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Evaluation of Potential Botanical Extracts against Root-Knot Nematode Infestation in Date Palm

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ABSTRACT

The date palm (*Phoenix dactylifera* L.) is an important and popular palm tree from the *Palmaceae* family. The production of date palms is impacted globally by several abiotic and biotic diseases. Globally, plant parasitic nematodes are the remarkable production constraints affecting yield and quality. By producing knots and galls in plant roots, root-knot nematode develops a particular bond with its host. To overcome this particular issue, comprehensive research has been conducted in the research area of the plant pathology department at the University of Agriculture, Faisalabad. The best control of *Meloidogyne* spp. was observed in a consortium of treatments with Neem, 10%, which showed the best results in the number of galls, root length, and number of nematodes present in soil. Garlic 2.5% extracts showed the best results for fresh root and shoot weight and dry root shoot and root weight in the presence of nematode. Treatment with 5% Moringa extracts showed the best result for several leaves. Treatment with chili 10% showed the best results for chlorophyll content and treatment with 10% aloe vera showed the best result in plant height. Plant extracts have been demonstrated to have biocontrol capabilities that can be utilized to prevent nematode infestation and reduce plant microbial infections on date palms. Further investigation on biological control strategies should be done on the infected plants as these are eco-friendly.

Keywords: *Phoenix dactylifera* L, *Meloidogyne*, Antagonistic, Botanical extracts, Infestation, Consortium.

INTRODUCTION

Date palm, or *Phoenix dactylifera* L., is a member of the *Palmaceae* family and mainly cultivated for its delicious fruits and attractive beauty, not only fruit, but also has some other valuable byproducts, like fodder and fuel wood, leaves for basketry, and house wood (Tengberg, 2012). Dates are essential for human nutrition due to their high amount of critical nutrients and the fact that they offer millions of farmers in dry regions of the world stable rural living. Dates are produced worldwide from 1.8 million tons in 1962 to more than 8.0 million tons today. Global warming-related climate change has had an impact on flora and fauna all across the planet, particularly in arid regions (Moradpour *et al*., 2019). Date palm, or *Phoenix dactylifera* L., is a member of the *Palmaceae* family and mainly cultivated for its delicious fruits and attractive beauty, not only fruit, but also has some other valuable byproducts, like fodder and fuel wood, leaves for basketry, and house wood (Tengberg, 2012). Dates are essential for human nutrition due to their high amount of critical nutrients and the fact that they

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Over the past ten years, the production of date palms has increased by almost 18%, reaching 8.53 million tonnes on 1.11 million hectares of cultivated land. The age of the tree and cultivar affect the date palm's susceptibility to water, salinity, temperature challenges, and pathogens (Alotaibi *et al*., 2023).Date palm plays a vital role in the desert ecosystem as their cluster provides niches to the native wildlife which dramatically depends upon them. They can tolerate a minimum temperature of 5℃ and a maximum of 50℃ but they grow best at a temperature of 12.7-27.5 ℃. Date requires enormously low moisture, very little rainfall, and hot summers (Kruger, 2021). Wherever the date palm is grown, it is susceptible to several diseases and pests. Except for *Meloidogyne* and *Pratylenchus*, other plant parasitic nematodes have not been extensively investigated (Youssef, 2014). *Meloidogyne* spp*.,* a plant parasitic nematode, is among the most destructive parasites that affect date palms (Carpenter, 1964).The most widespread nematode on the entire planet is *Meloidogyne* spp. (Van Den Hoogen *et al*. 2019). Due to *Meloidogyne* spp. Infection, around 30 to 50 % of the yield is lost which is estimated to be 100 billion U.S. dollars (Perpétuo *et al*. 2021). When the growing portion of the cotyledonary sheath becomes infected, it causes severe losses and even can kill the seedlings.

