Irradiation effect on Antimicrobial properties, Nutritional values and some Physico-chemical characteristics of *Nigella sativa*

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Summary: Effect of different doses of gamma radiation (2 kGy, 4 kGy, 6 kGy, 8 kGy and 10 kGy) on microbiological, biochemical and some important physico-chemical parameters of *Nigella sativa* (black seed) has been studied. The irradiated samples showed lesser fungal and bacterial counts than the unirradiated control samples. Moreover the gamma radiation brought insignificant change in the nutritional values and active antimicrobial properties of black seed. Doses of 4 kGy and 6 kGy were found more effective for inhabiting the fungal and bacterial growth respectively, as compared to lower doses. Irradiation up to 6 kGy did not cause any significant change in chemical constituents studied, total acidity and moisture percentage.

Thus disinfestations of black seed can be achieved by gamma rays up to 4-6 kGy. This dose has no any adverse effect on nutrients, external and internal quality characteristics of *Nigella sativa* seed.

Introduction

Plants are invaluable sources of new drugs. Many medicinal plants and their purified constituents have been shown beneficial therapeutic potentials.

Nigella sativa L. (Ranunculaceae family) is an indigenous herbaceous plant. In South Asia, it is called kalonjii, its Arabic name is Habat-ul-Sauda and its English name is black coumin [1]. Usually, there is a common Islamic belief that blackseed is a universal remedy for all ailments, but cannot prevent aging or death. Blackseed is also recognized as the beneficial black cumin in the Holy Bible and is described as *Melanthion* by Hippocrates and Dioscorides and as *Gith* by Pliny [2]. In many Arabian, Asian and African countries, its seeds, have been employed to promote health and fight disease for centuries [1, 3]. During the last two decades many studies have been conducted, on the effect of *Nigella sativa* L. seed extracts *in vitro* or *in vivo* on different biological systems. The pharmacological investigations of the seed extracts reveal a broad range of biological activities including antioxidant and antimutagenic [4] , the relaxant, anticholinergic and antihistaminic [5, 6], protective effect on gastric secretion and ethanol-induced ulcer [6] sciatic nerves tonic [7], immunopotentiation [8, 9] and anti-diabetic [10-12], analgesic & antiinflammatory [13], antimicrobial [14, 15] antifungal [16], anti-cancer [2, 17, 18] and anti-hypertensive [19] . Many of these activities have been attributed to the quinone constituents of the seed [15].

Irradiation has a clear edge over other phytosanitary treatments, because of its versatility in controlling a variety of microorganisms and insects. Further, it also causes insignificant changes in the quality of treated items. Hence irradiation may work as phytosanitary treatment of herbs. Irradiation decontamination is an effective technique for the long-term storage of herbs. This research effort will pave the way for a new potential to control the herbs spoilage and pathogenic microorganisms and to save the natural and economic wealth of the country. It will provide a firm scientific footing to the herbal industry. This work will also enable the country to cope up with the international strict sanitary requirement of World Trade Organization (WTO) and as well as will save foreign exchange.

Results and Discussion

Nigella sativa seeds were treated at irradiation doses of 2 kGy, 4 kGy, 6 kGy, 8 kGy and 10 kGy. Results regarding the influence of irradiation

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on total bacterial count of *N. sativa* are given in Table-1. The total bacterial count of the control samples of *N. sativa* was 3.7 x 10^5. The level of viable microorganisms decreased with irradiation, depending upon the absorbed dose. Gamma irradiation at doses of 4.0 kGy was effective in reducing bacterial count to below a level of 10^3 microorganisms per gram. At the irradiation doses of 6.0 and 8.0 kGy the bacterial count dropped to non-detectable limits. The total fungal counts in control samples were 5.6 x 10^2. No fungal growth was observed after applying the radiation dose at level 4.0 to 8.0 kGy (Table-2).

