Hospital, Ain Shams University. One drop of vaginal washout sediment was examined microscopically for motile *T. vaginalis* trophozoites (Cheesbrough 1998). Few drops of sediment containing the trophozoites were inoculated into TYM (pH 6.0) at 37 °C, supplemented with 10 % heat inactivated horse serum (in a water bath at 56 °C for 30 min.), penicillin G sodium (1,000,000 IU/ml) and streptomycin sulfate (100,000 µg/ml) (Diamond 1957). Isolates were sub-cultured every 48 h in TYM medium and maintained in Parasitology Diagnostic and Research Unit, Faculty of Medicine, Ain Shams University. Cultured trophozoites were counted (Borchardt et al. 1997) in Neubauer cell-counter chamber to adjust the inoculating dose of *T. vaginalis* trophozoites, and to evaluate the effect of *N. sativa* (seed oil, aqueous and alcoholic extracts) and MTZ on their multiplication and motility. The starting concentration of the parasite in culture was adjusted in all tubes to be 2×10^5 trophozoites/ml culture.

Extracts of *N. sativa*

Plant materials and oil

N. sativa L. seeds and the oil were purchased from a local herb store. It was coded as *N. sativa* oil (NsO). Oil was dissolved in dimethylsulfoxide (DMSO) and then diluted in incubation medium to yield 250 µg/ml, 500 µg/ml, 1 mg/ml and 2 mg/ml of oil.

Crude (alcoholic) extracts

N. sativa seeds were washed to remove any debris and air dried. Amount of 250 g seeds were ground to powder and soaked in 85 % aqueous-methanol (1/10 w/v) for 24 h. The extract is filtered through a Buchner funnel. The plant residue is re-extracted with 50 % methanol for additional 2 h. After filtration of the slurry, the two extracts are combined and concentrated under reduced pressure on a rotatory evaporator below 40 °C until most of the methanol has been removed (Houcher et al. 2007). The brownish black crude extract, yielding about 27 %, was coded as *N.*

Sativa crude extract (NsCr). The present study evaluated four doses of NsCr: 500 µg/ml, 1 mg/ml, 5 mg/ml and 10 mg/ml.

Aqueous extract

N. sativa seeds were washed, and then 250 g of seeds was boiled in distilled water (1,000 ml) for 90 min and filtered through muslin. The filtrated water extract was evaporated under reduced pressure and lyophilized to give an aqueous extract (El Wakil 2007). The aqueous extract was dissolved in distilled water. The extract was sterilized by filtration using Acrodisc (Gelman, 0.22 µm size) and then preserved in the deep freezer (−20 °C) till it was used. Aqueous extract was coded as *N. Sativa* aqueous extract (NsAe). The present study evaluated four doses of NsAe: 500 µg/ml, 1 mg/ml, 5 mg/ml and 10 mg/ml (Al-Heali and Rahemo 2006).

Determination of fatty acids composition in *N. Sativa* crude extract (alcoholic) and oil

Methylation of fatty acids for gas–liquid chromatography analysis was performed in the manner adopted by Sink et al. (1964). Then, 1 µl of fatty acid methyl ester was injected into a 6 feet × 1/8 inch internal diameter column packed with 20 % diethylene glycol succinate (DEGS) on chromosorb 60–80 mesh by using Hewlett-Packard (model: HP-GC-MS) according to the following conditions; GLC Model Shimadzu-8 A (PFE), F.I.D. detector, Column 5 % DEGS on 80/100 Chromo Q. Chart speed: 2.5 mm/min., H₂ flow rate: 75 ml/min., N₂ flow rate: 20 ml/min., Air flow rate: 0.5 ml/min. and Sensitivity: 32 × 10². The fatty acid composition of the samples was expressed as a percentage of the total fatty acids resolved.

Metronidazole (MTZ)

It was supplied as 250 mg tablets (Sanofi Aventis, Egypt). Tablets were dissolved in distilled water, and then diluted in incubation medium to yield 12, 25 and 50 µg/ml.

Growth inhibition assays

Concentrations of NsAe, NsCr and NsO were selected after several trials aiming to reach that which would produce total inhibition of growth after 24 h. The dose was then decreased to reach 500 µg/ml, 1 mg/ml, 5 mg/ml and 10 mg/ml of NsAe and NsCr; and 250 µg/ml, 500 µg/ml, 1 mg/ml and 2 mg/ml of NsO. The growth was observed after 24, 48, 72, 96 h. at 37 °C. In addition, growth was observed in tubes containing metronidazole diluted in incubation medium forming 3 concentrations 12, 25 and

50 µg/ml. All drugs were tested in duplicates. Cultures treated with MTZ, and non-treated control cultures of the parasites (containing the parasite only), were submitted to the same procedure used for the extracts cultures. Four tubes were used for every concentration of each extract, MTZ, and the non-treated control cultures.

