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Biological Activity of Seed Aqueous Extract of *Nigella sativa* (L.) on Germination and Seedling Growth of *Vigna radiata* (L.)

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Abstract: Seeds aqueous extract of *Nigella sativa* were evaluated for their effect on germination and seedling growth of *Vigna radiata*. Two different methods were used to prepare the seed aqueous extract. The aqueous extracts prepared were found to affect germination percentage and seedling growth of *V. radiata*. The germination percentage and seedling growth of *V. radiata* decreased as the concentration of the seeds aqueous extracts of *N. sativa* increased. Severe toxicity was observed at high concentrations and moderate toxicity at low concentrations in comparison with water control. Seeds aqueous extract significantly inhibited root length more than shoot. The aqueous dried powdered extract soaked for 4 days with or without boiling had inhibitory effect on germination and seedling growth of *V. radiata*, but method 2 (with boiling) was the most effective one specially on root length.

Key words: Germination, seedling growth, aqueous extract, biological activity, *Nigella sativa*, *Vigna radiata*

INTRODUCTION

Nigella sativa seed (Habbat Albarakah) also sometimes referred to black cumin seed. It belongs to Ranunculaceae family. It is an annual flowering plant, native to southwest Asia (Zohary, 1973). It is planted in the Winter and it was flowering in July and the seeds ripen in September. It has a bitter taste. It is included in the list of natural drugs according to tradition. Recent research has provided evidence that most illnesses arise because of an imbalanced or non-functional immune system, which cannot perform its primary function of defending the body optimally (El-Kadi and Kandil, 1987). So, *N. sativa* seed contains an ability to significantly boost the human immune system if taken over time. In general the endogenous chemical components of the *N. sativa* seed are unsaturated fatty acids. hormones, saponins, alkaloids, volatile oil and essential amino acids. It also has traces of calcium, iron, sodium, potassium and crude fiber. Ethanolic extract of *N. sativa* seeds had cytotoxic immunopotiating effects as shown by Swamy and Tan (2000). There are many of secondary metabolic products in plants and many are known to be phytotoxic (Modallal and Al-Charchafchi, 2006). Biological activity of these substances are often observed to occur early in the life cycle of the plant, causing inhibition of seed germination and seedling growth (Bewley and Black, 1994). The compounds exhibit a wide range of mechanisms of action, from affects on DNA, chloroplast and mitochondrial function, phytohormone activity, ion uptake and water balance (Castro *et al*., 1984). Interpretations of mechanisms of action are complicated by the fact that individual compound can have multiple phytotoxic effects (Williams and Hoagland, 1982). Some research was focused on the biological activity of some plant species on the others (Atoum *et al*., 2006). This research furthers the possibility of using the endogenas chemical substances as growth regulators and natural pesticides (a number of them are either commercially available or in the process of large-scale manufacture) to promote sustainable agriculture.

It was seen from the literature that the aqueous extract of *N. sativa* seed used in a very narrow field and little is known about it and no remarks on its components.

The aqueous extract of *N. sativa* seed may show activity against plant growth or it may act on metabolic reaction through germination and seedling growth of other plant that grows or cultivates near the *N. sativa*. This action may be due to endogenous chemical constituent of seed or other parts of the *N. sativa*. These endogenous compound(s) may be acts as stimulator or inhibitor. The kind of compound(s) depends on seed harvest time, seed storage conditions and the environmental conditions in which the plant grows (Redha *et al*., 1994).

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To test the hypothesis of germination and seedling growth, aqueous extract of *N. sativa* seed was prepared in different concentrations by using 2 different methods and tested their effect on germination and seedling growth of *V. radiata*. This plant species one of the most valuable plant species, because of its high nutritive value and palatability, moreover it was warm resistance. So it becomes one of the promising species to cultivate in hot region.

MATERIALS AND METHODS

Materials

Plant material: *N. Sativa* and *V. radiata* seeds were purchased from a market. Both of them were newly harvested.

N. sativa seed were ground and kept in glass jar at 5°C until use for extraction.

Preparation of extracts from *N. sativa* seeds

Method 1: Dried powder of *N. sativa* seeds (50 g) were extracted with 300 mL autoclaved boiling distilled water and allowed to stand 4 days under laboratory conditions. The supernatant was taken and centrifuged at 3000 rpm for 15 min, this would be full strength concentration (100%). Then it was kept in a refrigerator at 5°C until used. Series of dilution were prepared from the stock solution (75, 50, 30, 25, 15, 10 and 5%) and were tested for their effects on germination and seedling growth of *V. radiata*.

