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Effect of pesticide stress on seed priming and germination traits of *Nigella sativa* under laboratory conditions

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Key words: Malathion, ROS (reactive oxygen species), stress germination percentage, seedling, seed viability index.

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Abstract

Laboratory experiments were completely randomized was conducted at Seed of *N. sativa* to study the effect of malathion pesticide in University of Hail 2013. To create pesticide stress 0.02 0.04 0.06 and 0.08 µM of 58% of malathion concentration prepared in double distill water with no stress serve as control. Time period of the experiment was 10 days from the sowing of seeds under various conc. of pesticide. Stress resulted in the loss of seedling length, 21,18,and 10mm on comparing with control 25mm. Germination percentage (62 ,42,and 16%, control 94%) and seed viability index (6.5, 7.56, and 1.6 control 23.50) respectively, concentration of 0.08 µM proved to be lethal for all the growth parameters as seeds became dead. It can be concluded that pesticide stress significantly decreased germination and growth parameters of seedlings of medicinal plant *Nigella sativa*.

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Introduction

The seeds of *Nigella sativa* (family: Ranunculaceae), commonly known as Black Seed, Black Cumin, or "*Habbatul Barakah*", have long been used in folk medicine in the Arabian Gulf region, Far East Asia, and Europe Black Seed is recommended for a wide range of ailments modern day medicine In the Unani Tibb system of medicine, black cumin (*Bunium bulbocastanum*) is regarded as a valuable remedy for a number of diseases. Sayings of the Islamic prophet Muhammad underline the significance of black cumin. According to a hadith narrated by Abu Hurairah, he says, "I heard Allah's Apostle saying, "There is healing in black cumin for all diseases except death. An authentic saying of the Prophet Muhammad (Peace Be upon Him) about black seed isalso quoted in AlBukhari (Al Bukhari, 1976).

Pesticides play an important role in the effort to increase food production in today's agriculture but, at the same time, they cause environmental hazards because of their toxicity (Berglöf, 2002). It was reported that the oxidative stress is the principle manifestations of metalaxyl-induced toxicity (Kaloyanova F,1991). Oxidative stress was reported to lead to increase in the production of reactive oxygen species(ROS). If ROS formation exceeds the capacity of antioxidant, ROS can react with macromolecules such as lipid, protein, and DNA which lead to cell dysfunction and damage. The germinated seeds can fulfill the requirements of the modern nutritional science for whole-food compared to seeds, Germination and seedling establishment are critical stages in the plant life cycle. In crop production, stand establishment determines plant density, uniformity and management options (Cheng and Bradford,1999).Seed germination is first critical and the most sensitive stage in the life cycles of plants (Ahmed, 2009) and the seeds exposed to unfavorable environment conditions may have to compromise the seedlings establishment (Albuquerque and Carvalho, 2003). The germination of seeds is inhibited under stress (Abid *et al*, 2011, Li *et al*., 2011, He *et al*., 2011) Razmjoo *et al.* (2008) found that increasing of stress decreased almost all of growth parameters in *Nigella sativa*.The aim of this study was to evaluate the effects of various concentration of pesticide stress on *Nigella sativa*,on its different growth parameters *viz:* seedling length, germination percentage, and seed viability index.

Material and methods

Collection of Nigella sativa seeds

In order to study the effect of different concentration of malathion on germination of the seeds of *Nigella sativa* ,the seeds were procured from local grocery shop in Ha,il and the malathion was also purchased from the local nusery,of Hail, ., an experiment was conducted in College of Applied medical science, Department of Clinical laboratories (female branch) University of Hail in 2013.The experiment was a completely randomized one with three replications.

Preparation of solution

The experimental treatments were composed of four different levels of concentration 0.02, 0.04 0.06 and 0.08 µM of Malathion solution prepared in double distill water.The seeds were disinfected by alcohol 70% (for 10 seconds), sodiumhypochlorite 10% (for 60 seconds) and benomyl 2:1000 (for one minute) (Seghatoleslami, 2010). Afterwards, the seeds were rinsed with distilled water twice. Each experimental unit included a sterilized petri dish with the diameter of 12 cm in which 15 seeds were inoculated in each petri plates under aseptic conditions on damp filter paper moistened with different concentration of malathion solution at (27±2°C) and incubated in dark till the initiation of sprouting (3 days),. In order to check the proper seedling development in the petri plates, experimental samples were examined every alternate day and it was confirmed that the seedlings developed normally without drying or displacement of the sprouted seeds or entangling of root during seedling development.