Root-knot nematodes in heavily infested soil kill about 90% of the date palm seedlings (Griffith and Koshy, 1990). The presence of *F.oxysporum* with the *Meloidogyne* spp. in the soil and their association make these pathogens more severe. This type of relationship enhances the severity of the disease and causes plant wilting (Dean *et al*., 2012). Among the Phytoparasitic nematodes, Meloidogyne spp. has been ranked first (Sasser, 1989). The release of effector proteins from *Meloidogyne* spp. Esophageal gland cells through their stylets into host roots are a crucial characteristic of stationary plant-parasitic nematodes. A preliminary image of the intricacy of sedentary nematode parasitism at the molecular level is now beginning to emerge as a result of the ongoing reporting of host targets and biological activities of several worm effectors. These mechanisms control everything from the host plants' vulnerability to the induction of defensive reactions (Ereno, 2012). *Meloidogyne* spp. are widespread and economically significant plant pathogens. Root-knot nematodes show sexual dimorphism that is males are vermiform, whereas the females are pyriform or saccate (Escobar *et al.,* 2015). It's still a long way off to controlling root-knot nematode populations by switching them to communities of men with few or no females through the practical induction of sex reversal (Eisenback and Triantaphyllou, 2020). Plant parasitic nematodes are very difficult crop pests to be controlled (Agrios, 2005). Around the world, various strategies have been used to manage the nematode problem. Chemical nematicides are noteworthy among them (El-Ansary and Al-Saman, 2018). Herbicides, insecticides, fungicides, and nematicides are routinely employed to reduce pest pressure to fulfill the expanding food demand (Sarkar *et al*., 2021). Pesticides and fertilizers, both organic and inorganic, are now often employed in agricultural production (Baweja *et al*., 2020). For the first time, chemical-based insecticides like Organochlorine (OCl) were utilized to manage pest populations in 1940. However, because most pesticides are harmful to other animals, bioaccumulate, and are not environmentally friendly, they are not ideal. Alternative control strategies for the management of nematodes must be developed because of the toxic compounds of chemicals and their effect on beneficial flora and fauna present in the soil (Forghani and Hajihassani 2020). Numerous studies have shown the nematicidal effects of plant extracts on plant-parasitic nematodes (Bello *et al*., 2006). There have also been reports of several plant parts having nematicidal properties that efficiently killed RKNs (Yahia *et al*., 2020). Keeping in view the importance of plant extracts and their role in toxic residue-free residual management approval, it was planned to apply plant extracts against root-knot nematodes in date palms.

MATERIALS AND METHODS

Collection and cultivation of date palm seedlings and inoculation

The current experiment was conducted in Randomized Complete Block design (RCBD). Seedlings of date palms were collected from different research areas. Forty-seven (47) Seedlings were grown on the pots. The sample for nematode inoculum was collected from the roots and shoots of affected date palms by using the Baermenn funnel method in the laboratory of nematology.

Preparation of plant extracts

The five plant extracts were prepared by grinding fresh leaves of neem, moringa, gel of Aloe vera, fruits of chili, and tuber of garlic to control the *Meloidogyne* spp. After preparation extracts were sterilized. Standard concentrations (2.5%, 5%, and 10%) of the plant extracts were used. 20 ml of each extract was applied to date palm seedlings. Neem, Garlic, chili, moringa,

and Aloe vera extracts (Table 1) were used to check their efficiency against *Meloidogyne* spp.

Inoculum preparation and pathogen identification

Infected soil and root samples were used to isolate the respective nematodes as inoculum. For this purpose, the isolation of nematodes was done from roots and soil samples by following the Whitehead and Hemming tray method and the Baermann funnel method. After isolation, the species of nematodes were recognized by using the perennial pattern of female nematodes. Based on the perennial pattern the purity of culture was assured.

Table 1: Pesticidal activity of plants.

S. No	Common Name	Botanical Name	Compounds having pesticidal activity
1	Neem	*Azadirachta indica*	Nimbin, *azadiradione,* Azadirachtin *meliantriol*
2	Aloe vera	*Aloe barbadensis miller*	lupeol, sulfur salicylic acid, phenols cinnamonic acid, and, urea nitrogen,
3	Garlic	*Allium sativum*	Trisulfide, diallyl disulfide, diallyl sulfide, diallyl tetrasulfide, and,2-dithiin
4	Moringa	*Moringa oleifera*	catechol tannins, saponins gallic tannins, flavonoids, alkaloids, anthraquinones, steroids
5	Chili	*Capsicum annuum*	Capsaicin, dihydrocapsaicin, polyphenols

Application of extracts

Application of available extracts was done in earthen pots by using the soil drench method. After two weeks of transplanting, different extracts were applied to have three replications for each treatment. The concentration of each extract was prepared according to recommended doses.

