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EVALUATION OF NEEM PRODUCTS AGAINST DAMPING OFF DISEASE OF TOMATO

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ABSTRACT

Tomato damping off is one of the most serious and widespread disease in Pakistan and the world, due to the harmful effects of synthetic chemicals to humans, environment and animals, this study aims to manage them. The use of plant extracts has been recognized as one of the most encouraging and safe options for eco-friendly disease management. Therefore, this study was conducted to check the efficacy of different neem products e.g. Neem seed, Neem seed coat, Neem seed without coat. Dry neem leaves, Saw dust and Bio-neem compost on the tomato seeds, by dipping for 3 and 5 minutes and then sown in steam-sterilized soil artificially infested with *Fusarium oxysporum* f.sp. *lycopersici*. The maximum seed germination, root length (cm), shoot length (cm), root weight (g) and shoot weight (g) were recorded when the seeds were dipped for 5 minutes in neem seed decoction, followed by bio-neem compost and neem seed decoction for 3 and 5 minutes respectively. Seed germination and whole plant growth were reduced when untreated seeds were sown in artificially infested soil followed by seeds dipped in dried neem leaves decoction for 3 minutes and neem seed decoction (without coat) for 3 minutes. The seedling mortality was decreased as a time of posture of dipping of seeds was increased, and that was devoted to uninoculated soil. The seedling was increased as the age of tomato plants increased. The mortality was decreased after 30 days of sowing followed by 45 days of sowing, and when the seeds were treated with a decoction prepared from neem sawdust and neem seed decoctions as well as bio-neem compost, respectively. Interestingly, neem products are considered as a potential plant for controlling tomato damping off for the first time in the current study. According to the results, it can be used as a potential plant source for eco-friendly control of tomato damping off.

Keywords: Botanical control, Disease management, *Fusarium oxysporum*, *lycopersici*, Neem extract.

INTRODUCTION

Tomato, *Lycopersicon esculentum* (Mill.) are rich in vitamin C, mineral salts and are recommended for patients with constipation, diabetes, heart, and body diseases (Alajrami and Abu-Naser, 2020). Tomato is the second largest vegetable produced in Pakistan (Sajjad *et al.*, 2011). It is also an economically important crop of Hyderabad, Badin, Thatta, Mirpurkhas, Nawabshah, Karachi, Naushahro Feroze, Larkana and Sukkur districts of Sindh (Pakistan). It is a relatively long season vegetable crop of the tropical world and is a major source of income (Khokhar, 2013).

It is one of the most susceptible vegetable crops causing 70 to 95% yield losses due to diseases in tropical regions (Lukyanenko, 1991). The world over reported diseases of tomato are about 59, including 17 viral, 28 fungal, 4 bacterial, 3 plant-parasitic nematode, and 4 miscellaneous diseases or disorders (Jones *et al.*, 2015). Among these diseases, damping-off of seedlings and wilt of the adult crop (*Fusarium oxysporum* f. sp. *lycopersici*, *Rhizoctonia solani*, *Pythium* spp. and *Verticillium albo-atrum*), fruit rot (*Alternaria tenuis*) are the most common and destructive disease of tomato (Figure 1)

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(Gunasekaran *et al.*, 1994; Jiskani *et al.*, 2021; Lucas and Campbell, 2012).