Method 2: Dried powder of *Nigella sativa* seeds were extracted with 300 mL autoclaved distilled water. The mixture was boiled for 5 min and allowed to stand for 4 days under laboratory conditions. The supernatant was taken and centrifuged at 3000 rpm for 15 min, this would be full strength concentration (100%). Then it was kept in a refrigerator at 5°C until used. Series of dilution were prepared from the stock solution (75, 50, 30, 25, 15, 10 and 5%) and were tested for their effects on germination and seedling growth of *V. radiata*.

Germination and seedling growth test: Twenty five seed of *N. sativa* were germinated in Petri dishes on Whatman filter paper with 5 mL of seed aqueous extracts or distilled water as control. Three replicates were incubated in a randomized complete block design at 20°C in an incubator with fluorescent light. Germination criteria were the emergence of the radical through the pericarp. Germination percentages were recorded every day and total seedling length was measured after 3 days of incubation using five seedlings taken randomly from each dish.

Statistical analysis: ANOVA test was used to determine the level of significance within the *Vigna radiata* regarding the effect of *N. sativa* seed aqueous extract on germination and seedling growth of *V. radiata*. Significance of differences was accepted when $p \leq 0.05$.

RESULTS

Germination and seedling growth: After the first day of imbibition (method 1), germination percentage of *V. radiata* seed were gradually deceased significantly as the concentration of the aqueous seed extract of *N. sativa* increased (Fig. 1). While in the second and third day of imbibition the inhibition of germination was observed only at the higher concentration in comparison with water control.

After 3 days of imbibition, the total seedling length of *V. radiata* was significantly decreased with increased the concentration of the *N. sativa* seed aqueous extract in comparison with water control (Fig. 2). Moreover, the inhibition of root length was more than shoot length specially at the higher concentrations (Fig. 3).

After the first day of imbibition (method 2), germination percentage of *V. radiata* seed were gradually deceased significantly as the concentration of the aqueous seed extract of *N. sativa* increased (Fig. 4). While at the 2nd and 3rd day of imbibition the inhibition of germination was observed only at the higher concentration in comparison with water control.

The total seedling length of *V. radiata* was significantly decreased with increased the concentration of the *N. sativa* seed aqueous extract in comparison with water control (Fig. 5). Moreover, the inhibition of root length was more than shoot length specially at the higher concentrations (Fig. 6).

Seed aqueous extracts prepared either by method 1 or 2 was inhibited germination and seedling growth of *V. radiata*. But method 2 was the most effective one specially in root length.

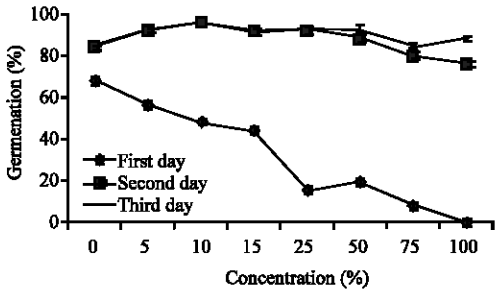

Fig. 1: Effect of different concentration of *N. sativa seed* aqueous extract on germination percentages of *V. radiata* (Standard Error had been shown)

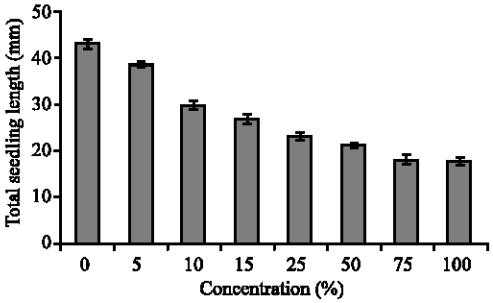

Fig. 2: Effect of different concentrations of *N. sativa* aqueous extracts on total seedling length (mm) of *V. radiata* (Standard Error had been shown)

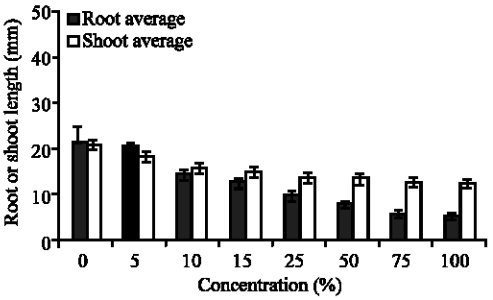

Fig. 3: Effect of different concentration of *N. sativa* aqueous extracts on root or shoot length (mm) of *V. radiata* (Standard Error had been shown)

