E3 Journal of Biotechnology and Pharmaceutical Research Vol. 1(1). pp. 009-013, October, 2010
Available online at http://www.e3journals.org/JBPR

Full Length Research Paper

Potential of *Nigella sativa* L. seed during different phases of germination on inhibition of bacterial growth

Aisha Kamal[1], Jamal Mohammad Arif[2] and Iffat Zareen Ahmad[1*]

[1]Department of Biotechnology, Integral University, Dasauli, Kursi Road, Lucknow-226026, Uttar Pradesh, India
[2]Department of Biochemistry, College of Applied Medical Sciences University of Hail, P.O. Box 2440, Hail, KSA.

Accepted 4 October 2010

Nigella sativa **has been used traditionally as spice, carminative, condiment, aromatic, stimulant, diuretic, stomachic, liver tonic and digestive. The rate at which the bacterial strains are becoming resistant to the available antibiotics has necessitated the demands for a renewed effort to investigate for new antimicrobial agents.** ***Nigella sativa*** **seed, in various germinating stages, was studied for antibacterial activity against various pathogenic bacteria resistant to a number of available antibiotics. The antibacterial activity of** ***Nigella sativa*** **in various germinating stages was studied on five pathogenic bacteria. The highest antibacterial activity was seen on 9d, 10d and 11d of germination while the seed at 0d showed no activity. The zone of inhibition ranges from 15 to 20mm. It can be concluded that there was moderate antibacterial activity. The results showed day-dependent activity and not the dose-dependent activity. During germination, there is always an increase in volume due to water uptake by the cells. This results into the initiation of numerous metabolic activities. During growth, the metabolic processes reach their optimum level. As a consequence there is an increase in biomass rapidly. These variations in the metabolic activity might lead to the alteration seen in the antibacterial activity.**

Keywords: *Nigella sativa*, germination phases, metabolites, antibacterial activity.

Introduction

Nigella sativa Linn. is commonly known as black seed which belongs to the botanical family of Ranunculaceae. *N. sativa* seeds have been used for nutritional and medicinal purposes in many Middle Eastern countries and other parts of the world (Al-Ghamdi, 2001; El-Dakhakhny et al, 2000). *N. sativa* is considered a natural food additive and a condiment. Also, it had been used for medicinal purposes as a natural remedy in many ancient cultures, as those of Egypt's Greece and Rome (Al_Haider et al., 1993). The *N. sativa* is the medicine for every disease except death (Ghosheh et al., 1999; Takruri, 2003). Seeds of *N. sativa* are frequently used in folk medicine in the Middle East and some Asian countries for acquiring good health and treating of many ailments including fever, common cold, headache, asthma, rheumatic diseases and various microbial infections and to expel worms from the intestine(Akhtar and Riffat, 1991; Al-Jassir, 1992, Al-Ghamdi, 2001).

The anti-microbial effects of *N. sativa* seeds against different pathogenic microbes were investigated. The diethyl ether extract was found to cause concentration dependent inhibition of *Staphylococcus aureus, Pseudomonas aeruginosa*, *Escherichia coli* and a pathogenic yeast *Candida albicans* (Hanafy and Hatem, 1991). The methanol and chloroform extracts have high inhibitory effects against *S. aureus*, *P. aeruginosa* and *C. albicans* (Mashhadian and Rakhshandeh, 2005). The essential oil of the seeds have also dose-dependent anti-bacterial effects on Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria (Hosseinzadeh, H. et al, 2007). The volatile oil of *N. sativa* seeds, prepared by steam distillation, was proved to be more effective against many strains of bacteria, including those known to be highly resistant to drugs (Salman, M.T. et al, 2008.)

The developing microbial resistance to the existing anti-microbial agents has become a serious problem.