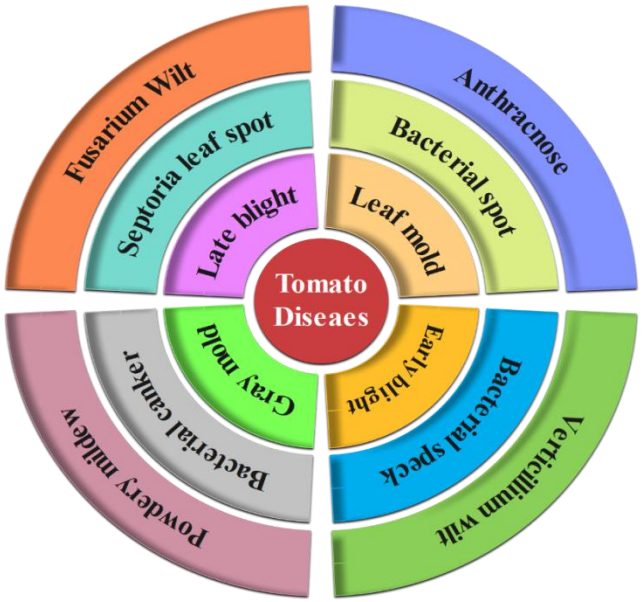

Figure 1. Most common diseases of tomato

Seed treatment provides an excellent anti-damping-off effect in the process of plant growth and development and can promote plant growth for a long time (Salman and Abuamsha, 2012). The use of fungicides as seed treatment remained dominant throughout the world, with a 68% share, followed by insecticides at 11% in the market (Schwinn, 1994). Several synthetic fungicides have been used in the control of commercially important crop diseases. However, their continued use in the agricultural system has brought some side effects to the agricultural ecosystem and the health of consumers (Pandey *et al.*, 2016). Seed dressing fungicides are quite dangerous for both human as well as animal health, and even their judicious uses make the environment polluted.

The literature available on the disease indicated that very little work had been reported on tomato damping-off disease in Sindh (Pakistan), hence keeping in view the losses caused by the disease and various health hazards due to non-judicious use of pesticides, the present research work was conducted to assess the effect of different neem products against damping-off of tomato seedlings caused by *Fusarium oxysporum* f. sp. *lycopersici*.

MATERIALS AND METHODS

Studies on the effect of different neem products (Figure 2) against *Fusarium oxysporum* f. sp. *lycopersici* causing tomato damping-off, seedlings were carried out in the Department of Plant Pathology, Sindh Agriculture University, Tandojam (Pakistan).

Collection of diseased samples and isolation of fungus: Tomato plants with damping-off disease symptoms were collected from different fields surrounding Tandojam. The diseased specimens were taken in plastic bags and carried to the laboratory of Department of Plant Pathology, Sindh Agriculture University, Tandojam for isolation of fungus.

The isolation was done through standard methods by cutting small pieces from the infected samples and treating with 0.01% $HgCl^2$ solution for 1 minute each. The surface-sterilized five tissues were kept in Petri dishes containing sterilized potato-dextrose-agar (PDA) medium. All the plates were incubated at room temperature (30±10°C) for 7-10 days. The dominantly isolated fungi were sub-cultured and multiplied from time to time throughout the entire research experiment.

Identification of the causal fungus: The predominantly isolated disease-causing fungus was purified and identified by studying typical colony characteristics, mycelial growth, and the presence of conidia, with the help of microscope observations using standard diagnostic keys.

Effect of different neem products on seed germination, plant growth, and damping-off disease: Before sowing, tomato seeds were dipped in various neem products (Figure 2) to observe their effects on tomato seed germination, plant growth, and tomato damping-off.

Preparation of stock solution: The 5% solution of each neem product except bio-neem compost was prepared by thoroughly washing and grinding. 50g of each were macerated individually in the blender with 250ml of distilled water, and then 1.0g of washing powder was added. The extracted solution was kept for 16 hours and, after that, added more 50 ml distilled water to each neem extract product to get 5% concentration.

Seed treatment: Seed treatment was made separately with various neem products (Figure 2) at different dipping timings (3 and 5 minutes). 50 seeds/pot, treated and untreated, were seeded in pots containing 2 kg of steam-sterilized soil infested with a fresh culture of Fusarium oxysporum f. sp. lycopersici. The untreated seeds on infested and un-infested soil were served as control.

