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Analysis of Anti-Mosquito and Antimicrobial Activities of The Extract of *Nigella sativa* (Black seeds)

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ABSTRACT

Nigella sativa (Black seed) is an annual flowering plant, belongs to the Ranunculaceae which has tremendous usage in medicine. In the treatment of digestive tract disorders, including gas, colic, diarrhea, dysentery, constipation, and hemorrhoids, the black seed is used. It is also used for asthma, allergies, cough, bronchitis, emphysema and other respiratory conditions. The seeds (50 g) were crushed, extracted with 70% methanol and filtered through filter paper. Antibacterial activity of the extract was assessed using the spread plate method against the *Candida albicans*. Different concentration of the extract i.e., 20, 40, 60, 80, 100% made and applied against mosquito larvae to determine the larvicidal activity of the extract. The results showed that the extract exhibited significant antimicrobial activity against *C. albicans* on the culture plate as compared to control. Different dilution showed larvicidal activity in a dose-dependent manner; however, 50% of larvicidal was noticed in all dilutions after 24 h. However, maximum activity was recorded in 60, 80, and 100% dilutions after 72 h. The results showed that the extract was extremely effective against the microbes even at the concentration of 50%. Similarly, the extract was very effective against microbes and could be used as an antimicrobial agent.

KEY WORDS: NIGELLA SATIVA, BACK SEEDS, ANTI-MOSQUITOES, LARVICIDAL, ANTIMICROBIAL.

INTRODUCTION

Nigera sativa (Black seed) is a herbaceous plant, grows annually; however, is also cultivated in different parts of the world, including Asia, Africa, and America. Black seeds are derived from the common fennel flower plant (*Nigella sativa*) of the Kennebug family (*Ranuncula family*) (Ahmad et al., 2021; Soltane et al., 2021). Black seeds have long been known and used for medicines (Ali and Meitei, 2011). The black seeds have been also used and recommended for the treatment of minor disorders such as skin conditions and allergies (Prashar et al., 2004). Prophet Muhammad (PBUH) illumined in the medicinal importance of the black seed. A large number of studies have been reported in a detailed review on the medicinal use of super foods of plant origin, based on the recommendations of Prophet Muhammad, (PBUH) by Ali et al., (2018).

In recent years, research in the field of agriculture has focused on the economy of plants influenced by selective minor elements. Secondary metabolites from plant extract acts as reducing and stabilizing agents to produce new metallic nanoparticles through bio-reduction reaction (Jang et al., 2020). Comparatively, using a biological method for synthesizing nanoparticles is superior to a

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non-biological (chemical, physical) one, the former is inexpensive easy, and eco-friendly while the latter is highly toxic and hazardous for living organisms, (Bharali et al., 2013 Ullah et al 2020).

The mosquitoes are the carrier of several diseases known as vector-borne diseases. The diseases are including yellow fever, malaria, dengue fever, and many others. Black seeds have reported being toxic against the larvae as well as adults of the *T. castaneum* even at extremely lower concentrations (Dagli et al., 2015). Moreover, plants source are a potent source for treating several diseases such as Cancer, Malaria, Human immunodeficiency virus (HIV), Hepatitis, and many other serious ailments (Dris et al., 2017). it has been mentioned in one study about the punctuality and application of plant extract in different fields including medicine, nutrition, and energy (Enan, 2005).

On the other side, the application for curing different bacterial and viral diseases in pharmacological industries is a remarkable advantage (Sabbah et al., 2020; Helaly et al., 2020). Several studies have demonstrated the antibacterial activity of black seed oil against a variety of microorganisms (Ait Said et al., 2015). This effect is shown against *Salmonella* spp, *E. coli, Shigellas* pp. The biological method is cost-effective than others due to the abundant availability of biological entities easy procedure, hence highly explored for syntheses of nanoparticles (Zuzarte et al., 2012). The present study was designed to determine the larvicidal and antimicrobial activities of the extract of black seeds.

MATERIAL AND METHODS

Methanolic extraction: The black seeds were rinsed in distilled water for removing surface dust particles. After washing the seeds were kept under sunlight for drying. The seeds were crushed with the help of liquid nitrogen and mortar. The powered seeds were transferred to the flask containing 70% methanol. After homogenization, the mixture was incubated on a magnetic stirrer at room temperature for two days. The mixture was filtered by using Whatman filter papers no.1. Furthermore, the filtrate was dried and was dissolved in deionized distilled water.

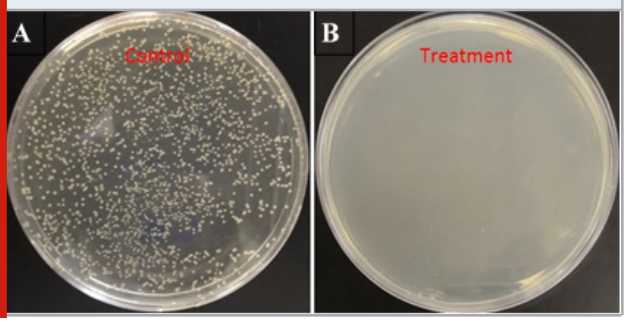
Antimicrobial activity of the extract: The fungicidal activity of the biogenic silver nanoparticles was assessed against the infectious fungi *Candida albicans*. This activity was determined using the colony-forming method. The *C. albicans* was cultured on PDA (potato dextrose agar) at 37°C, for 48 hours. Potato dextrose agar media were prepared and autoclaved. The autoclave media was transfer to the Petri plates and wait until solidification. The extract was applied on the plates followed by the transfer of *C. albicans* culture. The plates were incubated for 48 hours at 37°C.