Morphological observations

Morphological observations were made on each day. Fifty seeds were taken at random from three replications of petri plates. The following parameters were observed after every alternate day till 10 days to

evaluate the germination behavior.

Determination of Length of seedlings

Length of seedlings was measured in (mm) after every alternate day till 10th day.

Germination Percentage

Total number of germinated seeds in each petri dish until the 12th day was regarded as the germination percentage.

Seed viability index

Seed viability index was determined by the following equation (Seghatoleslami, 2010).

Seed viability index = germination percentage *average seedling length (mm) /100

Results and discussion

Effect on seedling length

A significant and regular increase in the seedling growth was observed in the control (Fig. 2) from day first to last 10th day, in contrast to the seeds grown under stress. Stressed seeds on day 4, with 0.06 and 0.02µM showed the same seedling length (12mm) but later variation were observed (21 and 18mm on 10th day) in their growth length. Late germination of seedling was observed under 0.06 µM stressed seeds, indicating adversely affected by high concentration of stress as the seedling length remain same on 8th and 10th day (10mm) the chemicals damaged cells, blocked photosynthesis, and stunted growth in varying ways, this was supported by the findings of Kobbia, I. A.(1991) as his findings include specific growth rate, cell number, chlorophyll a level, and dry weight yield significantly decrease with increasing pendimethalin concentrations. However 0.08 µM stress proved to be the lethal dose for the seeds as no germination was observed, till the 10th Day. Pesticides have breakdown products (metabolites) that are more toxic than the parent compound Similar reduction in plant growth was also reported by Prodgers and Inskeep, (1981).

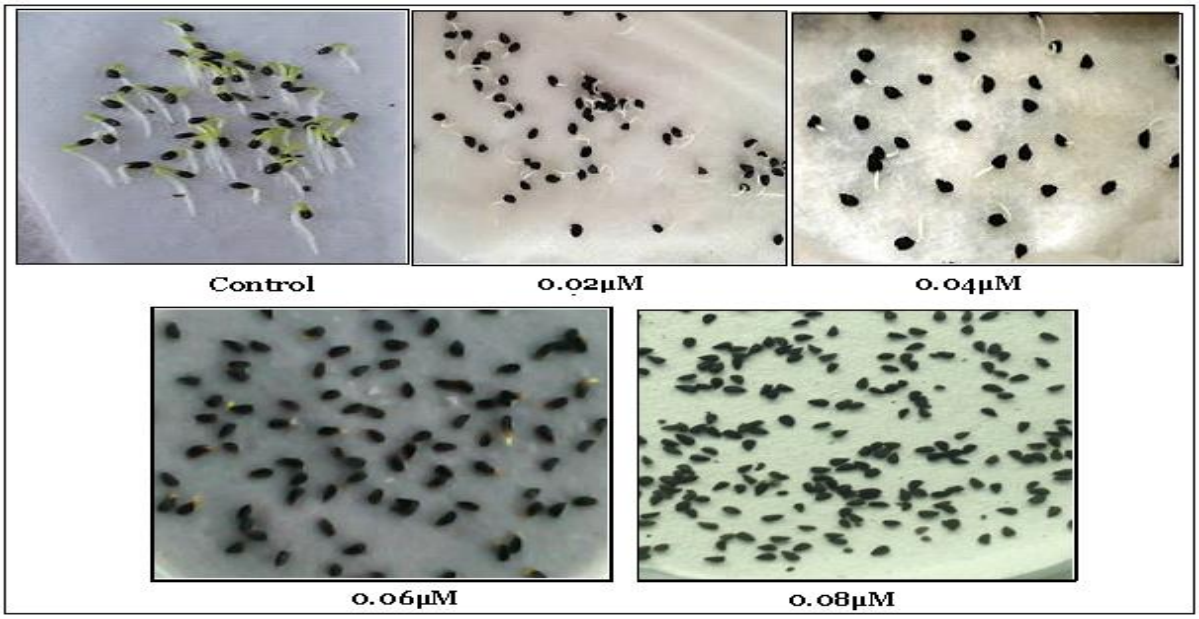

Fig. 1. Germination of *Nigella sativa* seeds under various conc. of malathion stress on 10th day.