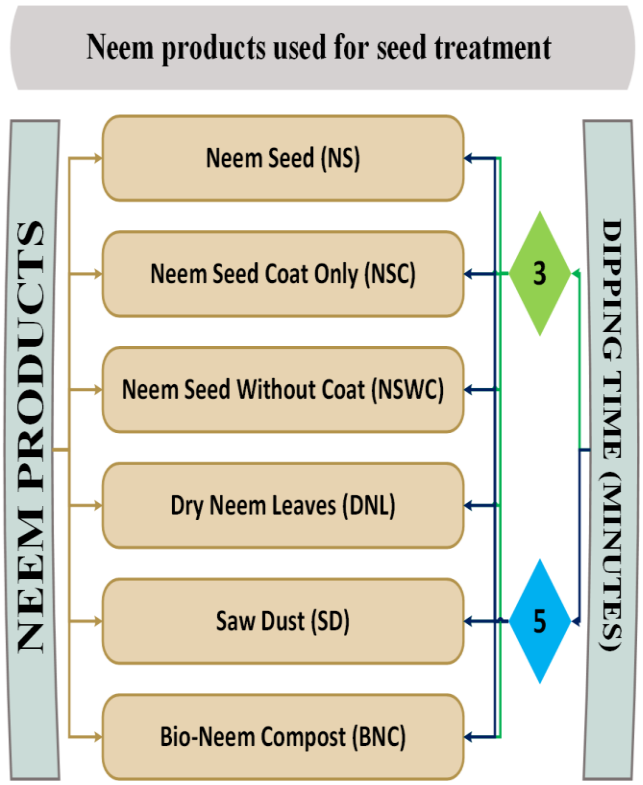

Figure 2. Various neem products used for seed treatment

* Obtained from LRSDA, Ratanabad, Mirpurkhas.

Observations: The observations were recorded on germination percent after 08 and 10 days of sowing. The mortality percentage was calculated by calculating the number of dead plants 30 days and 45 days after sowing. The data of shoot and root length (cm), weight (g), and development of the disease were recorded.

Randomized complete block design (RCBD) was used in the experiment with 4 replicates. The data were statistically analyzed with the computer software "Student Edition of Statistix" $P < 0.05$, and the analysis of variance was performed.

RESULTS AND DISCUSSION

Symptoms of the disease: The damping-off disease affected the seed, seedling, and roots of the tomato plants. Seeds grown on infested soil fail to germinate. Young seedlings became died (pre-emergence damping-off). Seedlings that have previously appeared also damaged at roots and stems nearly at or below the soil line. The infected plants also showed yellowing of leaves become stunted and gradually cover large root areas. The entire root gets rotted and finally dried up. Similar symptoms are reported by Zulfikar *et al.* (2018), who stated that post-emergence damping-off attacks seedlings as they emerge above ground, pinching off the stem tissue at or just below the soil line. Black *et al.* (1991) reported that *Rhizoctonia solani*, *Pythium* spp. and *Fusarium* spp. are worldwide distributed, causing damping-off and root rot in tomato. Plants become stunted due to root rots or stem collar rot. Under severe conditions, the stems become girdled, followed by drooping and dying of plants. The diseases can occur either before or after emergence of seedlings.

Identification of the causal fungus: The infected root pieces showed brownish discoloration of the fungus. The fungus produced white cottony mycelial growth with a large quantity of single-celled micro-conidia and curved macro-conidia. Therefore, the fungus was identified as *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.). Wong (2003) by studying colony characteristics as described by (Barnett and Hunter, 1972; Ellis, 1971) morphology of micro and macroconidia as reported by Gunasekaran *et al.* (1994); Kuprashvili (1996) and Lucas and Campbell (2012) also reported that damping-off of tomato seedlings and wilt of adult crop caused by *Fusarium oxysporum* f. sp. *lycopersici*. El-Shami *et al.* (1993) and Gunasekaran *et al.* (1994) also observed the association of *Fusarium oxysporum* f. sp. *lycopersici* from tomato seedlings causing a high level of damping-off disease.

Effect of different neem products on seed germination: The germination percent and germinated seeds of tomato plant was significantly increased with the usage of neem products as compare to control. The number of germinated seeds/pot was noted higher in NS after 8 and 10 days (35.67 and 41.67) with germination percent of 71.33 and 83.33% (Table 1) respectively, when seeds were dipped in neem seed decoction for 5 minutes. It was followed by BNC (33.00 and 39.00) with seed germination percent of 66.00 and 78.00%, as compared to seeds dipped in neem seed decoction for 3 minutes (32.67 and 37.00) with germination percent of 65.33 and 74.00%. The germinated seeds/pot and germination percent was lower in untreated and inoculated seeds (19.33 and 25.67 seeds/pot with 38.67 and 51.33%) followed by Untreated uninoculated seeds (28.33 and 31.67 seeds/pot with 56.67 and 63.33 germination percent) on 8 and 10 days after sowing.