Data collection of different plant-pathogen parameters plant characters

The number of leaves was counted manually for each plant. Leaves were counted with the break of at least one week as the data for every single plant were recorded. Then we observed how many leaves were produced between the two consecutive observations of data recorded. To measure the height of the plant, a measuring scale was used. It was measured from the base of the plant to the top of the plant after every week. Chlorophyll content was measured after a week with a chlorophyll meter (Minolt SPAD-502 DL Meter) from the leaves according to standard procedures (Wellborn, 1994). The root length of date palms was measured in centimeters with the measuring scale and compared the root length of the plant was different treatments. After recording all parameters, harvesting was done and the roots were thoroughly cleaned then the weight of the root and shoot were weighed on a weighing balance. The weight of dry root and shoots was done by wrapping the roots and shoots in wrapping paper and placing them overnight at 70°C. The next day weight of dry shoots and roots was weighed on a weighing machine and data were recorded. The number of galls was counted from infested date palm roots followed by the applications of different treatments according to (Din *et al*. 2018).

Statistical analysis of data

The (ANOVA) analysis of variance was done in (RCBD) Randomized Complete Block design and the means of all treatments were compared by using the (LSD) least significant difference test with a 95% level of confidence (Steel *et al.,* 1960).

RESULTS

Evaluation of pathogen parameters

Plant roots ware observed for collection of galls, number of galls was counted and data were recorded. The results exhibited highly significant difference among the treatments for number of galls. The mean comparison showed the treatment with 10 neem in the presence of nematode show minimum value for No. of galls (Figure 1). Whereas, treatment with garlic 2.5% presence of nematode revealed maximum no. of galls.

The mean comparison of various treatments showed significant effect on number of nematodes. The treatment with Neem 10% in presence of nematode showed minimum number of nematodes in the soil where treatment with aloe vera 2.5 % had highest numbers of nematodes in soil (Figure 2).

Evaluation of growth parameters

Chlorophyll contents were measured for 8 weeks with the help of a chlorophyll meter from leaves, and data were recorded. The results showed highly significant behavior for chlorophyll contents for all treatments ($P < 0.0001$). The mean comparison

showed that the treatment with 2.5% garlic Aloe vera in the presence of nematode showed a minimum value for chlorophyll content (Figure 3). Whereas, treatment with chili 10% in the presence of nematode revealed maximum value for chlorophyll content (Figure 3). Every week, plant height was measured with a measuring scale, and data was recorded. The results exhibited in figure 4 showed highly significant differences among the treatments for plant height. The mean comparison showed that the treatment with garlic 2.5% in the presence of nematode showed a minimum value for plant height (P = 0.0001). Whereas, treatment with aloe vera 10% in the presence of nematode revealed maximum value for plant height (Figure 4).