Table-1: Effect of different doses of radiation on the total bacterial count of *N. sativa.*

Replication	Control	2kGy	4kGy	6kGy	8kGy	10kGy
R1	$3.7x10^5$	$9.3 x 10^4$	$2.1 x 10^2$	ND	ND	ND
R2	$2.9x10^5$	$8.4 x 10^4$	$1.9 x 10^2$	ND	ND	ND
R3	$4.3x10^5$	$10.1 x 10^4$	$3.2 x 10^2$	ND	ND	ND

ND. Non detectable
The values are average of three determinations

Table-2: Effect of different doses of radiation on the total fungal count of *N. sativa.*

Replication	Control	2 KGy	4 KGy	6 KGy	8 KGy	10 KGy
R1	$5.6 x 10^2$	1.1 x 10	ND	ND	ND	ND
R2	$4.9 x 10^2$	0.9 x 10	ND	ND	ND	ND
R3	$6.3 x 10^2$	1.5 x 10	ND	ND	ND	ND

ND: non detectable.
The values are average of three determinations

The antimicrobial properties of irradiated and control samples were checked against certain important bacteria and fungi grown on nutrient agar media. It was noted that ethanolic extract of *N. sativa* seed shows activity against *Bacillus cereus*, *Escherichia coli* (800), *Pseudomonas aeruginosa* (79), *Salmonella typhimurium* (704), *Salmonella typhimurium* (714), *Shigella boydi*, *Shigella sonnei*, *Staphylococcus aureus* (56), *Staphylococcus aureus* (76) and *Candida albicans* (Table-3). The active or antimicrobial constituents of plant remained undegraded after irradiation. Further the seeds samples of the plant were found to be free from all types of insects (Table- 4).

N. sativa seeds are frequently used as a natural remedy for various health disorders. A lot of work has been done on the pharmacological activities of these materials[9, 13-15]. This study reports the effect of irradiation on the proximate nutrients composition and physio-chemical characteristics of *N. sativa* seeds that remained insignificant up to dose level of 4KGy (Tables-5, 6).

Table-3: Effect of different doses of gamma radiation on the antimicrobial properties of the ethanolic extract (400 μg/ml) of *N. sativa.*

PATHOGENS (BACTERIA)	CONTROL	Radiation (KGy) 2.0	4.0	6.0	8.0	10.0
Bacillus cereus	S	S	S	S	S	S
Escheriachia coli (799)	S	S	S	S	S	S
Escheriachia coli (800	S	S	S	S	S	S
Pseudomonas aeruginosa (79)	S	S	S	S	S	S
Escheriachia coli (704)	R	S	S	S	S	S
Escheriachia coli (714)	R	R	R	R	R	R
Staphylococcus aureus (56)	S	R	R	R	R	R
Staphylococcus aureus (76)	S	S	S	S	S	S
Shigella boydi	S	S	S	S	S	S
Shigella sonnei	S	S	S	S	S	S

R resistant
S susceptible

Table-4: Effect of different doses of gamma radiation on the antimicrobial properties (antifungal) of the ethanolic extracts (400 μg/ml) of *N. sativa.*

PATHOGENS (FUNGI)	CONTROL	Radiation (kGy) 2.0	4.0	6.0	8.0	10.0
Aspergillus flavus	S	S	S	S	S	S
Aspergillus niger	S	S	S	S	S	S
Candida albican	S	S	S	S	S	S
Epidermophyton flocossum	R	R	R	R	R	R
Trichoderma viride	S	S	S	S	S	S

R = resistant
S = susceptible

Table-5: Effect of different doses of gamma irradiation on mean proximate composition nutrients of *N. sativa.*

Treatment	Water	Crude Proteins	Crude Fats	Minerals	Crude Fiber	Carbohydrates
Control	4.83	18.54	35.46	2.67	6.75	31.73
2KGy	4.79	18.54	35.48	2.60	6.60	31.97
4KGy	4.78	18.05	35.54	2.76	6.24	32.62
6KGy	4.78	17.79	35.61	2.89	5.92	32.99
8KGy	4.74	17.72	36.44	2.97	6.00	32.11
10KGy	4.73	17.64	36.60	3.04	5.63	32.34

Table-6: Effect of different doses of gamma radiation on Physico-chemical Characteristics of *N. sativa* seeds.