Table 1. Effect of neem products on seed germination of tomato seedlings

Neem products	Dipping time (Minutes)	Germinated seeds		Germination %	
		8 Days	10 Days	8 Days	10 Days
NS	3	32.67	37.00	65.33	74.00
	5	35.67	41.67	71.33	83.33
NSC	3	22.00	28.00	44.00	56.00
	5	29.33	34.00	58.67	68.00
NSWC	3	21.33	26.67	42.67	53.33
	5	24.67	30.33	49.33	60.67
DNL	3	21.00	26.00	42.00	52.00
	5	23.00	29.67	46.00	59.33
SD	3	28.67	32.67	57.33	65.33
	5	31.67	36.33	63.33	72.67
BNC	3	30.00	35.67	60.00	71.33
	5	33.00	39.00	66.00	78.00
Untreated inoculated	-	19.33	25.67	38.67	51.33
Untreated uninoculated	-	28.33	31.67	56.67	63.33
P < 0.05	-	3.92	4.79	-	-

Effect of neem products on plant height and length (cm) of tomato seedlings: All the products showed a highly significant difference in shoot length, but their response was non-significant regarding root length. The maximum shoot and root length with a total plant height of tomato were recorded in 45 days after sowing with 5 minutes dipping time (5.25 cm and 9.75 with 15.00 cm plant height) in NS (Table 2), followed by BNC (5.00 and 9.13 cm with 14.13 cm plant height). The maximum shoot and root length with a total plant height of tomato were recorded in 45 days after sowing with 3 minutes dipping time (4.88 and 8.88 cm with 13.75 cm plant height) in NS, followed by BNC (4.63 and 8.57 cm with 13.25 cm plant height). Whereas, the minimum shoot and root length (3.63 and 6.48 cm with 10.25 cm plant height) were recorded in untreated inoculated treatment.

Table 2. Effect of neem products on plant height (cm) of tomato seedlings

Neem products	Dipping time (Minutes)	Length (cm)		Height (cm)
		Root	Shoot	
NS	3	4.88	8.88	13.75
	5	5.25	9.75	15.00
NSC	3	3.75	7.25	11.00
	5	4.38	8.50	12.88
NSWC	3	3.75	7.13	10.88
	5	4.00	7.63	11.63
DNL	3	3.63	7.13	10.75
	5	3.75	7.50	11.25
SD	3	4.25	7.88	12.13
	5	4.75	8.75	13.50
BNC	3	4.63	8.57	13.25
	5	5.00	9.13	14.13
Untreated inoculated	-	3.66	6.48	10.25
Untreated uninoculated	-	4.25	8.25	12.50
P<0.05	-	N. S	1.14	-

N. S= Non-Significant

Effect of different neem products on tomato plant weight: The root and shoot weight were also increased (0.12 and 0.22 g) in seeds when dipped for 5 minutes in NS with 0.34 g total plant height (Table 3), followed by BNC for 5 minutes (0.11 and 0.21 g with 0.31 g total plant weight, NS for 3 minutes (0.10 and 0.20 g with total 0.30 g plant weight) as compared to a SD for 5 minutes (0.10 and 0.20 g) with 0.30 g plant weight, BNC for 3 minutes (0.10 and 0.19 g, with 0.29 g plant weight), and un-treated and un-inoculated seeds (0.010 g and 0.19 g with plant height (0.28 g) after 45 days of sowing. The minimum shoot length (0.07 and 0.13 g) with plant height 0.20 g was observed in un-treated inoculated seeds followed by seeds dipped DNL decoction for 3 minutes (0.07 and 0.14 g, shoot weight with 0.22 g plant weight), a decoction of NSWC for 3 minutes (0.07 and 0.16 g with plant height 0.25 g), NSC for 3 minutes (0.08 and 0.18 g, with 0.26 g plant weight), seeds dipped in DNL for 5 minutes (0.09 and 0.18 g, having plant weight 0.26 g) NSWC for 5 minutes (0.09 g root and 0.19 g shoot weight with 0.28 g plant weight) and a decoction of SD for 3 minutes (Table 3).

Table 3. Effect of neem products on plant weight of tomato seedlings

Neem products	Dipping time (Minutes)	Weight (g)		
		Root	Shoot	Total Plant weight
NS	3	0.10	0.20	0.30
	5	0.12	0.22	0.34
NSC	3	0.08	0.18	0.26
	5	0.10	0.19	0.29
NSWC	3	0.07	0.18	0.25
	5	0.09	0.19	0.28
DNL	3	0.07	0.14	0.22
	5	0.09	0.18	0.26
SD	3	0.09	0.19	0.28
	5	0.10	0.20	0.30
BNC	3	0.10	0.19	0.29
	5	0.11	0.21	0.31
Untreated inoculated	-	0.07	0.13	0.20
Untreated uninoculated	-	0.10	0.19	0.28
$P < 0.05$	-	N. S	N. S	-

