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ENHANCEMENT ANTIFINGAL ACTIVITIY OF ZINC OXIDE NANOPARTICLES (ZNO-NPS) AND SESAME RESISTANCE AGAINST ROOT ROT CAUSED BY *MACROPHOMINA PHASEOLINA* (TASSI.) GOID., USING MIXTURE OF HYDROGEN PEROXIDE (H_2O_2) AND ACETIC ACID.

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Abstract

Six typical isolates that showed microscopic characters of *Macrophomina phaseolina* were isolated from sesame root rot tissue collected from sesame plantations' growing in El-Minya governorate. Pathogenicity tests revealed that isolate M3 was the most virulent in this topical. Thus, further identifications process were applied on isolate M3 using DNA profile. Phylogenic analysis assured that M3 isolate is *M. phaseolina*. An exogenous utilization of zinc oxide nanoparticles (ZnO-NPs) and/or peroxy acetic acid (PAA) were concerned for study their efficiency to inhibit sesame root rooting fungus *M. phaseolina*, sesame vegetative growth and their potential to control sesame root rot. Either ZnO-NPsor PAA showed antifungal activity. however, ZnO-NPs was more active against *M. phaseolina* than PAA. Sesame vegetative growth showed vigor index (VI) enhancement for single treatment of each compound tested and ZnO-NPs was better than PAA. Combining ZnO-NPs (0.1g/l) with PAA (0.1 g/l AA+1 g/l H_2O_2) was superior to increase vegetate growth. Moreover, substation sesame root rot reduction was addressed by single application of ZnO-NPs or PAA. Combining ZnO-NPs(0.1g/l) with PAA (0.1 g/l AA+1 g/l H_2O_2) gave the greatest efficiency than single application of each compound tested.

Keyword: Sesame root rot – *Macrophomina phaseolina* - Zinc oxide nanoparticles- Peroxy acetic acid (PAA).

INTRODUCTION

One of the oil crops that is thought to be prolific under high-yielding desert soil conditions is sesame (Sesamum indicum L.) **(Bedawy and Moharam, 2018)**. In Egypt, The area cultivated with sesame was 34000 ha, yielding 44000 tonnes of product (**FAOSTAT, 2020).**

Sesame plants are infected by multiple diseases at different stages of their development. One of the most common diseases affecting this crop in Egypt and around the world is charcoal root rot, which is brought on by the fungal pathogen *Macrophomina phaseolina* (Tassi.) Goid (**Mohsen and Ahmed, 2015; Raner *et al*.**, **2023 and Preeti Vashisht *et al*., 2023**). **Vyas (1981)** reported that this disease is a very deadly and destructive disease that causes yield losses ranging from 5 to 100% in all places where sesame is grown. An estimated yield loss of 57% at roughly 40% of disease incidence was reported by **Maiti *et al* (1988).**

Applications of chemical fungicides resulted in hazards towards environmental and human health. Thus, to avoid this problems environmental pollution and human health, must using safety alternatives compounds such as nanoparticles **(Omara *et al.*, 2020 and Elamawi *et al.*, 2016).** Introducing a nano technology in agricultural sector has taken more attention **(Servin *et al*., 2015).** It has been reported that zinc oxide nanoparticles ZnONPs gave an antifungal activity towards plant pathogens **(Elmer and White, 2018).** Peroxyacetic acid (PAA), a more environmentally friendly fungicide substitute, is created by combining acetic acid and hydrogen peroxide (H_2O_2) **(Buschmann and Del Negro, 2012). EL-Ashmony *et al*. (2017), Galal (2017), and Galal (2018)** demonstrated its effectiveness in fostering fungal development and its utility in controlling a variety of plant diseases. H_2O_2 and an organic acid - most often acetic acid - which functions as a "activator" to create the compound peroxyacetic acid are mixed to create an activated peroxy acetic acid disinfectant (Fig. 1), which is made up of H_2O_2 and peroxyacetic acid in solution.

$$H_2O_2 + CH_3COOH \overset{H^+}{\rightleftharpoons} CH_3\overset{\overset{O}{\|}}{C}OOH + H_2O$$

Figure (1): Production of peracetic acid (Buschmann and Del Negro, 2012).

Accordingly, we aimed to 1) isolate the causal root rot pathogen, 2) test the pathogenicity of isolated pathogens, 3) identify the most virulent *Macrophomina phaseolina* isolate and 4) investigate the role of (ZnO NPs) and / or (PAA) on sesame vigor index, *M. phaseolina* growth and sesame root rot infection.

MATERIALS AND METHODS

Samples collection

Samples of sesame plants (*Sesamum indicum* var. Shandawil-3), naturally infected roots were collected (Figure, 2) from different locations in El-Minya governorate. Samples were placed in a paper bags and stored in a cooler.

Isolation and identification of the causal organism(s):

Sesame rooted roots were washed in running tap water, cut into small pieces about 0.5 cm long, surface disinfected with 0.5% $NaOCl_3$ for 3 min, rinsed several times with sterile distilled water (SDW) then dried on sterile towel paper. The prepared pieces (5 mm) were transferred onto Petri dishes containing (PDA) medium then incubated at 25^{o}C for 5-7 days.The isolated fungi were further purified using both hyphal tip or single spore techniques (**Hildebrand**, **1938**) and identified according to **Dhingra and Sinclair (1973**). Six fungal isolates that choose of *Macrophomina phaseolina* were selected in this study.