Evaluation of efficacy of different extracts and MTZ on cultured trophozoites

1. Daily examination of the culture tubes using inverted microscope to assess the multiplication and motility of the trophozoites.
2. Counting the number of trophozoites using the haemocytometer (Neubauer cell-counter chamber).
3. Calculation of the percent of inhibition of multiplication according to the equation:

$$\text{Percent inhibition of growth} = \frac{a - b}{a} \times 100$$

where;

a = Mean number of trophozoites in control tubes.

b = Mean number of trophozoites in test tubes (Palmas et al. 1984).

4. Calculation of the minimal lethal concentration (MLC) of NsAe, NsCr, NsO and metronidazole as: the lowest concentration of the tested extracts and MTZ, at which no organism was observed i.e. complete growth inhibition (Meingasser and Thurner 1979).

Statistical analysis

Data management and analysis were made using SPSS version 15.0 for windows. Numbers of trophozoites in non-treated control cultures and treated cultures after different time intervals were calculated as mean ± SD and percent of growth inhibition. The mean numbers were compared at the same time interval using Student's *t* test. *P* values <0.001 were considered as statistically highly significant.

Ethical consideration

An informed consent was taken from all patients before taking vaginal samples.

Results

The present study was carried out to investigate the in vitro activity of NsO, NsCr and NsAe on the growth of *T. vaginalis*, compared to the standard drug metronidazole.

Table 1 Mean count $\pm$ SD and percentage of growth inhibition of *Trichomonas vaginalis* per culture after exposure to various concentrations of NsO (*Nigella Sativa* oil) in comparison to NTC (non-treated control) and DMSO control

Dosage of treatment	After 24 h			After 48 h			After 72 h			After 96 h		
	Mean $\pm$ SD	(%)	Sig.	Mean $\pm$ SD	(%)	Sig.	Mean $\pm$ SD	(%)	Sig.	Mean $\pm$ SD	(%)	Sig.
NTC	1.3 $\pm$ 0.13	0.00		2.35 $\pm$ 0.08	0.00		4.67 $\pm$ 0.85	0.00		2.20 $\pm$ 0.13	0.00	
DMSO	1.2 $\pm$ 0.15	6.84	0.591	2.3 $\pm$ 0.15	2.13	0.725	4.04 $\pm$ 0.16	13.60	0.478	2.13 $\pm$ 0.18	3.41	0.679
NsO	0.3 $\pm$ 0.16	77.19	0.021	0.6 $\pm$ 0.18	73.56	0.024	0.83 $\pm$ 0.18	82.33	0.087	0.00 $\pm$ 0.00	100.00 ^a	0.002
250 μ g/ml												
500 μ g/ml	0.1 $\pm$ 0.11	90.87	0.011	0.2 $\pm$ 0.18	89.13	0.004	0.00 $\pm$ 0.00	100.00 ^a	0.016	0.00 $\pm$ 0.00	100.00	0.002
1 mg/ml	0.0 $\pm$ 0.04	97.34	0.035	0.0 $\pm$ 0.00	100.00 ^a	0.001	0.00 $\pm$ 0.00	100.00	0.016	0.00 $\pm$ 0.00	100.00	0.002
2 mg/ml	0.0 $\pm$ 0.00	100.00 ^a	0.005	0.0 $\pm$ 0.00	100.00	0.001	0.00 $\pm$ 0.00	100.00	0.016	0.00 $\pm$ 0.00	100.00	0.002

^a MLC (minimal lethal concentration) of NsOe = 250 μ g/ml at 96 h and 500 μ g/ml at 72 h and 1 mg/ml at 48 h and 2 mg/ml at 24 h

Table 2 Mean count $\pm$ SD and percentage of growth inhibition of *Trichomonas vaginalis* per culture after exposure to various concentrations of NsCr (*Nigella sativa* crude extract) in comparison to NTC (non-treated control)