Corresponding Author Email: iffat77@rediffmail.com, iffat77@gmail.com Tel No.: +919919273517, Fax: +91-522-2890809

Therefore, production of new potent agents is urgently needed, especially for hospitals and health centers, keeping in mind that studies on strains of pathogenic microbes are scarce. So, the present study was the first to investigate the antimicrobial effect of successive germinating phases of *N. sativa* crude aqueous extracts against five pathogenic bacterial strains.

Materials and Methods

Collection of *Nigella sativa*

Seeds of *N. sativa* were procured in the month of February, 2009 from a herbal shop in Lucknow, India.

Germination of seeds

Seed lots used for the different experiments showed germination capacities ranging from 80 to 98%. For germination studies, seeds were placed on four folds of damp filter paper at 25°C and incubated in the dark till the initiation of sprouting after which they were placed at a light intensity of 100 µmol m^{-2} $s^{-1}$and a 14/10 h (day/night) photoperiod till the complete plantlet with two leaves were obtained. Germination, defined as 1 mm radicle emergence, was followed for 11d; no contamination by microorganisms was observed during this time.

Extraction method

A modification of reflux extraction and wetting procedure by Mashhadian and Rakhshandeh (2005) was used. 500 mg of *N. sativa* seeds in 20 mL of water were incubated for 48 hours at 25°C with at least 5 times vibration per day. The viscous mixture was centrifuged for 10 min at 12000 rpm to collect the supernatant. The extracts were filtered using Whatman filter paper and evaporated using rotary distillation apparatus. The extracts were further dried in a 50°C oven for 4 h, dissolved in DMSO and finally kept at 4°C until further testing.

Microbial strains used for the study

The aqueous extracts of different germinating stages of *Nigella sativa* seeds were tested against five standard microorganisms which included Gram positive strain *Staphylococcus epidermidis* (NCIM 2493) and Gram –ve bacteria *Pseudomonas aeruginosa* (NCIM 5029), *Klebsiella neumonia*(NCIM 2957) *Enterobacter aerogenes* (NCIM 5139) *Salmonella typhymurium* (NCIM 2501). These strains were obtained from National Chemical Laboratory (NCL), Pune.

Inoculum preparation

The test microorganisms were maintained at 4°C on nutrient agar slants. Active cultures for each bacterial species were prepared by transferring a loopful of cells from the stock cultures to test tubes of nutrient broth. The inoculated tubes were incubated without agitation for 24 h at 37°C. The cultures were diluted with fresh nutrient broth to achieve optical densities corresponding to 10^6 cfu mL^{-1} (Duraipandiyan, V., M. Ayyanar and S. Ignacimuthu, 2006).

Determination of in vitro anti-microbial effect

Broth dilution assay

The minimum inhibitory concentration (MIC) values were determined by using a modified macro-broth dilution technique (Ibrahim et al., 1997). Overnight culture of bacteria grown in nutrient both cultures were diluted 100 folds in NB (100µl bacterial cultures in 10ml NB which contained 10^5 cfu of bacteria). Gradually increasing volumes of the extracts were added to test tubes containing the bacterial cultures to know the inhibitory concentration in a particular tube inhabiting the bacterial growth. The tubes were incubated at 37^0C for 18-24 hours. The tubes were examined for visible turbidity and optical density of cultures was determined at 620nm using NB as control. The lowest concentration that inhabited visible growth of the tested organisms was recorded as the MIC.