N. S= Non-Significant

Effect of different neem products on mortality of tomato seedling: The mortality of tomato seedlings due to damping-off disease caused by *F. oxysporum* f. sp. *lycopersici* were varies from one to another product. The mortality was decreased as the period for dipping of seeds was increased; there was no mortality when untreated seeds were sown in uninoculated (uninfested) soil, but the mortality was increased as the age of seedlings was increased. However, there were significant differences among the treatments at $P < 0.05$ (Table 4) when the seeds were treated by dipping for 3 and 5 minutes and sown in artificially infested soil. The maximum mortality (5.33 and 8.33 with 20.78 and 32.47%) on 30 and 45 days after sowing was recorded when untreated seeds were sown in artificially infested soil (Table 4). The minimum mortality (1.00 to 3.33 died plants with 2.40 to 12.82%) was recorded on 30 days after sowing, followed by 45 days after sowing (2.00 to 4.33 died plants with 5.51 to 16.66%) seed treated with SD and seed decoction as well as BNC showed better performance by reducing seedling mortality as compared with other neem products.

Table 4. Effect of neem products on mortality of tomato seedlings

Neem products	Dipping (Minutes)	No. of germinated seeds (10 days after sowing)	30 days after sowing		45 days after sowing	
			No. of died plants	Mortality (%)	No. of died plants	Mortality (%)
NS	3	37.00	1.00	2.70	2.33	6.31
	5	41.67	1.00	2.40	2.33	5.60
NSC	3	28.00	2.67	9.52	3.67	13.09
	5	34.00	1.67	4.90	2.67	7.84
NSWC	3	26.67	3.00	11.25	4.00	15.00
	5	30.33	2.33	7.69	3.33	10.99
DNL	3	26.00	3.33	12.82	4.33	16.67
	5	29.67	2.67	8.99	4.00	13.48
SD	3	32.67	2.33	7.14	3.00	9.18
	5	36.33	1.00	2.75	2.00	5.51
BNC	3	35.67	1.00	2.80	2.67	7.48
	5	39.00	1.00	2.56	2.33	5.98
Untreated inoculated	-	25.67	5.33	20.78	8.33	32.47
Untreated uninoculated	-	31.67	0.00	0.00	0.00	0.00
P < 0.05		4.79	0.93	-	0.77	-

Meanwhile, the effect of different neem products on seed germination, plant growth (height and weight) and on mortality of tomato seedling due to damping-off varies from one to another product, when the seeds were treated by dipping for 3 and 5 minutes and sown in artificially infested soil. There were significant differences among the treatments at P <0.05. The maximum seed germination, shoot and root length and heaviness (total plant height and weight) were recorded when the seeds were sown in artificially infested soil after dipping for 5 minutes in NS, followed by dipping in BNC for 5 minutes and NS for 3 minutes. Significantly minimum seed germination, shoot and root length, and heaviness (total plant height and weight) were recorded when untreated seeds were sown in artificially infested soil followed by dipping seeds in DNL decoction for 3 minutes and NSWC decoction for 3 minutes. Whereas, the mortality was decreased as the period for dipping of seeds was increased; there was no mortality when untreated seeds were sown in uninoculated (uninfested) soil, but the mortality was increased as the age of seedlings was increased. However, the maximum mortality was recorded when untreated seeds were sown in artificially infested soil. The minimum mortality was recorded on 30 days after sowing followed by 45 days after sowing and when the seeds were treated with a decoction prepared from SD and NS as well as BNC represent better performance with reference to minimum mortality due to damping-off of tomato seedlings as compared with other neem products. Ali *et al.* (1992) also tested neem oil, leaf extract, and pericarp dust from (*Azadirachta indica*) against isolates of *Penicillium italicum, Alternaria alternata,* and *Aspergillus niger* and found effective in checking the growth of these fungi. Walia *et al.* (1994) reported that soil amended with neem (*Azadirachta indica*) leaves significantly increased tomato plant growth and decreased the incidence of both *Rhizoctonia bataticola* (*Macrophomina phaseolina*) and *R. solani* as compared with the unamended control. Sharma (1995) reported that neem (*Azadirachta indica*) leaf powder/extracts protected tomato fruits from fungi compared with untreated fruit. Khan and Saxena (1997) reported the greatest improvement in tomato plant growth in neem-cake amended soil. Amrendra *et al.* (1997) also reported that the effects of different combinations of 10 organic amendments and 3 chemicals on the growth and yield of tomato (*Lycopersicon esculentum* Mill.) were studied and results showed that the plant height, girth, leaf/plant, branch/plant and spread of tomato were significantly increased by the combination of 10 organic amendments and 3 chemicals. Results represented by Kumaran *et al.* (1998) showed that a mixture of Farmyard manure (FYM), neem cake, Azospirillum, phosphobacteria, and NPK gave the best results in terms of growth and yield. Bhonde *et al.* (1999) stated that different preparations of