Pathogenicity tests

The pathogenicity of the obtained isolates through inoculation of sesame plants was performed. The inoculum was grown in flasks (500 ml) sterilized natural medium containing mixture of (60g barely grains, 40g washed sand and 40 ml water). The flasks were inoculated with uniformed agar discs (5 mm in diameters) of desired fungus and incubated at 25ºC±2 for two weeks to obtain sufficient inocula. Healthy apparent sesame seeds cv. Shandawil-3 were used in this study. The test was carried out in pots (30 cm in diameters) in the green house. Pots were sterilized by soaking in formalin solution (5 %) for 5 min, then pots were aerated for 15 days after pot disinfection solution, the disinfested pots were filled with autoclaved clay soil (121ºC for 30 min) mixed with the desired fungal inoculum growing on barely grains at 2.5% (w/w). In check treatment equal amount of the un-inoculated substrate was added. After 7 days, sesame seeds sterilized by soaking in 0.5% $NaOCl_3$ for 3 min and then washed thoroughly three times with sterilized water then sown. The experiment was designed as a complete randomized plots with 3 replicates (3 pots/ replicate) each contains 25 seeds/pot.

Disease assessment:

The disease severity in which was determined by applying the arbitrary (0-5) disease scale as described by **Abd Elrazek *et al.* (1974).** There are five possible infection levels: 0 = no infection, 1 = 1–20% infection, 2 = 21–40% infection, 3 = 41-60% infection, 4 = 61–80% infection, and 5 = 81–100% infection. The severity of the disease was calculated using the following equation:

$$\text{Disease severity (DS, \%)} = \frac{0A+1B+2C+3D+4E+5F}{5T} \times 100$$

Where A, B, C, D, E and F are the numbers of plants corresponding to the numerical grades 0, 1, 2, 3, 4 and 5 respectively, and 5T is the total number of plants (T) multiplied by maximum disease grade 5 **(Sharma *et al.*, 2006).**

Molecular identification of fungal isolate:

The most pathogenic fungal isolate (M3) was subjected for molecular identification, the fungal isolate that was grown in sterile Petri plates containing autoclaved PDA medium and incubated for 7 days at 25°C **(Pitt and Hocking, 2009)**.However, DNA profile was performed as described by **white *et al.* (1990)**

Nanoparticles

Zinc oxide nanoparticles (ZnO-NPs) were purchased from Nanotechnology & Disease survey Research Department, ARC, Giza, Egypt. According to the source, the particles size was 5.95-14.2 nm and the shape is spherical. The characterization and properties of ZnONPs were confirmed through Transmission electron microscope (TEM) (Central Laboratory for Micro analysis and Nano technology, Minia University).

Effect of ZnONPs and/or PAA on *M. phaseolina* mycelial dry weight (MDW):

Unless the otherwise state, a complete randomized plot experiments were designed with three replicates, and each experiment was repeated two times. The efficiency of peroxide acetic acid (PAA), with various concentrations were tried in this study. *Macrophomina phaseolina* (isolate M3) were grown in 250-ml conical flasks, each containing 50 ml of medium. Concentrations were prepared in sterile distilled water and aliquots were pipetted to medium to obtain final concentration of PAA(0.05 g/l AA+0.5g/l H_2O_2), (0.1 g/l AA+1 g/l H_2O_2) and (0.2 g/l AA+2.0g/l H_2O_2) in case of ZnONPS (0,025 g/l, 0.5 g/l and 0.1 g/l) were used. while combined PAA(0.1 g/l AA+1 g/l H_2O_2) with ZnO -NPs 0.0 25 g/l , PAA(0.1 g/l AA+1 g/l H_2O_2) with 0.5g/l and PAA(0.1 g/l AA+1 g/l H_2O_2) with 0.1 g/l ZnONPs. For check treatment, conical flasks containing medium without test compound. One disc of a 4 day old cultures grown on PDA provided the inoculums of each flask; the disc floated. Cultures were incubated at 27ºc and growth was determined by weight after 10 days. Mycelial mats were removed from the solutions, rinsed with distilled water and oven-dried for 24 hr at 75-80ºc prior to autoclaving Three replicates (plates) were used and the experiment was repeated twice. to calculate percentage inhibition of fungal growth was conducted as follow: **(Sutton and Starzyk, 1972)**.

$$\text{Inhibition \%} = \frac{\text{MDW of the control} - \text{MDW of the treatment}}{\text{MDW of the control}} \times 100$$

Seed inoculation and treatments:

The fungal isolate, *M. phaseolina* (M3) was tested to inoculate the seeds of sesame cv. Shandawil -3. The sesame seeds were surface sterilized and washed as mentioned before, then dried on sterile filter paper.

For inoculation, 25 surface-sterilized sesame seeds were placed in each 9 cm diameter Petri dish, and 10 ml of fungal propagules suspension (1×10^4 propagules /ml) of *M. phaseolina* was added **(Hayden and Maude, 1992 and Southwood et al., 2015).** The infected seeds were allowed to dry for an hour at room temperature after the propagules suspension was poured out an hour later. Next, the seeds underwent the following. Ten treatments were ZnONPs (0.025, 0.5 and 0.1g/l), PAA (0.05 g/l AA+0 .5 g/l H_2O_2, 0.1 g/l AA+ 1.0 g/l H_2O_2) and (0.2 g/l AA+ 2.0g/l H_2O_2) while combined ZnONPs (0.025 g/l) with PAA (0.1 g/l AA+ 1.0 g/l H_2O_2), ZnONPs (0.5g/l) with PAA (0.1 g/l AA+ 1.0 g/l H_2O_2) and ZnO NPs (0.1g/l) with PAA

(0.1 g/l AA+ 1.0 g/l H_2O_2). Ten ml of the respective mixed chemicals were added into each Petri dish containing fungal infected seeds and incubated for one hour **(Behrani *et al*., 2015).** For one hour, seeds in the control treatment were just steeped in distilled water that had been sterilised.