Agar well diffusion assay

The agar well diffusion method was used to test the anti-microbial effect of *N. sativa* crude extracts in different stages of germination. (Okeke et al., 2001; Perez et al, 1990). All media plates (9 cm in diameter) were prepared with nutrient agar. After agar solidification, the well (7 mm in diameter) was cut from the agar to produce a total of four wells per agar plate. For test, three doses of extract (25, 50, 100 µg/well) were prepared using 99.5% analytical Dimethyl Sulphoxide (DMSO) as an organic solvent. Streptomycin (30 µg), gentamycin(10µg), doxycycline (30 µg), ampicillin (10 µg), penicillin (10 µg), tetracycline (10ug)were used as positive standard antibiotics. 100 µL (10^5 cfu) of each diluted microbial suspension were inoculated on nutrient agar plates using sterile cotton swab. The inoculums were allowed to dry for 5 min. Then, 100 µL of each extract solution, blank (DMSO) and positive control was added separately to each well of agar plate and allowed to diffuse at room temperature for 15-20 min. After incubation at 37°C for 24h, all plates were examined for any zones of growth inhibition and the diameter of these zones was measured. The assay was repeated three times for each extract. The anti-microbial effect was recorded as the mean diameter of the resulting inhibition zones of growth in millimeter.

Results and Discussions

Qualitative analysis of primary metabolites showed that after the imbibition and sprouting, there was a little decrease in the content of soluble sugars till 6d after which a sharp increase was observed till 10d. After 10d, the amount of sugars remained constant till 11d when complete plantlet was formed. These results clearly demonstrate that the contents of soluble sugars rise after the initiation of solubilization of complex sugars to liberate water soluble sugars which could be easily assimilated by the growing embryo. The changes of biomolecules such as chlorophyll, reducing sugar, starch, protein, proline and an enzyme activity peroxidase in shoot and root at 15th day seedlings has been reported in three species of *Vigna under* NaCl stress (Arulbalachandran et al, 2009). It could be deduced that there was modification of the aldose sugars by the addition of either ester group (Takashi et al, 2007) or any other group during later stages of germination which might be responsible for the antimicrobial activities. Antimicrobial compounds such as

Table 1. Preliminary screening of primary and secondary metabolites from *N.sativa*

Day	Soluble sugars	Fructans	Proteins	Alkaloids	Tannins	Flavonoids
0	++	+	+	+++	-	+
1	++	+	+	++	-	+
2	+	+	++	++	-	+
3	+	+	++	++	-	+
4	+	+	+++	++	-	+
5	+	+	+++	++	+	++
6	++	++	++	++	+	++
7	+++	++	++	++	+	++
8	++++	+	++	+	++	++
9	++++	+	++	+	++	++
10	++++	+	++	-	++	+++
11	++++	+	++	-	++	+++

carbohydrates, proteins, tannins and flavonoidal glycosides have been reported in the extracts of *Lantana indica* Roxb. (Venkataswamy, 2010).

In case of total fructans, it remained uniform till day 5 after which there was a sharp increase till day 8. After day 8 there was a decline in the amount of fructan till day 11. Therefore it could be deduced that fructans are not responsible for the antibacterial activity.

In case of proteins, there was a sharp increase on day 4 and day 5 after which it showed uniform content till day 11. The antibacterial activity can also be due to the synthesis of some bioactive peptides during later stages of germination. Proteins with antimicrobial activity were first reported by Balls et al (1942). Moreover, the aqueous extract of medicinal and food plants have been previously reported to show antifungal activity (Schamourlo, et al, 2005). Generally, higher levels protein and carbohydrate content of the extract had better antimicrobial activities (Wafaa, et. al.2007). During the hydrated part of seeds life, i.e. maturation and germination, seeds are very sensitive to viruses, fungi and bacteria. Many proteins are involved in the microbial defense mechanism of plants. Puroindoline is the main component of a new family of proteins that has been suggested to exert an antimicrobial activity in plant seeds (Dhatwalia et al., 2009). Two peptides with antimicrobial activity, designated p1 and p2, were purified nearly to homogeneity from Scots pine seedlings (Kovaleva et al, 2009).

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols *etc*. Recent studies have shown that the secondary metabolite content varies during germination of seeds. A highly significant antibacterial effect of *Allium roseum* L (bulb, leaf, seed and flower) extracts on *S. aureus*, *B. subtilis*, *B. cereus*, *E. faecalis, E. coli*, *P. aeroginosa, S. typhimurium* and *C. albicans* strains.has been shown (Najjaa H, 2009). The extracts of seeds of *Nigella sativa* in different germination stages have revealed the presence of alkaloids, tannins and flavonoids (Table 1).Thus the preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development.