Azadirachta indica (neem) extracts could be used as potential fungicides against many plant pathogenic fungi. However, Mohammad (2000) reported that treatment with all concentrations of neem-based commercial product and neem seeds solvent extract at various periods of dipping time were not toxic to the tomato seedlings, whereas, improvements of plant weight and height were correlated to the reduction of nematode infestations. Babu *et al.*, 2000a,b assessed the effects of *Azadirachta indica* products (leaf, neem seed kernel, and neem cake) on *Alternaria solani* in field and pot experiments using tomato cv. PKM-1, results showed that the incidence rate of disease decreased compared with the control group. Bowers and Locke (2000) treated the soil infected by *Fusarium oxysporum* f. sp. *chrysanthemi* with 90% *Azadirachtin* neem oil and reported that it reduces the number of pathogens and increases the healthy plant community reveals that the extract played an important role in the biological management strategy of controlling Fusarium wilt diseases. Faruk *et al.* (2001) reported that the treatment of soil with Azadirachtin leaf powder 10 grams per pot, 5 kg of pit soil, and 0.5 tons / ha can significantly reduce disease and improve plant growth (shoot length and weight, root weight). Khan and Siddiqui (2001) reported that *Azadirachta indica* had the greatest effect on the growth and weight of tomato and was the most effective. In other studies, Khan and Rathi (2001) reported that neem cake was the best at 25 q/ha and improved tomato plant growth and yield in comparison to the untreated control. Randhawa *et al.* (2001) also reported a significant increase in height and weight of tomato seedlings when used neem cake, fresh leaves of *Melia compacta, Melia azadirachta* [*Azadirachta indica*], and other amendments. Seedling height in neem cake treatments was increased by 43.2 % and the increase in seedling height was also observed in *Melia compacta* (8.63 cm) and *Melia azadirachta* (8.46 cm) treatments but was non-significant. Agbenin *et al.* (2004) stated that the attractiveness of using chemicals is declining due to health effects. Moreover, in most developing countries in the world, the chemicals needed are often out of reach of farmers. Therefore, they also searched for an alternative mode of control of Fusarium wilt and root-knot, the important diseases of tomato. Field plots without treatment and Furadan treatment were used as control. Neem seed powder significantly reduced the severity of Fusarium Wilt and root-knot disease in the greenhouse and field. The results showed that *Azadirachta indica* seed powder might be used to control root-knot nematode and fusarium wilt syndrome. Abbasi *et al.* (2005) investigated the effects of neem cake on plant-parasitic nematodes, Verticillium Wilt, and seedling damping-off caused by *Rhizoctonia solani* and *Pythium aphanidermatum* showed that neem cake had no direct toxicity to the damping-off. Still, neem cake might have produced a kind of bioactive substance, and the climate during incubation could inhibit the disease.

CONCLUSION

In the present study, it is concluded that the infected root pieces showed brownish discoloration. The fungus produced white cottony mycelial growth with a large quantity of single-celled micro-conidia and curved 3-5 septate macro-conidia. Therefore, the fungus was identified as *Fusarium oxysporum* f. sp. *lycopersici*. Whereas the effect of different neem products on seed germination, plant growth (shoot and root length and weight), and mortality due to damping-off of tomato seedlings varied from one to another product when the seeds were treated by dipping for 3 and 5 minutes and sown in artificially infested soil. All the treatments showed a highly significant difference from one another at $P < 0.05$ and that the tested neem products found to be the most effective against tomato seedlings damping off caused by *Fusarium oxysporum* f. sp*. lycopersici* and enhanced seed germination and plant growth.

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