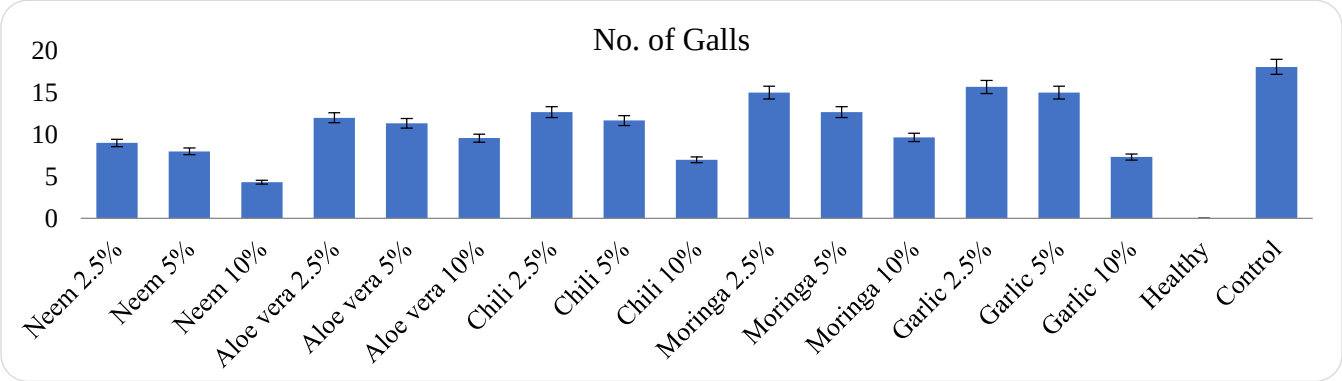

Figure 1: Effect of botanical extracts on gall formation in date plants.

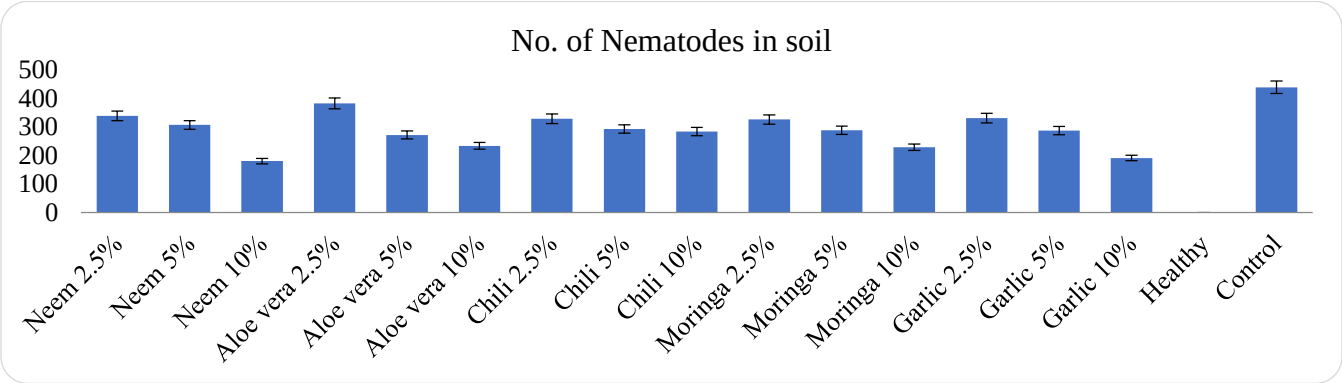

Figure 2: Effect of botanical extracts on nematode population in soil of date plants.

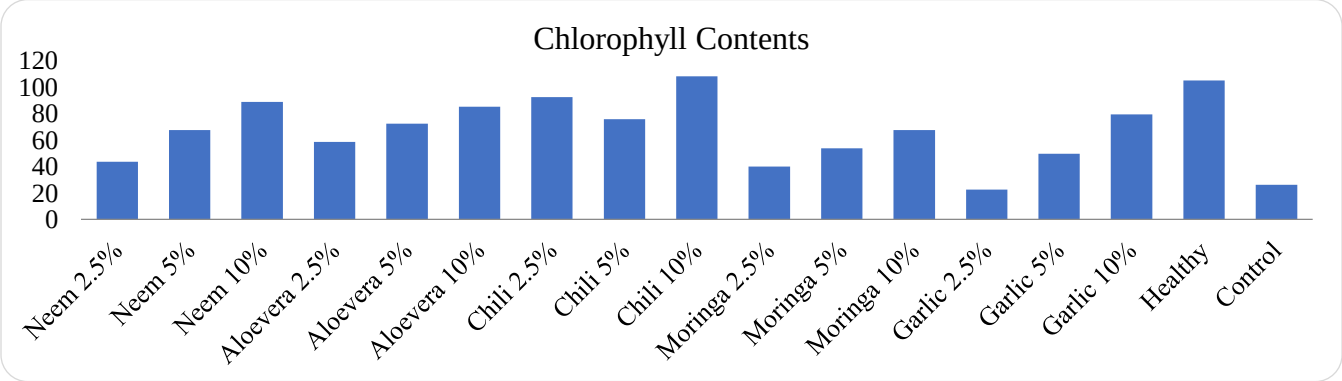

Figure 3: Effect of botanical extracts on chlorophyll contents in date plants.