In this study, we had also investigated the antibacterial effects of aqueous extracts of successive stages of the germinating seed on standard gram positive and gram negative bacterial strains. The results of antibacterial test indicated that different extracts of *Nigella sativa* showed different degrees of growth inhibition depending on the day of germination and bacterial strains. *Nigella sativa* is frequently used as an active ingredient in certain medicines and reported to possess a number of pharmacological effects to treat different human ailments (Bonjar et al., 2004). Several investigations have been directed towards their antibacterial properties (Voravuthikunchai et al., 2005). The preliminary assessment of the in vitro antimicrobial effect of different germinating stages of *N. sativa* crude extracts revealed some basic outcomes. First, the aqueous extracts of seed of *Nigella sativa* did not show any significant inhibitory effect during earlier germination stages but they showed inhibition against all standard strains in later stages of germination (Table 3). Second, the extracts showed highest activity from 8d to 11d of germination (Table 2).

Conclusion

In the light of these results we can conclude that level of antimicrobial activities of the aqueous extracts was compared with the chemical composition of extract to determine the chemical composition-activity relationship of extract. The alkaloid content showed a decrease while the tannin and flavonoid contents have shown an increase from day 8 of germination. High tannin and

Table 2. Minimum Inhibitory Concentration of aqueous extract of *N. sativa* seed in different germination phases.

S.No	Pathogen	Minimum Inhibitory Concentration (mg/ml) Day of germination								
		Day 0	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11
1	*E. aerogenes*	-	8±0.43	10±0.37	10±0.39	9±0.42	5±0.57	7±0.43	7±0.47	67±0.51
2	*K. pneumoniae*	-	9±0.77	8±0.56	9±0.48	7±0.66	6±0.75	6±0.48	6±0.49	6±0.57
3	*P. aeruginosa*	-	-	-	-	-	8±0.59	8±0.67	7±0.43	6±0.42
4	*S. epidermidis*	-	9±0.29	10±0.37	10±0.77	7±0.57	7±0.67	6±0.47	6±0.57	6±0.32
5	*S. typhimurium*	-	-	-	-	-	8±0.59	9±0.54	8±0.39	7±0.56

Table 3. Zone of Inhibition shown by aqueous extract of *N. sativa* seed in different germination phases

Pathogen	ZONE OF INHIBITION (mm)														
	Days									Antibiotics					
	0	4	5	6	7	8	9	10	11	ST	GN	DO	AM	PN	TC
E. aero	-	10±0.47	10±0.53	10±0.52	10±0.39	12±0.33	12±0.54	12 ±0.41	14±0.35	23±0.37	18±0.43	12±0.39	-	-	19±0.44
K. pneu	-	10±0.81	10±0.65	10±0.49	11±0.35	13±0.72	14±0.75	13±0.59	13±0.61	21±0.57	18±0.49	19±0.33	-	-	25±0.59
P. aeru	-	-	-	-	-	12±056	12±0.37	12±0.41	13±0.52	26±0.38	18±0.62	12±0.58	-	-	10±0.39
S. epi	-	10±0.30	10±0.27	10±0.61	11±0.57	14±0.44	13±0.59	14±0.81	13±0.77	23±0.58	18±0.49	12±0.69	-	-	19±0.43
S. typhi	-	-	-	-	-	12±0.37	12±0.42	12±0.31	14±0.29	22±0.75	18±0.38	12±0.58	-	-	10±0.39

*Data is a mean of three replications, * Antibiotics - positive control, *10% DMSO - Negative control, *"-" No inhibition observed,
* ST : Streptomycin(30 µg) , GN : Gentamycin(10 µg), DO : Doxycycline (30 µg), AM : Ampicillin (10 µg), PN : Penicillin (10 µg), TC : Tetracycline (10ug) OBSERVATION: All the bacterial strains are resistant to Penicillin and ampicillin.

flavonoid content might also be responsible for the antibacterial activity in later stages of germination (Table 1).