Dosage of treatment	After 24 h			After 48 h			After 72 h			After 96 h		
	Mean $\pm$ SD	(%)	Sig.	Mean $\pm$ SD	(%)	Sig.	Mean $\pm$ SD	(%)	Sig.	Mean $\pm$ SD	(%)	Sig.
NTC	1.32 $\pm$ 0.13	0.00		2.35 $\pm$ 0.08	0.00		4.67 $\pm$ 0.85	0.00		2.20 $\pm$ 0.13	0.00	
NsCr 500 μ g/ml	0.59 $\pm$ 0.13	55.13	0.031	1.05 $\pm$ 0.17	55.22	0.029	1.65 $\pm$ 0.18	64.67	0.112	0.62 $\pm$ 0.16	71.82	0.009
1 mg/ml	0.34 $\pm$ 0.17	74.14	0.027	0.66 $\pm$ 0.18	71.86	0.025	0.84 $\pm$ 0.17	82.01	0.089	0.00 $\pm$ 0.00	100.00 ^a	0.002
5 mg/ml	0.06 $\pm$ 0.07	95.44	0.007	0.35 $\pm$ 0.18	85.07	0.005	0.00 $\pm$ 0.00	100.00 ^a	0.016	0.00 $\pm$ 0.00	100.00	0.002
10 mg/ml	0.00 $\pm$ 0.00	100.00 ^a	0.005	0.00 $\pm$ 0.00	100.00	0.001	0.00 $\pm$ 0.00	100.00	0.016	0.00 $\pm$ 0.00	100.00	0.002

^a MLC (minimal lethal concentration) of NsCr = 1 mg/ml at 96 h and 5 mg/ml at 72 h and 10 mg/ml at 24 h

Table 3 Mean count $\pm$ SD and percentage of growth inhibition of *Trichomonas vaginalis* per culture after exposure to various concentrations of NsAe (*Nigella sativa* aqueous extract) in comparison to NTC (non-treated control)

Dosage of treatment	After 24 h			After 48 h			After 72 h			After 96 h		
	Mean $\pm$ SD	(%)	Sig.	Mean $\pm$ SD	(%)	Sig.	Mean $\pm$ SD	(%)	Sig.	Mean $\pm$ SD	(%)	Sig.
NTC	1.32 $\pm$ 0.13	0.00		2.35 $\pm$ 0.08	0.00		4.67 $\pm$ 0.85	0.00		2.20 $\pm$ 0.13	0.00	
NsAe 500 μ g/ml	1.20 $\pm$ 0.07	8.75	0.396	1.73 $\pm$ 0.18	26.44	0.045	2.11 $\pm$ 0.16	54.82	0.137	1.26 $\pm$ 0.17	42.73	0.029
1 mg/ml	0.84 $\pm$ 0.17	36.12	0.096	1.34 $\pm$ 0.14	42.86	0.013	1.44 $\pm$ 0.18	69.27	0.105	1.13 $\pm$ 0.18	48.86	0.025
5 mg/ml	0.25 $\pm$ 0.14	80.99	0.016	0.41 $\pm$ 0.16	82.52	0.004	0.83 $\pm$ 0.16	82.33	0.089	0.37 $\pm$ 0.13	83.18	0.005
10 mg/ml	0.38 $\pm$ 0.16	71.1	0.025	0.00 $\pm$ 0.00	100.00 ^a	0.001	0.00 $\pm$ 0.00	100.00	0.016	0.00 $\pm$ 0.00	100.00	0.002

^a MLC (minimal lethal concentration) of NsAe = 10 mg/ml at 48 h

Results are presented in Tables (1, 2, 3, 4 and 5) and Figs. (1, 2, 3 and 4).

Table (1) and Fig. (1) show that minimal lethal concentration of NsO, which caused 100 % inhibition of growth of the trophozoite, at 24 h was 2 mg/ml. Lower concentration of 1 mg/ml also showed a very high anti-*T. vaginalis* activity, reducing the parasite growth by 97 % after 24 h and 100 % later on. The lower concentrations of NsO 500 μ g/ml and 250 μ g/ml reduced the parasite growth by 73.56–100 % but they are less effective than the lower concentration of

MTZ (Table 4 and Fig. 4) 12.5 and 25 μ g/ml which reached a complete growth inhibition.

DMSO-treated cultures show only 2.13–13.6 % inhibition (Table 1 and Fig. 1), which indicates that the inhibitory effect of NsOe is attributed to the extract itself not to the DMSO solvent.