Effect of ZnONPs and/or PAA on sesame seed germination and vigor index:

The germination rate, root length, shoot length, and vigour index of sesame cv. Shandawil-3 infected with M. phaseolina were evaluated in relation to the ten treatments previously indicated. For every treatment, there were three duplicates. Ten seeds were placed on a sheet of water-soaked filter paper in each replicate, and the sheet was covered with another wet sheet of paper. The seeds were placed in a 500 mL glass beaker with 50 mL of water to keep the seeds moist and promote germination at room temperature. The two layers of filter paper with the seeds in between were then rolled into a scroll to give the seedlings enough oxygen. To verify the results, the experiment was conducted twice. Ten days later, the germination percentage was determined as follows:

$$\text{Germination percentage (G\%)} = \frac{\text{Number of germinated seeds}}{\text{Number of total seeds.}} \times 100$$

The length of either shoots and roots (cm) of 10 sesame seedlings replicate were assayed and used to calculate vigor index (VI) according to the formula described by **Abdul-Baki and Anderson (1973):**

Vigor index (VI) =(shoot length + root length)× germination percentage.

Effect of ZnONPs and/or peroxyacetic acid (PAA) on sesame root rot infection:

The study examined the impact of the ten treatments described earlier on the percentages of root rot in sesame cv. shandawil-3 that were infected with *M. phaseolina*. Ten treated seeds were sown in a 30-cm pot in each of the three duplicates for each treatment, for a total of three pots per treatment. Sterilised water was used to inoculate the seeds in the control treatment. The entire randomised block design experiment was set up, and it was conducted twice in 2020 (Exp. I) and 2021 (Exp. II). In this investigation, the results were compared using the estimated means of the two trials. Disease assessment was determined as the percentages of root rot after 60 days of sowing.

Statistical analysis

The least significant difference (L.S.D) values at 5 % ($P< 0.05$) were used to test the variants among treatments **(Gomez and Gomez, 1984).**

Results:

Pathogenicity tests :

Data in Table (1) and Figure (2) showed that all isolates tested were infective to sesame plant causing pre-emergence damping-off, post - emergence damping-off and root rot , however pathogenicity was varied with isolate tested. Among six isolates, one isolate (M3) revealed the most virulent that caused the highest disease severity (55.8%) followed by isolate M6 (47.5%) and isolate M2 (42.4%) while isolate M1 induced the least disease severity (38.6%) followed by isolate M5 (39.2%).

To be noticed, all fungal isolates tested were caused an obvious Pre-EDO more than Post-EDO but the root rot severity was the greatest values for each isolates tested.

Fig. 2: Natural infected sesame plant showing root rot and artificial inoculated sesame plant by *Macrophomina phaseolina.* Infected (right) and non-infected (left) plants.

Table(1): Pathogenicity test of *Macrophomina phaseolina* on sesame, Pre EDO, Post EDO and root rot / wilt plants.

Fungal isolates	Pre EDO	Post EDO	RRS	DS(%)
M1	15.3 c [(1) (2)]	3.1d	20.2e	38.6d
M2	13.2e	4.1c	25.1c	42.4c
M3	20.4a	6.1a	29.3a	55.8a
M4	15.1c	4.2c	20.1e	39.4d
M5	14.3d	2.4e	22.5d	39.2d
M6	16.2b	5.1b	26.3b	47.5b
LSD 0.05	0.42	0.25	0.66	1.12

(1) Each reading is an average of 3 replicates, each one contain 25 plants.

(2) Each reading with the same letter is a not significant at 5%.

Molecular identification of the fungal isolate:

The fungal isolate M3, which appeared the most virulent, was subjected for identification. Analysis of its phylogenic (Figure 3) based on IT sequences of rDNA confirmed that M3 is *Macrophomina phaseolina* (Tassi.) Goid.

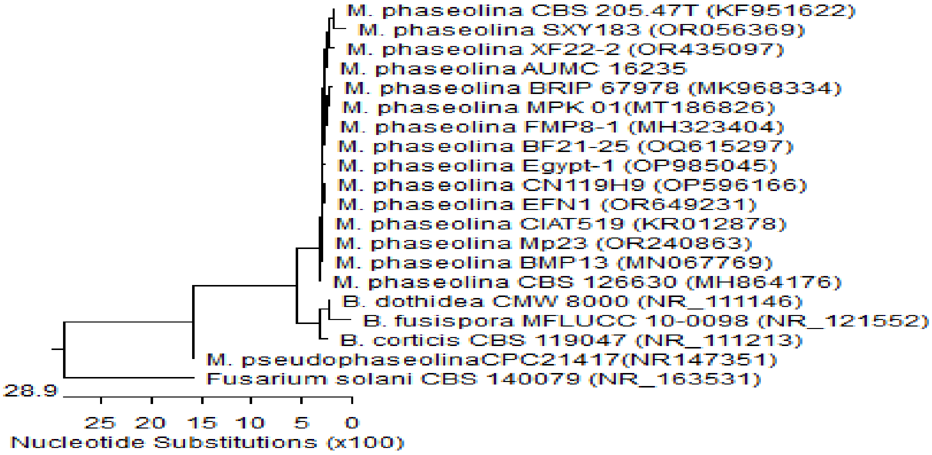

Figure (3): Phylogenetic tree based on IT sequences of rDNA of the fungal samples isolated in the present study (AUMC16235, arrowed) aligned with closely related strains accessed from the GenBank (M= *Macrophomina phaseolina* Accession number for isolate M3 is *OR89500*

Zinc oxide nanoparticles (ZnONPs)

Figures(4) and (5) showed that the particles used in this study were sphericals and their size ranged between 5.95 and 14.2nm.