Finally, the extract also revealed superior inhibitory effect over the standard drugs ampicillin (10 µg) and penicillin (10 µg), and tetracycline (10ug) (Table 3). Penicillin and ampicillin antibiotics are the inhibitor of cell wall synthesis (β-lactams). They are the inhibitors of the last step in cell wall synthesis, the cross-linking of different peptidoglycan strands. The extract of *N.sativa* showed the activity against the penicillin and ampicillin resistant pathogens on from day 8 to day 11 of

germination (Table 3). This might be due to the synthesis of some metabolites on these days of germination which could act by inhibition of cell wall synthesis, by inducing changes in membrane structure, by inhibiting bacterial protein synthesis or by binding to ribosomal 50S subunit and interfering with the peptidyl transferase activity. The depiction of the exact mechanism of inhibition by the extracts needs further investigation.

The results are in agreement with others which showed that *Nigella sativa* extracts produce antimicrobial activity against a broad range of microbes and especially on multiple antibiotic resistant bacteria. (Morsi et al., 2000). Further studies on the activity-directed fractionation for the isolation of respective pure compounds from the extracts may result in interesting results.

REFERENCES

Akhtar MS, Riffat S (1991). Field trial of *Saussurea lappa* roots against nematodes and *Nigella sativa* seeds against cestodes in children. J. Pakistan Med. Assoc., 41(8):185–187.

Al-Ghamdi MS (2001). Anti-inflammatory, analgesic and anti-pyretic activity of *Nigella sativa*. J. Ethnopharmacol., 76: 45–48.

Al-Haider A, Aqeel M, Hasan Z (1993). Hypoglycemic effect of volatile oil of *Nigella sativa* seeds. Pharma. Biol., 31:96-100.

Al-Jassir MS (1992). Chemical composition and microflora of black cumin (*Nigella sativa* L.) seeds growing in Saudi Arabia. Food Chem., 45:239-242.

Arulbalachandran D, Sankar GK, Subramani A (2009). Changes in Metabolites and Antioxidant Enzyme Activity of Three Vigna Species Induced by NaCl Stress. American-Eurasian. J. Agro., 2(2): 109-116.

Balls A, Hale WS, Harris TH (1942). A crystalline protein obtained from lipoprotein of wheat flour. Cereal Chem., 19: 279-288.

Bonjar GHS (2004). Screening for antibacterial properties of some Iranian plants against two strains of *E. coli*. Asian J. Plant Sci., 3(3): 310-314.

Dhatwalia VK, Sati OP, Tripathi MK, Kumar A (2009). Isolation, Characterization and Antimicrobial Activity at Diverse Dilution of Wheat Puroindoline Protein. World J. Ag. Sci., 5 (3): 297-300.

Duraipandiyan V, Ayyanar M, Ignacimuthu S (2006). Antimicrobial activity of some ethnomedicinal plants used by paliyr tribe from Tamil Nadu, India. BMC. complement. Alternat.Med., 6: 35-35.

El-Dakhakhny M, Mady NA, Halim MA (2000). *Nigella sativa* L. oil protects against induced hepatotoxicity and improves serum lipid profile in rats. Arzneimittel-forschung. 50: 832-836.

Ghosheh OA, Houdi AA, Crooks PA (1999). High performance liquid chromatographic analysis of the pharmacologically active quinones and related compounds in the oil of the black seed (*Nigella sativa* L.). J. Pharm. Biomed. Anal., 5:757-762.

Hanafy MS, Hatem ME (1991). Studies on the anti-microbial activity of *Nigella sativa* seed (black cumin). J. Ethnopharmacol., 34: 275-278.