Roots lengths were measured with a measuring scale and the data were collected. The variation among the treatments was tested by the analysis of variance (ANOVA) significant differences were shown among the treatments (P= 0.0005). The mean comparison showed that the treatment with Chili 5% with the presence of nematode application showed minimum root length

(11.6 cm) as shown in figure 5. Whereas, the treatment with Neem 10% in the presence of nematode shows the maximum value for root length (18.6 cm) (Figure 5).

All seedlings from the pots were uprooted and after that roots were cut with the help of a scissor their weight was measured by weighing balance and data were recorded. The ANOVA demonstrated significant differences for fresh root weight ($P<0.0005$). The mean comparison showed that the treatment with moringa 10% presence of nematode showed minimum value for fresh root weight (0.63 gm). Whereas, treatment with garlic 2.5% in the presence of nematode revealed maximum value for fresh root weight that was 4.0 gm (Figure 6).

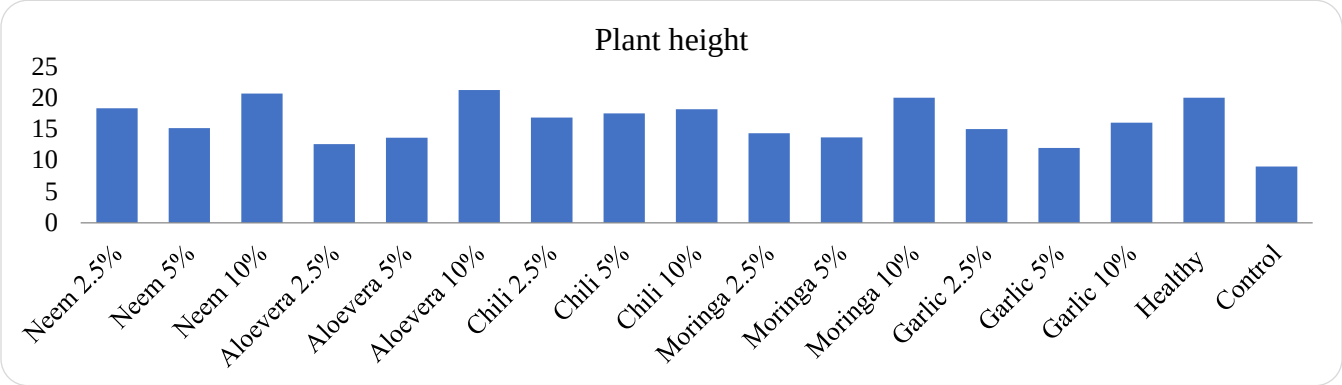

Figure 4: Effect of botanical extracts on plant height in date plants.