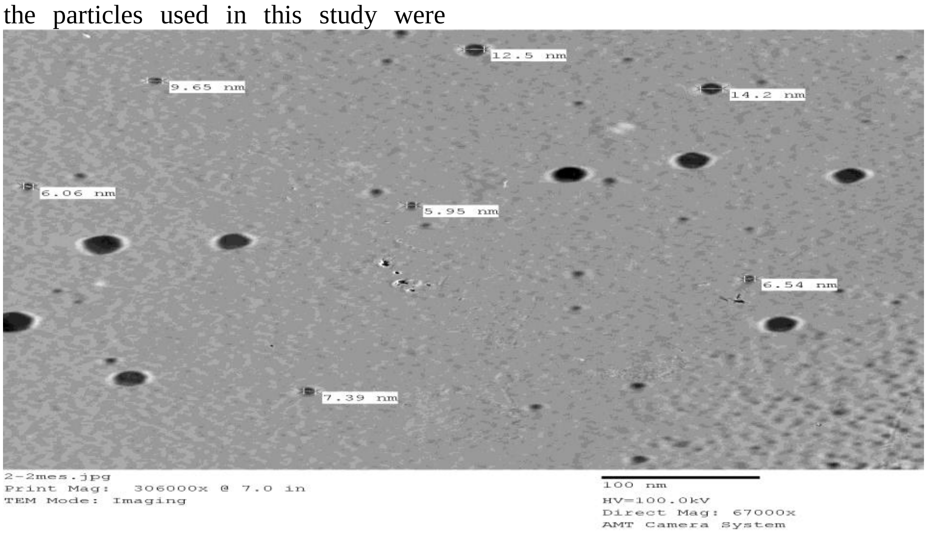

Figure (4): Transmission electron microscopic image of zinc oxide nanoparticles (ZnONPs)

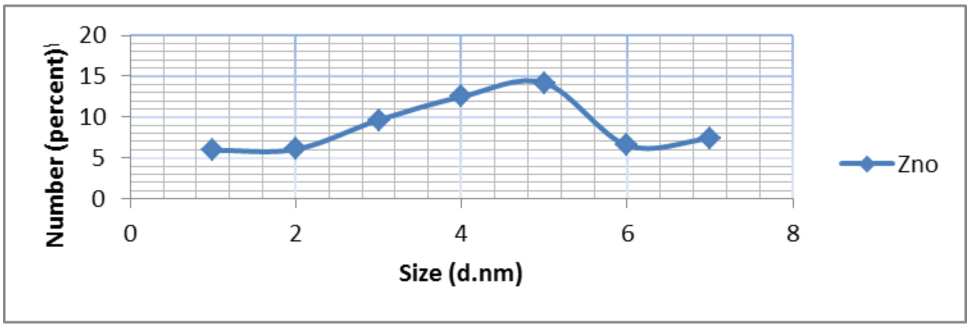

Fig.5: Particle size of zinc oxide nanoparticles (ZnONPs) (droplet size = 14.2 nm)

Effect of Zinc oxide nanoparticles (ZnONPs) and/ or peroxyacetic acid (PAA) on mycelial dry weight of *M. phaseolina*:

Mycelial dry weight (MDW) of *M. phaseolina* was significantly affected by ZnONPs and/or PAA tested (Table 2) and figures (6 and 7). Antifungal activity of ZnONPs or PAA was increased as concentration enhancement Application of (ZnONPs) showed inhibitor effect more than PAA. The greatest inhibitor effect (85.9% efficiency) was obtained by combining 0.1g/l ZnONPs with PAA (0.1g/l AA +1.0 g/l H_2O_2) compared to single application of each compound.

Table (2): Effect of Peroxyacetic Acid (PAA) and/or Zinc oxide nanoparticles (ZnO-NPs) on mycelial dry weight of Sesame root rot/wilt pathogen.

Comp.	*Conc.(g/l)*	**Mycelial dry weight (mg/50 ml liquid media)**			
		EXP.I	EXP.П	Mean	Efficiency%
ZnO-NPs	0.025	357[1]d	334d	345.5	41.6f
	0.5	297e	283e	290	51.0e
	0.1	199g	184h	191.2	67.7b
PAA	0.05AA+0.5H_2O_2	435b	437b	436	26.3h
	0.1AA+0.1H_2O_2	359c	368c	363.5	37.7g
	0.2AA+0.2H_2O_2	225fg	229g	227	61.6c
ZnO-NPs PAA	0.025+0.1	247f	251f	249	57.9d
	0.5+0.1	149h	147i	148	75a
	0.1+0.1	84i	82j	83	85.9a
control	0	595a[2]	589a	592	0i
LSD5%		14.8	6.08	12.55	2.12

[1] Each value in an average of 3 replicates (plates)

[2] Each reading with the same letter is a not-significant at 5%.

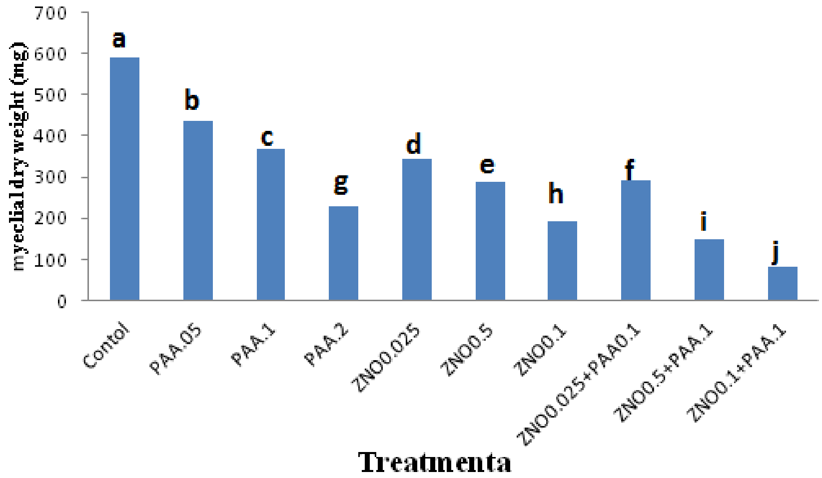

Fig. (6): Effect of peroxyacetic acid (PAA) and/or zinc oxide nanoparticles (ZnONPs) on mycelial dry weight (mg) of sesame root rot/wilt pathogen (The figure represents the average of the two experiments).