Doses KGy/hr	PH	EC us/cm	TTA
Control	6.8	1.3	1.1
2	6.8	1.5	1.3
4	6.9	1.6	1.5
6	7.0	1.6	1.2
8	7.2	1.7	1.5
10	7.4	1.8	1.4

EC = Electrical Conductivity
TTA = Total Titratable Acidity

Study of control and irradiated samples of *N. sativa* showed Ca, Mg, Na, K, S, P, Zn, Cu, Fe, Mn an, Cr to be sufficient in seed of the plant (Table-6). No significant effect on the quantity of these minerals is noted with the given radiation doses (Table-7).

Table-7: Effect of different doses of gamma radiation on Percent concentration of various elements in *N. sativa.*

Doses	Ca	Mg	Na	K	S	P	Zn	Cu	Fe	Mn	Cr
00000	23.20	13.9	8.25	29.5	1.82	16.2	0.0450	0.0021	0.2480	0.357	0.0042
2 kGy	22.80	13.5	8.20	29.5	1.85	15.7	0.0426	0.0018	0.2487	0.362	0.0051
4 kGy	23.40	13.8	8.32	29.8	1.74	16.3	0.0453	0.0024	0.2474	0.352	0.0043
6 kGy	23.25	13.7	8.27	29.3	1.90	16.5	0.0430	0.0021	0.2486	0.356	0.0047
8 kGy	23.20	13.8	8.26	29.3	1.80	16.3	0.0452	0.0022	0.2482	0.356	0.0042
10 kGy	22.86	13.5	8.22	29.4	1.84	16.5	0.0450	0.0020	0.2483	0.357	0.0046

Decontamination with radiation of dry herbs is more promising method than fumigation, heat treatment and other conventional methods for extracts (extraction at room temperature). However, decontamination using irradiation processing is not a substitute to Good Manufacturing Practices (GMP). Irradiation can only increase the premium of good quality material but could not improve the quality of sub-standard products, nor can it prevent contamination from improper handling after the irradiation. Thus, manufacturers should ensure that the herbal raw materials used are of good quality and processing and handling is in accordance to the GMP established by national and international authorities. Post harvest irradiation conditions that are suitable for one fresh herb may not be appropriate for another herb.

Our research work on the commercially available seeds of *N. sativa* indicated high initial microbiological counts (*i.e.* 10^3 to 10^8 micro-organisms/ gm). Local population customarily uses these seeds for cold/hot water extraction. These contaminated aqueous extracts may have negative effect on their health and if local industry uses the materials, it will cause the spoilage of the finished herbal products. Applying 6.0 kGy radiation doses from Cobalt-60 gamma radiation source, microbial counts of irradiated plant have reduced to non-detectable levels. Regarding the wholesomeness of irradiated edible materials, Irradiation treatment of up to 10 kGy has already been approved by IAEA/FAO/ WHO, however research on irradiation decontamination of pharmaceuticals is limited and need to be popularized. The ethanolic extracts of *N. sativa* showed high anti-bacterial activities against certain important pathogens and it also showed that irradiation does not affect the antimicrobial properties of the plant.

Pakistan has a very rich flora of medicinal plants but large portion of this floral wealth is being exported at a very low price due to raw and low quality of the materials and unawareness from irradiation's economic importance. Further to use an effective and environmental-friendly technique is needed more urgent than before. This research effort will pave the way with a new potential to control the microorganisms and to save the natural and economic wealth of the country. The increasing awareness by consumers of the risk from microbiological hazards will provide impetus to acceptance of irradiation to ensure herbs safety. In view of economic importance, hygienic concern, WTO challenges and quarantine standard of herbal raw materials, more research is needed to improve the hygiene, minimize the cost of storage and to boost up the commercial application of irradiation technology in the country.