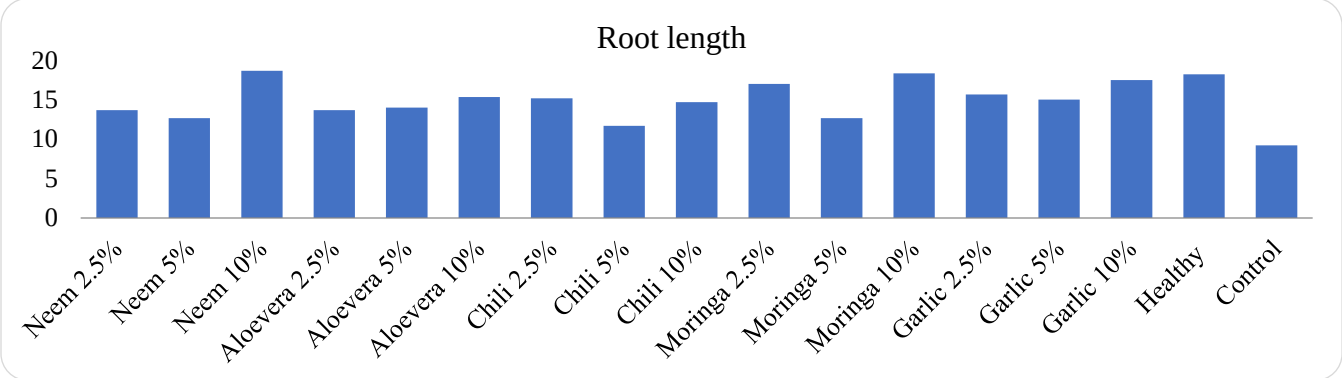

Figure 5: Effect of botanical extracts on root length in date plants.

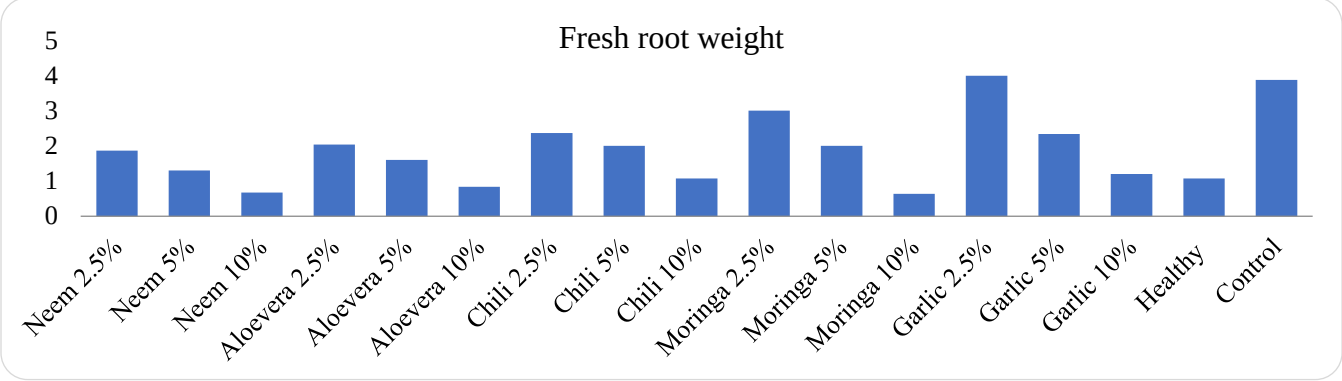

Figure 6: Effect of botanical extracts on fresh root weight in date plants.

Furthermore, the mean comparison of fresh shoot weight showed that the treatment with Neem 10% in the presence of nematode revealed minimum value for fresh shoot weight (1.83 gm). However, the treatment with Garlic 2.5% in the presence of nematode showed maximum value for fresh shoot weight that was 4.0 gm (Figure 7).

The roots were weighed using a balance after being dried in an oven, and the results were noted. The mean comparison showed that the treatment with Neem 2.5% in the presence of nematode showed minimum value for dry root weight (1.4 gm). However, on the other hand, the treatment with garlic 2.5% in the presence of nematode revealed maximum value for dry root weight that was 3.26 gm (Figure 8). Furthermore, the shoots were sun-dried and weighed by the weighing balance and the average comparison of the various treatments revealed no significance difference for dry shoot weight. In the presence of nematodes, treatment with 2.5% neem demonstrated the lowest value of dry shoot weight (1.93 gm). Conversely, the garlic treatment that included 2.5% nematode presence demonstrated the highest value for shoot dry weight that was 3.83 gm (Figure 9).