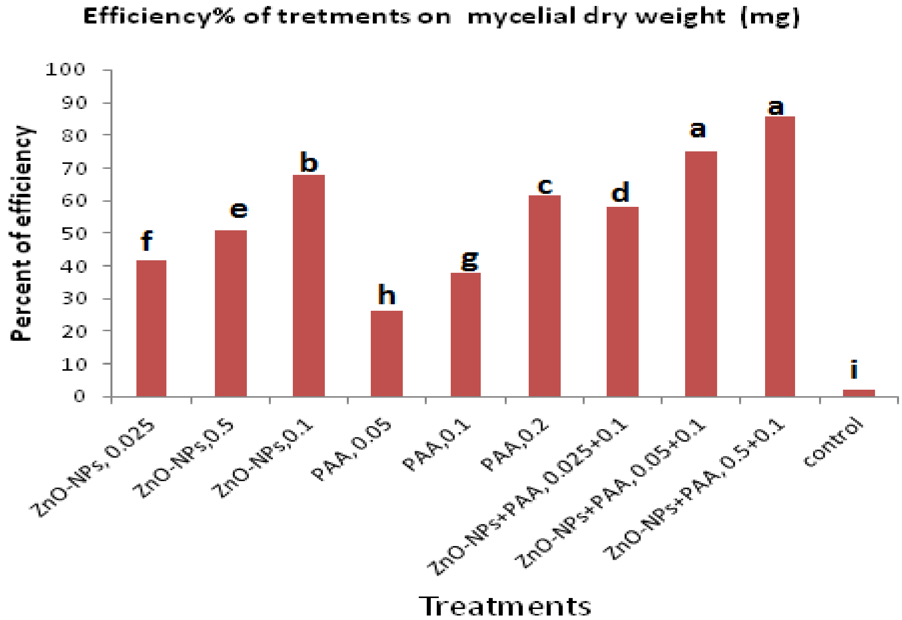

Figure (7): Efficiency% of treatments on mycelial dry weight (mg).

Viability of sesame seeds under *M. phaseolina* infection as influenced by Zinc oxide nanoparticles (ZnONPs) and/or peroxyacetic acid (PAA) treatment :

Viability of sesame seeds was assayed as germination % after ten days incubation at 25ºC. The recent data (Table 3) and Figures (8 and 9) showed insignificant effects for ZnONPs, PAA and ZnONPs combined with PAA. The highest germination % was provided with non-infected untreated seeds (control, 86.4%). The least concentrations of the tested compounds, however slight germination % reductions was expressed when compared with the control or with the highest concentrations of the tested compounds. As for *M. phaseolina* infected seeds the least germination % was addressed (64.4%) with infected untreated seeds. Meanwhile, treated–infected seeds was significant improved seed germination%. Increasing ZnONPs or PAA concentrations enhanced seed germination %. Combing 0.1g/l ZnONPs + PAA (0.1g/l AA+1.0 g/l H_2O_2) enhanced seed germination % better than single application of each compound. The highest seed germination %. Related *M. phaseolina* was obtained by 0.1g/l ZnONPS+ PAA(0.1g/l AA +1.0 g/l H_2O_2) highest 78,4% germination. Similarly shoot and root length were positively affected by ZnO-NPs and/or PAA practically at low concentrations tested even under *M. phaseolina* infection as compared to untreated infected seeds. However increasing concentration decreased either shoot or root length. Data related to seed viability, shoot length or root length reflected an improvement of VI values as result of treatments tested even upon *M. phaseolina* infection as compared to untreated infected seeds.

Table (3): Effect of Peroxyacetic acid (PAA), Zinc oxide nanoparticles (ZnONPs) on sesame germination.

Comp.	Conc. (g/l)	*Non-infected*				M. phaseolina				**Mean**	
		G%	SL (cm)	R l (cm)	VI	G%	SL (cm)	R l (cm)	VI	G%	VI
Zno	0.025	85.5*b	7.2b	8.1a	1308.1a	65.4h	6.4a	7.6a	915.6a	75.4e	1111.8a
	0.5	82.4d[1]	6.2e	6.8d	1071.2e	68.3f	5.6b	60.1cd	799.1cd	75.3e	935.1f
	0.1	81.8e	5.1h	6.2f	924.3h	78.4a	4.6d	5.6e	799.6cd	80.1a	861.9g
PAA	0.05	86.3a	6.8c	7.5b	1234.0c	68g	6.6a	6.7b	904.4ab	77.2c	1069.2c
	0.1	82.3d	5.4g	6.2f	954.6g	71d	5.1c	5.9de	781d	76.6d	867.8g
	0.2	80.5g	4.5i	5.4g	796.9i	76b	4.2e	4.8g	684e	78.2b	740.4i
Zno+ PPA	0.025+0.1	82.4c	7.4a	8.2a	1285.2b	63.4j	6.4a	7.5a	881.2ab	72.9f	1083.2b
	0.5+0.1	81.1f	6.4d	7.6b	1135.4d	69.6e	5.8b	6.4bc	849.1c	75.3e	992.2d
	0.1+0.1	80.4g	6.0f	7.0c	1045.2ef	74.1c	5.7b	6.4cd	896.6b	77.2c	970.9e
control	0	86.4a	5.5f	6.5e	1036.8f	64.4i	4.5de	5.4f	637.5f	75.4e	837.1h
LSD 5%		0.15	0.16	0.14	19.48	0.33	0.30	0.32	32.09	0.19	19.05

*Each reading is an average of 3replicates, each containing 10 seeds.

[1] Each reading with the same letter is a not-significant at 5%.

