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EFFECT OF BIO-FERTILIZERS AND GAMMA RADIATION ON THE ESSENTIAL OIL OF DILL AT THE VEGETATIVE, FLOWERING, FRUITING STAGES AND THEIR ANTIMICROBIAL ACTIVITIES

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Abstract

This study was carried out to investigate the response of dill plants to gamma radiation and bio-fertilizers on the essential oils and their constituents during vegetative, flowering and fruiting stages. The results showed that the most effective treatment on the volatile oil content in the three stages was 2 k-rad + bio-fertilizers. The major compounds were found to be α-phellandrene, limonene, β-phellandrene and p-cymene (vegetative stage). In the flowering stage, p-cymene, carvone, dillether and dillapiole. Whereas, carvone, dillapiole and limonene were major compounds (fruiting stage). The results showed those dill volatile oils at different developing stages exhibited strong and a variable degree of antimicrobial activity.

Key words: *dill (Anethum graveoleus L.) essential oil, gamma radiation, biofertilizer, anti-microbial*

1. INTRODUCTION

Dill is an annual herb, family *Apiaceae* is one of the first-known multi-purpose plants which have been used as a spice and medicine. Herb oil is mainly used in the food industry for flavouring and seasoning, perfumes, cosmetics, soaps, creams, lotions and in detergents. Also, herb and seed oil have also been widely investigated in respect of their antiseptic and exhibits anticarcinogenic activity, antimicrobial and antioxidant (Mahran *et al.*, 1992).

Bio-fertilization is generally based on altering the rhizosphere flora, by seed or soil inoculation with certain organisms, capable of inducing beneficial effects on a compatible host. These organisms may affect their host plant by one or more mechanisms such as nitrogen fixation, production of organic acids, enhancing nutrients uptake, synthesis of vitamins, amino acids, auxins and gibberellins which stimulate growth, or increasing the resistance against plant pathogens. (Sprenat, 1990).

Irradiated seeds with gamma rays induced biochemical, physiological and cytological changes, the biochemical contents, i.e, enzyme, protein and phytohormone were severely changed by exposing seeds to gamma rays. There is general agreement that the low dose irradiation of seeds stimulates the growth (Korosi and Pal, 1989 and Rabie *et al.*, 1996).Applications of the use of low dose (< 10 kGy) irradiation in controlling insect infestation, reducing microbial numbers (Bande *et al.*, 1990).

The aim of this investigation was to study the response of dill plants to bio-fertilizers and gamma rays on volatile oil and their composition at different developing stages and determine their anti-microbial.

2. MATERIALS AND METHODS

The experiment of this study was carried out at the Farm Station of National Research Centre, at Shalakan Kalubia Governorate during the seasons of 2000/2001 and 2001/2002. The dill seeds were sown in 15th October in the two seasons. The bio-fertilizers used in this study were Rhizobacterin (a mixture of *Azotobacter* and *Azospirillum*) and Microbein (a mixture of *Azotobacter*, *Azospirillum*, *Pseudomonas*, *Rhizobium* and *Bacillus*) produced by General Organization for Agriculture Equalization Fund (G.O.A.E.F.).

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Dry seeds of dill were irradiated with gamma rays doses at 2, 4, 8, 16, 32 and 64 k-rad and inoculation with bio-fertilizers (1kg Rhizobacterin + 1kg Microbein / feddan) added as inoculated the seeds and sowing. The source of irradiation is installed at the Middle Eastern Radioisotope centre for the Arab Countries, Dokki, Giza, Egypt.

The experimental design was factorial and planned in a complete randomized block design having three replications. The essential oil percentage was subjected to hydro-distillation for 3 hours using a modified Clevenger apparatus according to Gunther [5]. The data was statistically analyzed according to Steel and Torrie [6]. The resulted oil was dehydrated over anhydrous sodium sulphate and stored at freezer till used for gas liquid chromatographic (GLC) analysis.

Microorganisms and growth conditions

Microorganismes are five gram-positive bacteria such as *Bacillus cereus, Bacillus subtilis, Escherichia coli, Pseudomonas aeroginosa* and *Staphylococcus aureus* and two fungi such as *Aspergillus niger, Trichoderma* sp. and one yeast, *Candida albicans* were used to check the antimicrobial potential of essential oils of dill at different developing stages. These microorganisms were obtained from Microbial Chemistry Department, National Research Centre, and Egypt. The antimicrobial activity was determined by disc diffusion method (Ericsson and Sherris, 1971).