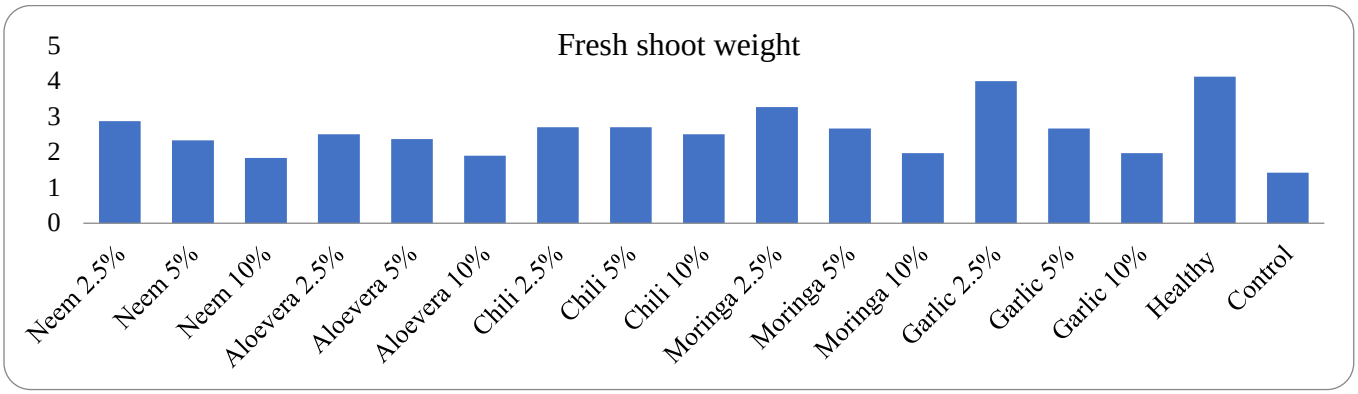

Figure 7. Effect of botanical extracts on fresh shoot weight in date plants.

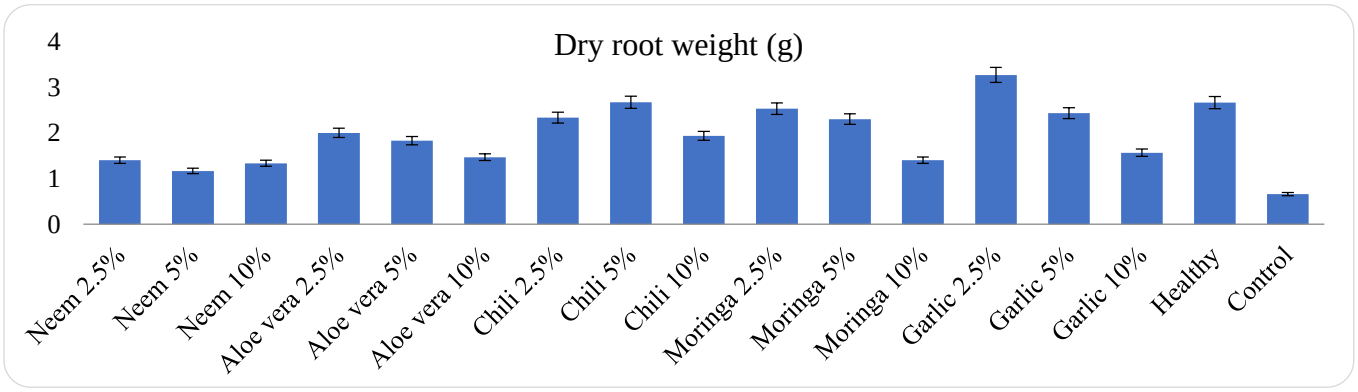

Figure 8: Effect of botanical extracts on dry root weight in date plants.