NsCr produced a lethal effect after 24 h with a dose of 10 mg/ml. Lower concentrations of 5 mg/ml showed complete inhibition only after 72 h incubation (Table 2 and Fig. 2) an effect which is less than that of NsO (1 mg/ml)

Table 4 Mean count $\pm$ SD and percentage of growth inhibition of *Trichomonas vaginalis* per culture after exposure to various concentrations of MTZ (metronidazole) in comparison to NTC (non-treated control)

Dosage of treatment ($\mu\text{g/ml}$)	After 24 h			After 48 h			After 72 h			After 96 h		
	Mean $\pm$ SD	(%)	Sig.	Mean $\pm$ SD	(%)	Sig.	Mean $\pm$ SD	(%)	Sig.	Mean $\pm$ SD	(%)	Sig.
NTC	1.32 $\pm$ 0.13	0.00		2.35 $\pm$ 0.08	0.00		4.67 $\pm$ 0.85	0.00		2.20 $\pm$ 0.13	0.00	
MTZ 12.5	0.56 $\pm$ 0.11	57.41	0.028	0.30 $\pm$ 0.07	87.21	0.001	0.00 $\pm$ 0.00	100.00 ^a	0.016	0.00 $\pm$ 0.00	100.00	0.002
25	0.30 $\pm$ 0.07	77.19	0.011	0.16 $\pm$ 0.10	93.18	0.002	0.00 $\pm$ 0.00	100.00	0.016	0.00 $\pm$ 0.00	100.00	0.002
50	0.00 $\pm$ 0.00	100.00 ^a	0.005	0.00 $\pm$ 0.00	100.00	0.001	0.00 $\pm$ 0.00	100.00	0.016	0.00 $\pm$ 0.00	100.00	0.002
100	0.00 $\pm$ 0.00	100.00	0.005	0.00 $\pm$ 0.00	100.00	0.001	0.00 $\pm$ 0.00	100.00	0.016	0.00 $\pm$ 0.00	100.00	0.002

^a MLC (minimal lethal concentration) of MTZ = 12.5 $\mu\text{g/ml}$ at 72 h and 50 $\mu\text{g/ml}$ at 24 h

Table 5 Fatty acid composition (mol %) of oil and crude (alcoholic) extract of *Nigella sativa* seeds. Data presented as mean $\pm$ SD

Fatty acid	<i>N. sativa</i> oil	<i>N. sativa</i> crude (alcoholic) extract
Lauric (C12:0)	0.509 $\pm$ 0.034	0.6099 $\pm$ 0.042
Tridecanoic (C13:0)	0.233 $\pm$ 0.015	–
Myristic (C14:0)	0.2058 $\pm$ 0.026	2.5018 $\pm$ 0.10
Palmitic (C16:0)	6.025 $\pm$ 0.093	5.2712 $\pm$ 0.027
Palmitoleic (Omega 7) (C16:1)	1.41 $\pm$ 0.052	–
Stearic (C18:0)	2.39 $\pm$ 0.064	2.3063 $\pm$ 0.083
Oleic (Omega 9) (C18:1)	25.76 $\pm$ 0.41	21.67 $\pm$ 0.53
Linoleic (Omega 6) (C18:2)	49.44 $\pm$ 0.58	41.184 $\pm$ 0.49
Linolenic (Omega 3) (C18:3)	1.6727 $\pm$ 0.026	9.653 $\pm$ 0.096
Arachidic (C20:0)	1.505 $\pm$ 0.014	1.434 $\pm$ 0.031
Eicosadienoic (Omega 6) (C20:2)	1.5132 $\pm$ 0.035	2.7034 $\pm$ 0.016
Eicosatrienoic (Omega 3) (C20:3)	2.410 $\pm$ 0.027	3.1692 $\pm$ 0.025
Behenic (C22:0)	1.4067 $\pm$ 0.063	1.529 $\pm$ 0.051
Total known	94.49 $\pm$ 1.43	92.032 $\pm$ 1.49
Unsaturated fatty acids	82.22 $\pm$ 1.12	78.38 $\pm$ 1.1
Saturated fatty acids	12.275 $\pm$ 0.309	13.65 $\pm$ 0.33
Ratio of unsaturated/saturated fatty acids	6.698	5.742

(Table 1) but similar to the lower dose of MTZ (25 $\mu\text{g/ml}$) (Table 4).

Concerning NsAe, it totally abolished the trophozoites growth starting at the concentration of 10 mg/ml after 48 h incubation (Table 3 and Fig. 3) but the lower concentrations failed to show complete inhibition.

Table (5) shows that oleic acid and linoleic acid were the major fatty acids detected in both *N. sativa* oil and alcoholic extract representing 75.2 and 62.85 % of the total fatty acid content in oil and alcoholic extract, respectively.