3. RESULTS AND DISCUTION

3-1: Essential oil production

The data presented in Table (1) showed that the different gamma ray doses and bio-fertilizer treatments had a generally favourable effect on the formation of essential oil. Combined treatment between gamma ray at 4 k-rad and bio-fertilizer gave the highest essential oil in herb at the vegetative stage.

In both seasons, increasing the gamma radiation doses from 2 to 64 k-rad resulted in a significant increase in the oil percent of herb at flowering stage as well as fruits. There were significant differences between the interaction treatments on essential oil percent of herb during flowering stage in the two seasons. Whereas, the doses 4, 8 and 16 k-rad resulted in significant in oil percent at vegetative stage in the first season. Also, the effect of bio-fertilizer treatments on oil percent was significant at vegetative stage and both seasons. The highest volatile oil percent in herb at flowering stage was obtained by 2 k-rad dose of irradiation in the two seasons, respectively.

The data in Table (1) also showed that the irradiation dose at 8 k-rad gave the highest values on the essential oil percent of fruits in the first and second seasons, respectively. There were significant differences between the interactions treatments on essential oil percent of fruits in both seasons. The stimulative effect of gamma radiation and bio-fertilizer on secondary production such as volatile oil percentage might be due to the beneficial effects on synthesis of metabolic carbohydrates in dill plants consequently used for volatile oil formation.

However, the oil content of dill increased very rapidly during the development of the plant, the umbels being the most important oil producing organs than leaves and stalks (**Houpalahti and Linko, 1983).** This increment of volatile oil might be due to the resultant of the increase in vegetative growth as the plant advanced towards maturity as well as the increase in the carbohydrate synthesis. **Penka (1978)** claimed that the formation and accumulation of essential oil was directly dependent on the perfect development and growth of plants. **Sefidkon *et al.*, (1999)** on *Thymus kotschyanus* found that the highest oil yield was obtained at the complete flowering stage than each of before flowering and at the beginning of flowering.

Randhawa and Singh (1991) on dill found that harvesting at the milk stage resulted in the highest oil content. The increase in oil percentage in plants fertilized with bio-fertilizer might be due to the beneficial effects of these ferttilizers on the synthesis of carbohydrates in plant tissues, consequently used for oil production. The yield and quality of essential oil produced depends on various intrinsic and extrinsic factors. Intrinsic factors comprise all internal hereditary characters. Extrinsic factors affecting the production of essential oil in plants are soil, nutrition and water supply, climate (light/temperature), maturity, post harvest treatment and distillation (**Varshney, 1991).** However, **Al-Humaid (2004)** emphasized the beneficial role of the inoculation of fennel seeds with Rhizobacterin as a bio-fertilizer on the metabolic process that is responsible for the essential oil content. The above results are in agreement with those obtained by **Jeliazkova *et al.*, (1997)** on fennel; **Khalil *et al.*, (2001)** on fennel and black cumin; **Khater (2001)** on *Carum carvi;* **Hassanein *et al.*, (2003)** on fennel; and **Kandeel *et al.*, (2004, a and b)** on dill and fennel.

3-2: Essential oil constituents

3-2-1: In the vegetative stage

The main constituents of the essential oils from fresh herb during vegetative stage were indicated in Table (2). The obtained data revealed that, α-phellandrene (32.4718- 61.0433%), limonene (10.8714 - 14.2536%), β-phellandrene (7.0882 -13.7149%) and p-cymene (11.6728 - 29.3774%) were the most abundant, but their quatitative contents varied to different extents.

With regard to bio-fertilizers and gamma radiation treatments, data presented in Table (2) show that, bio-fertilizers treatments increased α-phellandrene and limonene compared to control plants. On the contrary, bio-fertilizers treatments decreased β-phellandrene and p-cymene compared to control plants. Also, all treatments of gamma radiation (2 k-rad to 64 k-rad) caused a decrease in contents of limonene and p-cymene compared to control plants. The doses of 2, 8, 16 and 64 k-rad increased α-phellandrene and the doses 32 and 64 k-rad increased β-phellandrene.

3-2-2: In the flowering stage

In the Table (5) four main compounds were identified, such as p-cymene (10.93595 -33.4236%), carvone (7.61115- 28.3321%), dillether (6.9993-25.32085%) and dillapiole (4.1622-19.1895%). Bio-fertilizers treatments increased carvone, dillether, dillapiole, piperitone and carveol. All treatments caused increase dillapiole content. On the other hand, all treatments caused decrease p-cymene content. Dillether content was increased by treating with 8 and 32 k-rad alone as well as 2 and 64 k-rad plus bio-fertilizers.