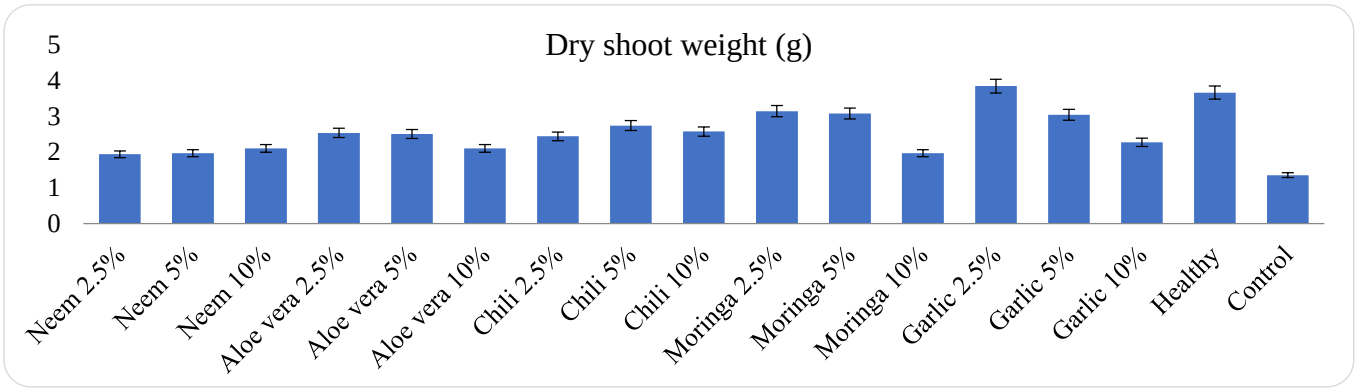

Figure 9: Effect of botanical extracts on dry shoot weight in date plants.

DISCUSSION

The date palm tree is a versatile crop that produces a wide range of goods with several advantages. The generated products

can be separated into biomass materials, which are regarded as secondary goods having a variety of uses, and date fruit, a primary product with nutritional and medicinal benefits whether ingested directly or processed into other products (Hussain *et al*., 2020). Date palms are susceptible to a variety of pathogens that cause significant reductions in the number of trees and their production. Several fungal bacterial and nematodal attacks on date palms have been recorded (Baraka *et al*., 2011). Among these pathogens, *Meloidogyne* species which are also known as root-knot nematodes, are widespread, destructive, and economically important parasites of the crop (Kayani *et al*., 2012). Globally, 5-43% yield losses occur due to RKN (*Meloidogyne spp*.) (Khan *et al*., 2014). And RKN has been recognized as a substantial threat to crop yields (Kinlock and, Sprenkel, 1994; Sasser and Kirby, 1979; Hussain *et al*., 2012). The interpreted results showed the similarity with the current work.

Pest revival is the quick resurgence of the pest population in dangerous numbers after the use of a pesticide (Skendžić *et al*., 2021). The primary cause of pest reemergence is thought to be the use of chemical pesticides, which can also destroy significant natural enemies (Zaller, 2020). In South Asia, carbofuran is frequently used to control nematodes, although it is persistent, has a half-life of 30 to 60 days, and is extremely poisonous to creatures other than the target. (Tomlin, 1997). The use of chemical substances must be reduced to its minimum level and alternative methods for the management of nematodes must be used such as cultural control and biological control (Folorunso *et al*., 2021). Biological control strategies are one of the most environment-friendly methods to control the nematode attack on plants. In place of chemical pesticides, plant extracts are increasingly widely utilized as environmentally acceptable methods for biological control of parasite pests, including root-knot nematodes (Haroon *et al*., 2018). Our research has focused on plant extract used as a biological control agent. Five plant extracts were used to check their efficacy on root-knot nematode control in date palms. Aqueous extracts of neem, moringa aloe vera, chili, and garlic were used at the concentration of 2.5%, 5%, and 10% for each to get the best result. Aquas extracts have reduced the nematodes and their effect on plants which shows that these plants have compounds that have toxic effects on root-knot nematodes and can strengthen plant defense mechanisms against nematodes or any other pathogenic microorganisms. In comparison to the control treatments, a significant rise in the plant parameters demonstrates that the extracts have a harmful effect on inhibiting the active juvenile nematodes (Bawa *et al*., 2016).

CONCLUSION

It was concluded that all botanical extracts have shown a positive result for the management of nematodes not only on the mortality but some treatment has a positive effect on plant growth, chlorophyll content, and height. The improvement in plant characteristics like plant height, chlorophyll content in the leaf, no of galls, and plant weight, points to a potential nematicidal effect of the extracts utilized in this study on the root-knot nematodes. Further investigation on biological control strategies should be done on the infected plants as these are eco-friendly.

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AUTHOR CONTRIBUTIONS

All authors contributed equally in the manuscript.

COMPETING OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this research paper.