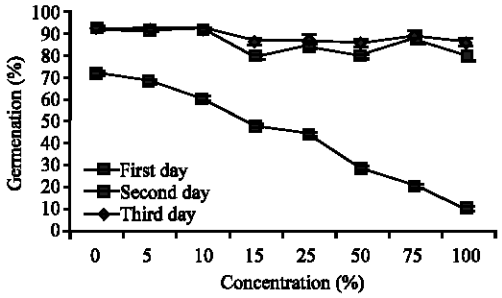

Fig. 4: Effect of different concentration of *N. sativa* aqueous extracts on germination percentage of *V. radiata* (Standard Error had been shown)

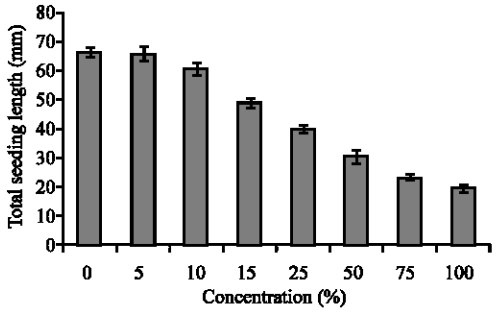

Fig. 5: Effect of different concentration of *N. sativa* aqueous extracts on total seeding length (mm) of *V. radiata* (Standard Error had been shown)

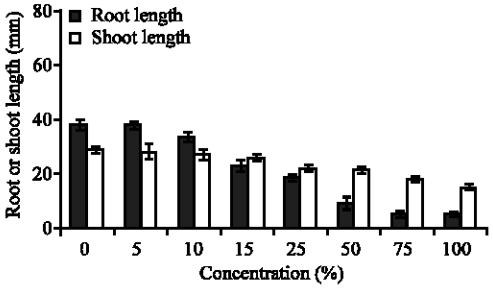

Fig. 6: Effect of different concentration of *N. sativa* aqueous extracts on root or shoot length (mm) of *V. radiata* (Standard Error had been shown)

DISCUSSION

Seed aqueous extract of *N. sativa* exerted inhibition effects on germination and seedling growth of *V. radiata*. Severe toxicity was observed at high concentrations and moderate toxicity at low concentrations in comparison with water control. There by *N. sativa* seed may contain some toxic substance (s) that inhibits germination and seedling growth of *V. radaita*. Aqueous extract of some plant species may contain some toxic substance (s) (Habib and Abdul Rehman, 1988). These substances probably inhibit the germination and seedling growth of other plants species (Al-Charchafchi *et al*., 1987), which was due to their interference with indol acetic acid metabolism, or synthesis of protein and ions uptake by the plants (Hussain and Khan, 1988).

The present results indicated that aqueous extract of *N. sativa* seeds capable of inhibiting root length more than shoot and the toxic compounds of the aqueous extract which in turn may inhibit cell division (Rietjens and Alink, 2003) which is highly active in meristimatic tissue in the growing root tip. These results were in agreement with that observed by Atoum *et al*. (2006) and Modallal and Al-Charchafchi (2006).

There was a significant correlation between seed aqueous extract concentration of *N. sativa* and the result of germination and seedling growth inhibition of *V. radiata*, indicated that toxic compound(s) was responsible agent for toxic effects of *N. sativa* on germination and seedling growth *V. radiata*. Previous investigation reported that aqueous extract of some plant species contains phenolic compounds (Atoum *et al*., 2006). These phenolics inhibit the germination and seedling growth of same plant species or others by their effects on metabolic processes of germination and growth (Castro *et al*., 1984). Therefore, some soluble allelochemicals such as phenolics might be released from seed aqueous extract of *N. sativa* to the environment

which had inhibitory effect on germination and seedling growth of *V. radiata*. This interpretation consists with that found by other researcher (Xu *et al*., 2003).

This research needs further investigation to determine the nature of chemical components of *N. sativa* seed aqueous extract by using thin layer chromatography or high performance liquid chromatography. Then test their activity against germination and seedling growth of *V. radiata*.

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