Hosseinzadeh H, Bazzaz BSF, Haghi MM (2007). Anti-bacterial activity of total extracts and essential oil of *Nigella sativa* L. seeds in Mice. Pharmacol., 2: 429-435.

Ibrahim MB, Owonubi MO, Onaolapo JA (1997). Antimicrobial effects of extracts of leaf, stem, and root-bark of *Anogiessus leicarpus* on *Staphylococcus aureus* NCTC 8190, *Escherichia coli* NCTC 10418 and *Proteus vulgaris* NCTC 4636. J. Pharm. Res. Dev., 2: 20-26.

Kovaleva V, Kiyamova R, Cramer R, Krynytskyy H, Gout I, Filonenko V, Gout R (2009). Purification and molecular cloning of antimicrobial peptides from Scots pine seedlings. *Peptides*, available at: ,http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T0M-4X0PBTJ-1&_user=10&_coverDate=12%2F31%2F2009&_rdoc=1&_fmt=high&_orig=search&_origin=search&_sort=d&_docanchor=&view=c&_searchStrId=1489794723&_rerunOrigin=google&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=fdb527ab6be6d9bc7012408c2432db95&searchtype=a., 30(12), 2136-2143.

Mashhadian NV, Rakhshandeh H (2005). Antibacterial and anti-fungal effects of *Nigella sativa* extracts against *S. aureus*, *P. aeruginosa* and *C. albicans*. Pakistan J. Med. Sci., 21: 47-52.

Morsi NM (2000). Antimicrobial effect of crude extracts of *Nigella sativa* on multiple antibiotics-resistant bacteria. Acta. Microbiol. Pol., 49: 63-74.

Najjaa H, Ammar E, Neffati M (2009). Antimicrobial activities of protenic extracts of *Allium roseum* L., a wild edible species in North Africa, J. Food. Ag. Environ., 7(3&4):150 - 154.

Okeke MI, Iroegbu CU, Eze EN, Okoli AS, Esimone CO (2001). Evaluation of extracts of the root of Landolphia owerrience for antibacterial activity. J. Ethnopharm. 78: 119-127.

Perez C, Paul M, Bazerque P (1990). Antibiotic assay by agar-well diffusion method. Acta Biol. Med. Exp., 15: 113-115.

Salman MT, Khan RA, Shukla I (2008). Anti-microbial activity of *Nigella sativa* Linn. Seed oil against multi-drug resistant bacteria from clinical isolates. Nat. Prod. Rad., 7: 10-14.

Schamourlo G, Mendonca-Filho RR, Alviano CS, Costa SS (2005). Screening of antifungal agents using ethanol precipitation and bioautography of medicinal and food plants. J. Ethnopharmacol., 96: 563-568.

Takruri HR (2003). Black cumin in prophet hadith and modern medicine. Presented in Symposium on Medicine in the Prophet Sunnah. Islamic Hospital , Amman.

Takashi W, Shihoko K, Mayu M, Yoichi H, Masaaki K (2007). Antibacterial Carbohydrate Monoesters Suppressing Cell Growth of *Streptococcus mutans* in the Presence of Sucrose. Curr. Microbiol., 41(3): , 210-213.

Venkataswamy R, Doss A, Sukumar M, Mubarack HM (2010). Preliminary phytochemical screening and antimicrobial studies of *Lantana indica* roxb. Indian J. of Pharm. Sc., 72 (2): 229-231.

Voravuthikunchai SP, Phongpaichit S, Subhadhirasakul S (2005). Evaluation of antibacterial activities of medicinal plants widely used among AIDS patient in Thailand. Pharm. Biol., 43(8): 701-706.

Wafaa AH, Hassan A, Nefisa MAE (2007). Biological and Anti-microbial Activities of Aqueous Extracts from Neem Tree (*Azadirachta indica* A. Juss., Meliaceae) J. of Appl. Sci. Res., 3(10): 1050-1055.