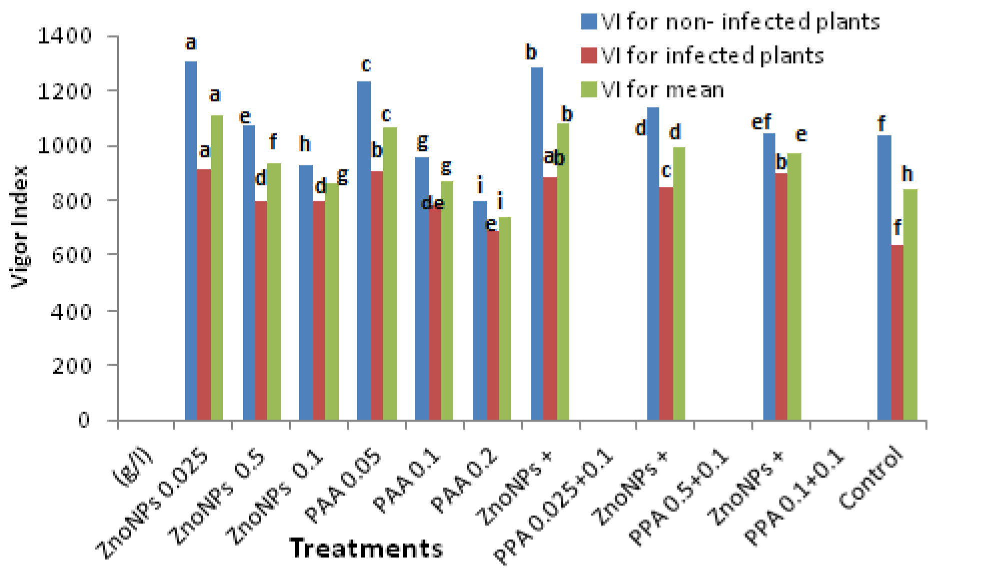

Fig. (8): Effect of Zinc oxide nanoparticles and/or PAA on Vigor index of sesame seedlings

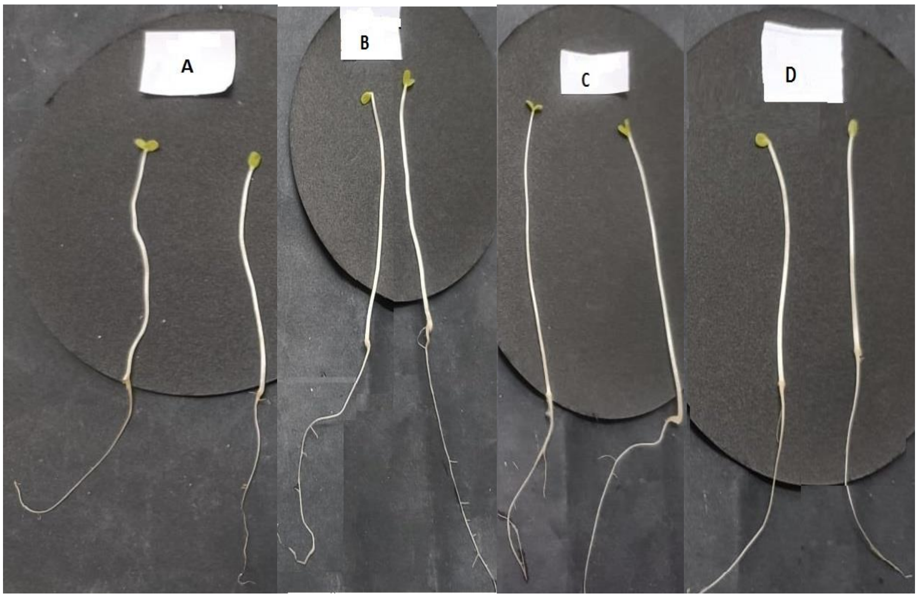

Fig. 9: Shoot and root length of sesame, 10 days old sesame seed length as affected by (A) untreated (control), (B) ZnO- NPs(0.1/gl) with PAA (0.1g/l AA +1.0 g/l H_2O_2), (C) ZnO- NPS(0.1/gl) and (D) PAA (0.1g/l AA +1.0 g/l H_2O_2).

Effect of Zinc oxide nanoparticles (ZnO-NPs) and/or peroxyacetic acid (PAA) on sesame root rot severity :

Either ZnONPs or PAA were effective to reduce sesame root rot severity caused by *M. phaseolina* infection (Table 4 and fig. 10 and 11). Increasing concentration of the test compounds raised their efficiency to reduce infection . Singly application of ZnONPs was more effective to reduce sesame root rot than using PAA). ZnONPs (0.1g/l) single gave greater efficiency (78.7%). Than PAA (0.2g/l AA +2.0 g/l H_2O_2) (61%) to be noticed, combining ZnONPs with PAA gave more efficiency than When those were used singly. Combining PAA(0.1g/l AA +1.0 g/l H_2O_2) with ZnONPs at (0.025-0.5-0.1 g/l) undividedly gave higher efficiency to reduces sesame root rot severity than they applied singly. The most efficiency to decrease sesame root rot infection was exhibited by combining 0.1g/l ZnONPs with PAA(0.1g/l AA +1.0 g/l H_2O_2) (84.6%) followed by 0.5g/l ZnONPs+PAA(0.1g/l AA +1.0 g/l H_2O_2) (66.8%) and 0.025 g/l ZnONPs+ PAA (0.1g/l AA +1.0 g/l H_2O_2)(49.5%)

Table (4): Effect of Zinc oxide nanoparticles ZnONPs and/or Peroxyacetic Acid (PAA) on sesame root rot/wilt .