Larvicidal Bioassay of the extract: The larvae of the mosquito were obtained from the Mosquito Research Laboratory, King Abdulaziz University Saudi Arabia. The larvae were reared in containers (25 × 35 × 6 cm) filled with up to the marked water supplemented with artificial food. The colony was maintained under the following conditions: 27°C ± 2°C, 70% ± 5% relative humidity. Different dilutions of an extract of black seed i.e., 20, 40, 60, 80, 100% (w/v) were used to assess the larvicidal activities. The larvae were transferred to each well of the six-well culture plates. The larvae were given standard larval food and the temperature was kept 28 ± 2°C. After 24 h of exposure, percentage mortality was noted. The experiment was repeated three times.

RESULT AND DISCUSSION

Antimicrobial activity of the extract: The spread plate method was used to assess the antimicrobial activity of the extract against *C. albicans*. The spread plate method is widely employed to check the susceptibility of chemical components of extracts against the microbes. The results revealed that the extract consisted of metabolites, responsible for antimicrobial activity against *C. albicans* (Fig. 1A and 1B). The surface of the plates i.e., control and treated showed the efficacy of the extract. No colony was observed on the plate treated with the extract. Hazardous effects associated with the usage of synthetic chemicals, it is important to introduce natural products, of plant origin. The plant origin compounds will reduce the harmful effects in the environment. The results of our study revealed that the extract showed 100% fungicidal activity against *C. albicans*. It has been reported that the aqueous extract of *N. sativa* exhibits an inhibitory effect against *C. albicans* (Ait Said et al., 2015). In the other study, Al-Jabra et al. reported that the ether extract of *N. sativa* seed showed inhibitory effects against *T. interdigitale*, *T. rebrand* (Zuzarte et al., 2012).

Figure 1: Antimicrobial activity of the extract of black seed against *Candida albicans* grown on potato dextrose Agar. (A) *Candida albicans* grown without application of extract represents control. (B) *Candida albicans* has grown with the application of extract.

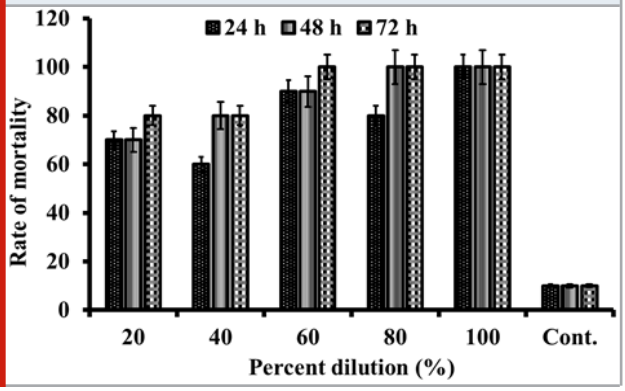


Larvicidal activity of the extract: The extract showed great larvicidal activity against the larvae of the mosquitoes. The rate of mortality shown in results (Fig. 2) represented the range of mortality in the mosquito larvae and revealed the insecticidal activity of ethanol extracts. The per cent mortality of the extract was directly related to the concentration of the extract as well as the time of exposure. The results exhibited that the maximum larvicidal activity was recorded by 60,

80, and 100% dilutions after 72 h of exposure. However, more than 50% mortality was recorded in all dilutions even after 24 h. In addition, the extract showed great larvicidal activity against the larvae of the mosquitoes in a dose-dependent manner.

The percent mortality of the extract was directly related to the concentration of the extract as well as the time of exposure (Abutaha et al., 2018). It has been reported that extracts of plants such as *Ageratum conyzoides* and *Cassia sophera* induced mortality in mosquitoes (Ghosh et al., 2012). Moreover, a *Mentha piperita* extract resulted in maximum insecticidal activity against *B. brassicas* with increased concentration and time exposure (Mersha et al., 2014). Similarly, essential oil from *Cinnamomum zeylannicum* was found to be effective against *B. brassicae*. The results showed that the extract was extremely effective against mosquitos and also showed antimicrobial activities.

Figure 2: Larvicidal activities of different dilutions of the extract of black seeds.



CONCLUSION

The results showed that the extract was extremely effective against mosquitos and also showed antimicrobial activities. The methanolic extract was dried and dissolved in different concentrations in water e.g., 20, 40, 60, 80, and 100%. All the dilutions were effective and killed the mosquito at their larval stages even within 24 h. similarly, the extract showed great antimicrobial activities against *C. albicans*.

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