Experimental

Sample Collection

Seeds of selected herbal plant (*N. sativa*) were purchased from the local market, Khyber Bazar in Peshawar, NWFP, Pakistan. The chemical analyses *i.e.,* proximate composition and irradiation effect on active chemicals and some important physico-chemical characteristics of black seeds were carried out in the laboratories of Nuclear Institute for Food and Agriculture (NIFA), Tarnab, Peshawar. Each analysis was made in triplicate.

Sample preparation

Plant samples (seeds) were washed with tap water to remove adhering soils, rinsed with distilled water and dried at room temperature. The dried plant materials were ground with the help of grinding mill and the ground sample was sieved from a 30 mm mesh sieve. The powdered samples were stored at 4^0C in plastic bags for subsequent biochemical analysis.

Gamma Radiation Treatment

The grounded powder samples of black seeds were irradiated in ambient conditions (20-35^{0}C relative humidity 65-70 %) at 2- 10 kGy radiation doses in a Cobalt-60 irradiator, Issledovatel (USSR), with a dose rate of irradiation 0.96 kGy/hour as determined with Fricke dosimeter. The doses level applied were 2.0, 4.0, 6.0, 8.0 and 10.0 kGy. Unirradiated (control) samples were also kept in each case. The samples were analyzed for total bacterial and fungal load in triplicate and for the presence of insects.

Biological Activities Test

The active ingredient of the selected plant was extracted with petroleum ether. The extract was concentrated and checked against different pathogenic microorganisms in order to see the effect of irradiation on the antimicrobial compounds and other active constituent of the plant. All the organisms used in the present study were clinical isolates and were obtained from the pathological laboratory, Khyber Medical College, Peshawar.

Antimicrobial Activities

Stock solutions of crude ethanolic extracts were prepared in sterilized water. These solutions were diluted into sterile molten nutrient agar to reach the final concentration of 400µg/ml separately. The Petri dishes were kept at room temperature for solidification. Sterilized distilled water was used as negative control. Pure cultures of selected pathogens were evenly spread. The incubation period was allowed to proceed for 24 hours at 37°C. After the incubation period, the pallets were examined for antimicrobial activities. For antifungal activities, Sabouraud Dextrose Agar (SDA) medium was used and after inoculation the plates were kept at 29°C for 5-7 days.

Enumeration of Microbial Count

The total aerobic bacteria were determined by the surface plate agar method with medium containing Difco-nutrient agar 23 g, Glucose 5 g, yeast extract 5 g and K_2HPO_4 2 gm per litre (pH 7.0). Two grams of each treated and control sample was added to 20 ml of 0.01% Tween-20 sterile water and mixed thoroughly. Each suspension was diluted 10^2 and 10^4 times with the same sterile water and then 0.2 ml aliquots were spread on the surface of agar plate. Total aerobic bacteria were counted after 3 days incubation at 30°C. Fungal count of the herbs was also determined by plate count method using potato dextrose agar. Plates were incubated at a temperature of 28 °C for 3-5 days and the number of colony-forming unit (cfu) counted. The samples were analyzed for microbial load in triplicate.

Physico-chemical Characterization

Irradiated and un-irradiated (controls) were processed for chemical constituents, proximate compositions, certain elements and ascorbic acid contents according to standard methods. Solubility, Electrical Conductivity (EC), pH, Total Titratable Acidity (TTA) values were also determined to see the effect of irradiation.

Proximate Composition

The proximate composition for both control and irradiated samples (seeds) were performed in triplicate in accordance with A.O.A.C. (20).

Mineral Analysis

For determination of selected trace elements such as iron (Fe), copper (Cu), manganese (Mn), zinc (Zn) and calcium (Ca), the method of Sandell [21] and Dell *et al.* [22], modified for macro levels [23] was employed by using Atomic Absorption (Hitachi model 170-10 with an air acetylene flame). Potassium and Sodium were determined by flame photometry technique using standard method [24]. Phosphorus was determined by Spectronic-20 at 470 nm, [25]. The data obtained from this study was analyzed according to standard statistical procedures (student t' test).

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