Comp.	Treatment	Conc.(g/l)	EXP.I	EXP. II	mean	Efficiency%
ZnO-NPS	ZnO-NPS	0.025	41.8(1)b(2)	39.7b	40.7	32.8i
	ZnO-NPS	0.5	26.1f	26.4f	26.2	56.7e
	ZnO-NPS	0.1	14.2i	11.7i	12.9	78.7b
PAA	PAA	0.05	40.9c	39c	39.9	34.1h
	PAA	0.1	37.5d	37d	37.2	38.6g
	PAA	0.2	22.7g	24.6g	23.6	61.0d
ZnO-NPS+PAA	ZnO-NPS+PAA	0.025+0.1	31e	30.3e	30.6	49.5f
	ZnO-NPS+PAA	0.5+0.1	20.1h	20.2h	20.1	66.8c
	ZnO-NPS+PAA	0.1+0.1	9.5j	9.1j	9.3	84.6a
Control(2)	Control(2)	0	61a	60.3a	60.6	0j
Control(3)	Control(3)	0	0k	0k	0	0j
LSD5%			0.68	0.68		1.14

(1)The reading is an average of 3 replicates, each containing 25 seeds

(2) Each value with the same litter in non-significant at 0.05%

Control(2): Soil infected with the pathogen

Control(3): Non- infected soil

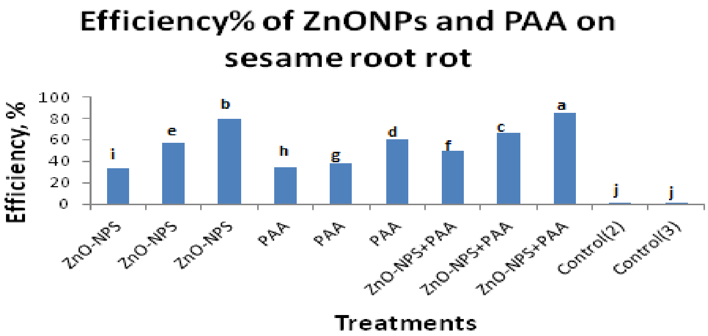

Fig. 10: Effect of Zinc oxide nanoparticles (ZnONPs) and/or Peroxyacetic Acid (PAA) on sesame root rot/wilt.

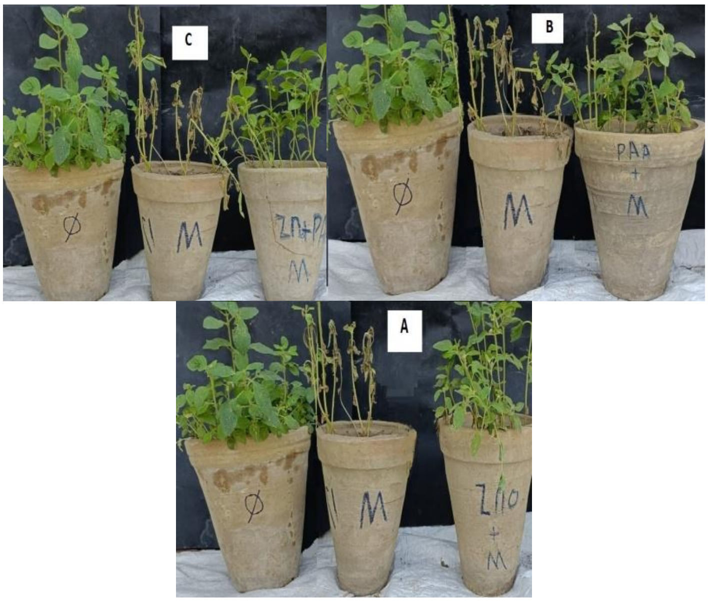

Fig.11: : Growth of sesame plant growing in non-infected (A,B,C) (control, left), Infected (A,B,C) untreated (medium) by *Macrophomina phaseolina* and treated infected (a) ZnONPs, (b) ZnONPs with PAA and (c) PAA.

DISCUSSION

Six *Macrophomina phaseolina* isolates were isolated from rotted sesame roots growing in El-minya governorate. The fungus *M. phaseolina* has been isolated from sesame root rot from several locations in Egypt **(Mohamed and Omar, 2006 ; Elewa *et al.*, 2011).** Among six isolates of *M. phaseolina*, the isolate M3 provided the most virulent to sesame plants (*Sesamum indicum* var. Shandawil -3).The most virulent isolate (M3) was conserved and subjected for molecular identification. The Phylogenetic analysis for isolate M3 sure that it make its identification as *Macrophomina phaseolina* (Tassi.) Goid.

According the application of sustainable disease management in agriculture sector, an eco-friendly control approaches became general trend to avoid environment pollution and human health problems **(Alallam *et al.*, 2023).** This, ZnO NPs and PAA were taken into consecration in the present study. Utilizing ZnONPs in agriculture as antimicrobial, plant growth promoter as well as plant resistance inducer has been reported **(Elamawi *et al.*, 2016; Gondal *et al*. 2011 and Hazem *et al*., 2023**). So far, PAA which consider as strong oxidizer has antimicrobial behavior, sanitizers and reacted as signally in plant aganist pathogens (**EL-Ashmony et al., 2017; Galal, 2017; Galal, 2018 and Abdelrhim *et al*., 2022)**.

The percent work revealed that ZnONPs which purchased are characterized as spherical shape with size 5.95-14.2 nm. Several reports showed that ZnONPs of small particle are more efficient than those large. **(Sirelkhatim *et al.*, 2015 and Yusof *et al*., 2022)**