Effect on germination

From Fig:3 germination percentage can be estimated, seeds grown under the normal distill water,Fig:3 showed germination rate up to 94%, without any sign of contamination, on the other hand seeds grown under stress showed decrease in germination on comparing with control 62% (0.02µM), 42% (0.04µM),and least 16% (0.06µM) . The decrease in seed germination of *N. sativa* can be attributed to the accelerated breakdown of stored food materials in

seed by the application stress. No germination takes place in the seeds soaked in 0.08µM since day one. , some pesticides have breakdown products (metabolites) that are more toxic than the parent compound. It seems that, decrease of germination percentage and germination pace is related to reduction in water absorption into the seeds at imbibitions and seed turgescence stages. (Hadas, 1977).

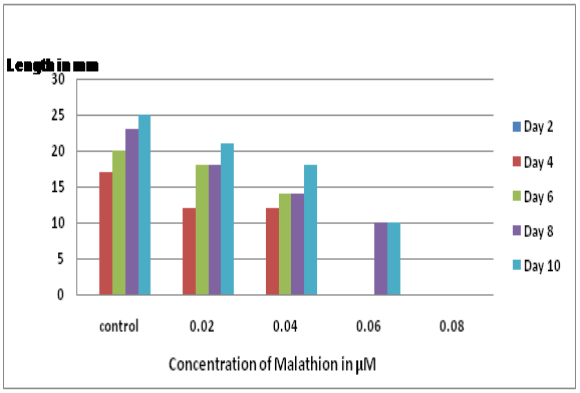

Fig. 2. Effect on the seedling length under various conc. of pesticide stress on medicinaI herb *N.sativa*.

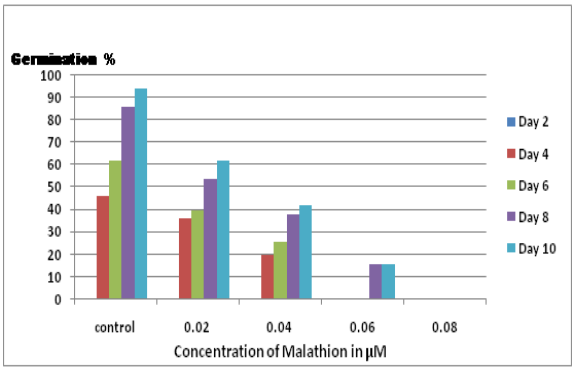

Fig. 3. Effect on the Germination under various conc. of pesticide stress on medicinal herb *N.sativa*

Seed viability index

The obtained results from Fig: 4 showed constant increase in the seed viability index grown under normal condition (control), Highly significant decrease in the seed viability index was observed in the seeds under stress on comparing with control (23.5%). Lowest seed viability index was observed in0.06µ M stressed conc. (1.6%) , 0.02 and 0.04µM stressed conc. Showed 6.5 % and 7.56% seed viability index.0.08 µM conc. Seemed to be lethal for seeds. The high concentrations of stress in the environment impede the seed germination by imposing toxicity in seeds (Rajabi and Postini, 2005, Atak *et al.*, 2006). Fig.1 shows the response of germination of *N.sativa* seeds towards malathion under various concentration on10th day.

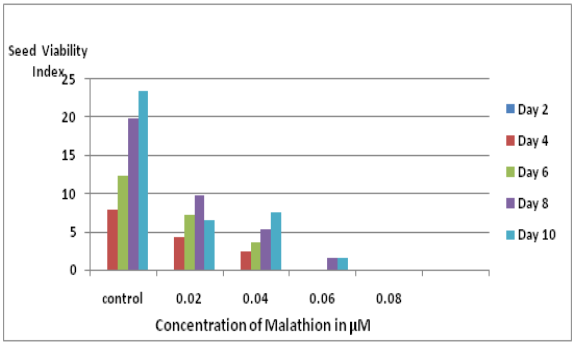

Fig. 4. Seed viability index under various conc. of pesticide stress on medicinal herb *N.sativa*.

Conclusion

Germination is a natural biological process that every higher plants exhibit, during which the seed at rest starts to grow under favorable environmental conditions. The sprouted seeds can fulfill the requirements of the modern nutritional science for whole-food. In the present study pesticide stress adversely affected the seedling length, germination percentage and seed viability index of *Nigella sativa* it can be concluded that pesticide stress significantly decreased all studied traits.

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