Discussion

Considering the impact of trichomoniasis in public health and the emergent number of resistant *T. vaginalis* isolates, it is necessary for new alternatives for the treatment of this infection. Medicinal plants or herbs could be good

alternative regimens to be inexpensive, effective and safe to use (El-Sherbiny and El Sherbiny 2011; Brandelli et al. 2013).

Among various medicinal plants, *Nigella sativa* (Family Ranunculaceae) is emerging as a miracle herb with a rich historical and religious background since many researches revealed its wide spectrum of pharmacological potential. The use of *N. sativa* against protozoal infections has been tested by many researchers. *N. sativa* alcoholic extract was found to be as effective as metronidazole in the cure of giardiasis (Bishara and Masoud 1992). Moreover, aqueous extract has demonstrated inhibitory effect against *Blas-tocystis hominis* (El Wakil 2007).

Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure, properties of a good solvent in plant extraction that induces ease of evaporation at low heat, promotion of rapid physiologic

Fig. 1 Mean count $\pm$ SD and percentage of growth inhibition of *Trichomonas vaginalis* per culture after exposure to various concentrations of NsO (*Nigella sativa* oil) in comparison to NTC (non-treated control) and DMSO control

Fig. 2 Mean count $\pm$ SD and percentage of growth inhibition of *Trichomonas vaginalis* per culture after exposure to various concentrations of NsCr (*Nigella sativa* crude extract) in comparison to NTC (non-treated control)

absorption of the extract, a preservative action and inability to cause the extract to complex or dissociate. The choice will also depend on targeted compounds. The most commonly used solvents for investigation of microbial activity in plants are methanol, ethanol, and water (Rojas et al. 2006). Consistent with this fact, this study used different extracts and detected variable results. Methanol (NsCr) and water (NsAe) extracts were used. Oil (NsO) was also used dissolved in dimethylsulfoxide (DMSO).

The present work was carried out to investigate the in vitro activity of three *N. sativa* extracts (aqueous, alcoholic and the oil) on the growth of *Trichomonas vaginalis*, compared to metronidazole which is so far the drug of choice for human

trichomoniasis. A literature survey revealed that previous studies have been dealing only with the effect of aqueous extract (Tonkal 2009), but not with the effect of either oil or alcoholic extract of *N. sativa* on *T. vaginalis*.

Dose-response and time course experiments showed that the degree of growth inhibition was dependent upon the concentration of the drug and incubation time.

The current results proved that *N. sativa* oil (2 mg/ml) and alcoholic extract (at a higher concentration of 10 mg/ml) were as efficient as the high concentration of metronidazole (50 µg/ml) with the added advantage of being natural products. All demonstrated the optimal anti-*T. vaginalis* activity inducing complete cytotoxicity (100 % of

Fig. 3 Mean count $\pm$ SD and percentage of growth inhibition of *Trichomonas vaginalis* per culture after exposure to various concentrations of NsAe (*Nigella sativa* aqueous extract) in comparison to NTC (non-treated control)

Fig. 4 Mean count $\pm$ SD and percentage of growth inhibition of *Trichomonas vaginalis* per culture after exposure to various concentrations of MTZ (metronidazole) in comparison to NTC (non-treated control)

growth inhibition) after 24 h incubation. However, the aqueous extract displayed the least effect which was similar to that detected by Tonkal (2009) against Saudi isolates.

This remarkable effect of NsO may be attributed to the fact that the active principles extracted from *N. sativa* seeds are mostly from its essential oil. Thirteen fatty acids were identified in *N. sativa* fixed oil, which represented 94.49 % of the total fatty acid and eleven fatty acids (represented 92.03 % of the total fatty acid) were identified in the alcoholic extract of *N. Sativa* (Table 5).

Oleic acid and linoleic acid were the major fatty acids detected in both *N. sativa* oil and alcoholic extract

representing 75.2 and 62.85 % of the total fatty acid content in oil and alcoholic extract, respectively. *N. sativa* oil were characterized by having markedly higher amounts of oleic acid and linoleic acid than those in the alcoholic extract which has higher amount of linolenic acid (9.65 %) compared to the oil (1.6727 %). Interestingly, tridecanoic acid and palmitoleic acid were only detected in *N. sativa* oil and were absent in the alcoholic extract. In addition, it was found that oil has a higher (6.7) total unsaturated/total saturated fatty acids value compared to the alcoholic extract (5.7).