Regarding *M. phaseolina* growth that monitor as mycelial dry weight, the current results revealed an antifungal activity for (ZnO-NPs) and/or PAA against *M. phaseolina* fungus. Antifungal activity of ZnONPs was more than PAA's. ZnONPs is shown to give effective pathogen growth inhibition. ZnONPs clearly outperform silver in pathogen suppression efforts due to their decreased toxicity and indirect benefits on soil fertility **(He *et al.*, 2011).** The antimicrobial effectiveness of ZnONPs has been explained by a number of different methods. The production of H_2O_2 on the surface of ZnONPs is thought to be a useful method for preventing fungal development **(He *et al.*, 2011)**. Another possible mechanism is the discharge of ZnONPs, which have the ability to interact with intracellular materials and harm cell membranes (Sirelkhatim et al., 2015). As a result, size-dependent features of nanomaterials include the morphological features of a substance, such as pores, phase inclusions, and grain or particle size, influence its qualities **(Andrievskii *et al*., 2009).** It has been established that the PAA mixture has antifungal efficacy against plant pathogenic fungi **(Mari *et al.*, 2004; and Elbouchtaoui *et al.*, 2015)**, *Sclerotinia sclerotiorum* **(EL-Ashmony et al., 2017), (Abdelrhim *et al*., 2022)** and **(Ali *et al*., 2022).** The obtained results show inhibitory effect for PAA against *M. phaseolina* growth. Inundation PAA enhanced the antifungal activity of ZnONPs. Energetic antifungal activity was explored when ZnO-NPs was combined with PAA and the maximum antifungal activate was pranced by combining (0.1g/l) ZnONPs with PAA (0.1g/l AA+1.0 g/l H_2O_2). **Ayoub *et al.* (2017)** demonstrated energetic activity for PAA when combined with cupper against *Botrytis cinerea*. In case of sesame seed viability, an obvious seed germination improvement was exhibited particular at low concentration tested for ZnONPs and/or PAA reduced seed germinations non-significant for non-infected seeds. Meantime, viability of *M. phaseolina* infected sesame seeds were positively affected by ZnONPs and/or PAA. Infected sesame seed gave the least germination %. Meanwhile, ZnONPs significant increase in germination % followed by PAA. The greatest germination % was achieved by combing ZnO-NPs with PAA. Generally, ZnONPs, PAA and (ZnONPs+PAA) provided growth promotion as shoot length and root length enhancement in uninfected or infected satiations, That reflected an improvement of VI values. The greatest VI values was explored when ZnONPs combined with PAA followed by ZnONPs and PAA. Data indicated that sesame growth promotion exhibited by ZnONPs more than PAA. The sesame growth promotion was reached the greatest VI values by combining ZnONPs with PAA. It has been shown that ZnONPs reacted as plant growth promoter **(Anu *et al*.2020).** Likewise, PAA showed plant growth promoter for either plant **(EL-Ashmony et al., 2020).** Additionally, the researchers showed that deep vegetative growth and physiological and biochemical tests, such as those involving antioxidant enzyme activities, could activate the plant's natural defence system (Elsharkawy et al., 2018).The recent work explored that ZnONPs as efficiency to reduce sesame root rot caused by *M. phaseolina.* The efficiency of ZnONPs to reduce sesame root rot, increased as concentrations function utilizing PAA gave significant disease reduction against several phatopathogeinc fungi (**EL-Ashmony et al., 2017; Abdelrhim *et al*., 2022 and Ali *et al*., 2022)** .The recent study showed signaling properties to sesame plants against *M. phaseolina.* Using PAA led to reduce sesame root rot put its efficiency was lower than ZnONPs. Combing ZnONPs (0.1g/l) with PAA (0.1g/l AA +1.0 g/l H_2O_2) achieved the height efficiency values than using each compound single. However, efficiency of ZnONPs was enhanced against fungi. Using PAA to enhance antifungal, sesame growth promotion and induce resistance activity of ZnONPs is needed to use low ZnONPs with low concentrations to avoid their toxicity to plants. In concluding, PAA that reacted as fungicide alternative or eco-friendly save compound in proper to be combined with ZnONPs to enhance ZnONPs activities against plant pathogen specially *M. phaseolina*, the sesame root rooting, improve sesame vegetative growth and reflected the height VI value. Furthermore, enhanced efficiency sesame root rot reduction was addressed through combining ZnONPs with PAA.

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الملخص العربى

زيادة فاعليه جزيئات أكسيد الزنك النانونية ZnONPs كمضـــاد فطرى فى مقاومة مرض عفن جذور السمسم الناتج عن الإصابة بالفطر*Macrophomina phaseolina* (Tassi.) Goid. باستخدام مخلوط من فوق أكسيد الهيدروجين (H_2O_2) وحمض الخليك (AA).

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امكن عزل ستة عزلات للفطر ***Macrophomina phaseolina* (Tassi.) Goid.** الممرض لجذور السمسم التى تم جمعها من مزارع السمسم بمحافظه المنيا. اظهر اختبار القدرة المرضيه للعزله M3 أنها الأكثر خطوره وبالتالي تم عمل اختبار تفاعل البلمره المتسلسل حيث اكد التعريف ان العزله هي ***Macrophomina phaseolina***. تم نقع بذور السمسم في محلول جسيمات اكسيد الزنك النانوية (ZnONPs) و/أو حمض البيروكس استيك (PAA) لدراسة كفاءتهما لتثبيط الفطر المسبب لعفن جذور السمسم ***M. phaseolina*** والنمو الخضري للسمسم وقدرتهم علي السيطرة علي مرض تعفن جذور السمسم، حيث اظهر كلاً من ZnO-NPs و PAA نشاط مضاد للفطر الممرض ولكن ZnONPs اظهر نشاط مضاد للفطر اكثر من ال PAA. اظهر النمو الخضري لسمسم تحسناُ في مؤشر القوه (VI) للمعاملة الواحدة لكل مركب تم اختباره وكان ZnONPs افضل من PAA، وكان الدمج بين ZnONPs (0.1g/l) وPAA (0.1 g/l AA+1 g/l H_2O_2) اكثر تفوقا في احداث نمو نباتي افضل علاوة علي ذلك تم الحد من مرض تعفن جذور السمسم من خلال تطبيق الخلط بين ZnONPs(0.1g/l) و PAA (0.1 g/l AA+1 g/l H_2O_2) والذي اظهر كفاءه اكبر من الاستخدام الفردي لكل مركب على حده.