Gamma ray at 2, 4 k-rad increased carveol content. Doses at 4 and 64 k-rad increased β-phellandrene. Whereas, piperitone content (6.70485%) increased with the treatment 32 k-rad. The treatment at 2 k-rad gave the highest content (4.28835%) of carveol and 64 k-rad + bio-fertilizers led to the highest content (13.6816) of β-phellandrene.

3-2-3: In the fruiting stage

From results in Table (6), it is clear that carvone (51.5096 - 68.8437%), dillapiole (17.1895 - 23.5798%) and limonene (5.3223 - 22.1267%) were identified as the major compounds. Application of bio-fertilizers alone led to increase the percentage of limonene (20.0850%) at the expense of carvone (57.9056%) when compared with that of control ones. All treatments except of 64 k-rad and 2 k-rad + bio-fertilizers increased limonene content. On the other hand 64 k-rad and 2 k-rad + bio-fertilizers increased carvone content. Furthermore, all treatments enhanced the content of dillapiole when compared to the other control.

It is clear that, treatments caused an increase of limonene content at the expense of carvone content.

Dillether was found to be the fourth main compound and ranged from 1.649% (control plants) to 2.3632% (16 k-rad treatment). All treatments increased dillether content compared to control plants.

Application of bio-fertilizers increased piperitone, carveol and dillapiole.With regard to gamma radiation, 2 k-rad treatment increased carveol, dillapiole and β-phellandrene, whereas, 8 k-rad treatment increased dillether, carvone and piperitone. Furthermore, 32 k-rad treatment enhanced the contents of p-cymene, dillether, carvone and piperitone.

Data in Table (3) showed that, gamma rays at 2, 8, 16 k-rad resulted in the highest carveol, carvone and dillapiole contents (8.4562%, 7.8371% and 24.4204%, respectively) compared to the other treatments. Inoculating the plants led to the highest piperitone content (10.0567%). Whereas, 2 k-rad treatment plus bio-fertilizer showed the highest content of β-phellandrene (18.4987%).

3-3: Antimicrobial activity

The results recorded in Table (97) indicated that volatile oils of dill plants distilled individually from fresh herb during vegetative and flowering stages and from the air dried seeds (fruits) throughout fruiting stage had a considerable biologically activities against some microorganismes of bacteria such as *Bacillus cereus, Bacillus subtilis, Escherichia coli, Pseudomonas aeroginosa* and *Staphylococcus aureus* and fungi such as *Aspergillus niger, Trichoderma* sp. and *Candida albicans.*

Basing the estimation on the values of growth inhibition zones (mm), it was clearly found that volatile oils from the fresh herb had a much stronger bacterial activity on *Bacillus cereus* and *Staphylococcus aureus* than that of volatile oil obtained from the seeds, because the two genus of bacteria were the most sensitive organisms to the action of volatile oil, while *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeroginosa* were the most resistant strains towards the volatile oils, because the tabulated values were absolutely more than less than 40 (mm) when inoculated with 12.5 and 25 ul/Disc. In this concern, fungi and yeast were markedly sensitive

organisms to the action of volatile oils distilled from herb and seeds with exception of *Candida albicans* that resistant strain towards the volatile oil from herb during flowering stage, thus susceptibility of microorganisms to volatile oils activity depended on strains of organisms, dill plant organs and stages.

The resulted given in Table (98) showed that the concentration of volatile oils (1 ul/ml) was a strong inhibitor on the growth of *Bacillus cereus* and *Candida albicans*, while concentration of 2.5 ul/ml was a strong inhibitor on the growth of *Pseudomonas aerogenosa* and *Trichoderma* sp., whereas, concentration of 3.5 ul/ml was a strong inhibitory on the growth of *Bacillus subtilis, Escherichia coli, Staphylococcus aureus* (bacteria) and *Aspergillus niger*. Therefore, the responses of the strains (*Bacillus cereus* and *Candida albicans)* were markedly similar in that, low concentration of volatile oils of each dill organs at 1ul/ml were the most effective comparing to that of the strains under various concentrations of volatile oils. Similar results have been reported by Leopold *et al.*, (2003) and Nevas *et al.*, (2004) on volatile oil of *Anethum graveolens* plants.

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