The mechanism of action for this potentiality of alcoholic extract and oil of *N. sativa* against *T. vaginalis* has

not been studied before but we suggested that the cell membranes could be the main target. We proposed that fatty acids may interact with cell membranes to create transient or permanent pores of variable size leading to leakage, reduction of nutrient uptake or inhibition of cellular respiration. Another explanation could be that when *T. vaginalis* is exposed to high fatty acids concentrations (mainly oleic acid and linoleic acid) this could generate inhibition of these fatty acids in *T. vaginalis* and with that affect the lipidic metabolic processes in the organism.

The increased anti *Trichomonas* activity of *N. sativa* oil over that of the alcoholic extract could be attributed to the increased quantities of fatty acids per ml in the oil over those in the alcoholic extract. Moreover, the combination of omega 3, 6, 9 fatty acids in addition to omega 7 (palmitoleic acid, that was not detected in the alcoholic extract) in the oil could have a synergetic effect against *T. vaginalis* and hence explain the strong antiprotozoal effect of the oil. In addition the higher (6.7) total unsaturated/total saturated fatty acids value in oil compared to the alcoholic extract (5.7) is in favor for more antiprotozoal affect as previous reports have confirmed that the unsaturation of the fatty acids can affect the biological activity and can mediate killing of protozoa (Rohrer et al. 1986; Sun et al. 2003). The key membrane-located process affected by FFAs is the production of energy caused by interference with the electron transport chain and the disruption of oxidative phosphorylation (Boyaval et al. 1995; Wojtczak and Więckowski 1999).

Adhesion is thought to play an important role in the pathogenesis of trichomoniasis, and investigations of the molecular basis of adhesion of *T. vaginalis* to human cells have identified several adhesion molecules. The antitrichomonas effects of *N. sativa* could be due to its power as anti-adhesion agent as described before for its efficiency against the adhesion of *Staphylococcus* strains to human epithelial cells (Shaaban et al. 2011). In their study, among the plant extracts tested for their anti-adhesion potency the highest effect was recorded to the extract of *N. sativa*. Fatty acids could also contribute in this anti-adhesion character as it was proved that saturated and unsaturated FFAs can prevent initial bacterial adhesion (Won et al. 2007; Stenz et al. 2008; Davies and Marques 2009).

N. sativa has been extensively studied for its biological activities and therapeutic potential and shown to possess wide spectrum of activities as diuretic, antihypertensive, antidiabetic, anticancer and immunomodulatory, analgesic, antimicrobial, anthelmintic, antiprotozoal, analgesic and anti-inflammatory, spasmolytic, bronchodilator, gastroprotective, hepatoprotective, renal protective and antioxidant properties (Ahmad et al. 2013). Its seeds are widely used in the treatment of various diseases like bronchitis, asthma, diarrhea, rheumatism and skin disorders. It is also used as

liver tonic, digestive, anti-diarrheal, appetite stimulant, emmenagogue, to increase milk production in nursing mothers to fight parasitic infections, and to support immune system (Abel-Salam 2012; Assayed 2010; Abdel-Zaher et al. 2011; Boskabady et al. 2010). Black seeds also used in food like flavoring additive in the breads and pickles because it has very low level of toxicity (Ahmad et al. 2013).

An advantage of *N. sativa* oil and alcoholic extract as a promising treatment for *T. vaginalis* is that they do not have toxic effect. In this respect, the oral administration of aqueous extracts of the seeds of *N. sativa* for 14 days has been shown to cause no toxicity symptoms in male Sprague-Dawley rats (Tennekoon et al. 1991). The safety of consuming *N. sativa* seeds was also reported by Al-Homidan et al. (2002) whereby the seeds did not affect the growth of 7-day-old Hibro broiler chicks when fed to them at 20 and 100 g/kg of the diet for 7 weeks. Also, the administration of *N. sativa* seed extract (50 mg/kg) intraperitoneally to rats for 5 days did not significantly affect the activities of several enzymes and metabolites indicative of hepatic and renal function (El Daly 1998). Only acute administration of mega doses (2 g/kg or more), caused hypoactivity and difficulty in respiration (Badary et al. 1998).

In conclusion, the results in the present study support that the two safe *N. sativa* extracts (alcoholic extract and oil) proved to be valuable agents as efficient as MTZ in treating *T. vaginalis* infection, and will form the basis for further investigation in the potential discovery of new natural bioactive compounds. Further experimental and clinical investigations are needed to evaluate and standardize the doses of these natural products to be safe and efficient.

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