Caspian Journal of Environmental Sciences
Online ISSN: 1735-3866
Print ISSN: 1735-3033

Controlling soybean root rot pathogens using some safe organic compounds

Sahar A. Elsayed

Department of Biology, Faculty of Science & Arts of Baljurshi, Al- Baha, Saudi Arabia & Institute of Plant Pathology, Agricultural Research Centre, Giza P.O. Box 12613, Egypt

ABSTRACT

Laboratory and greenhouse experiments were carried out during the summer season of 2023 to study the effect of some safe organic compounds i.e. humic acid (HA), amino acids (AA) and proline for controlling damping-off and root rot diseases compared to Rizolex-T50® Wp as a fungicide. *Rhizoctonia solani*, *Fusarium solani* and *Macrophomina phaseolina* were isolated from naturally-infected soybean fields and identified as a causal pathogen of damping-off and root rot. *R. solani* exhibited a highest frequent followed by *F. solani* while *M. phaseolina* comprised the least pathogen frequent isolates. In pathogenicity test under greenhouse experiment, *R. solani* isolate No 6, *F. solani* No 3 and *M. phaseolina* No 5 were the most aggressive isolates for causing damping-off and root rot diseases. In laboratory experiment, proline and humic acids displayed fungicidal activity against mycelia growth of root rot fungi. The high level of proline (300 ppm) was the most effective followed by humic acid at high level (3000 ppm). In artificially-infested soil with a mixture of pathogenic fungi under greenhouse, Rizolex-T50® Wp exhibited the highest reduction of pre, post-emergence damping-off, root rot and wilt diseases. Morover, from the application trails, the best protection against the causal pathogens of soybean damping-off, root rot and wilt was recorded by prolin at 300 ppm followed by HA at 3000 ppm. Herein, it is suggested that soaking of soybean seeds in proline 300 ppm or humic acid 3000 ppm before sowing could be considered as alternative safe methods for chemical fungicides to control root rot diseases.

Keywords: Soybean, Root rot, Organic compounds, Humic acid, Amino acids, Proline
Article type: Research Article.

INTRODUCTION

Soybean, *Glycine max* L. Merrill is considered as among of the foremost crucial annual crops grown around the nations. It is considered also a remarkable plant due to its ability to nourish the soil with nitrogen through nitrogen fixation. In addition, it is an environmentally benign crop, and contains plenty of phosphorous as well as calcium. Its protein content is up to 40 %, oil 20% and carbohydrate 30% (Ab El-Hai *et al.* 2010, 2016; Ghaleb 2021). Herein, it delivers dietary oil plus protein and industrial manufacture of many products. Soil-borne fungal diseases are a major hindering factor of soybean germination, outgrowth and productivity. Damping-off or root rot is one of the foremost devastating illnesses to soybean plants. These diseases are caused by a varius complex fungus-likes such as *Rhizoctonia solani*, *Fusarium solani* and *Macrophomina phaseolina* (El-Baz 2007; Abd El-Hai *et al.* 2016; Ghaleb 2021; Hussein 2023; EL-Saman *et al.* 2023). Rhizoctonia root rot is caused by *Rhizoctonia solani* wide-spreads across the globe under soil temperature 18 ℃ or normal soil moisture, leading to serious sickness. The signs of infection is reddish-brown, lengthened, cankerous lesions present on bottom hypocotyl and root. Brown sclerotia form on cankers. Young plants that are sick have crimson pith and may perish. *Fusarium* spp., the causative agent of Fusarium root rot flows by draining and irrigation water (Hagedorn & Inglis 1986; Rouhi *et al.* 2011). Disease signs are small, lengthen, or tan-red patches in the superior taproot and inferior hypocotyl. Stunted plants have illness and eventually will be perished. Symptoms of *M. phaseolina* appear in hot dry weather; leaves of infected plants become yellow, and all plants wilt then perish with the leaves retaining attached (Colyer 1989). The fungicidal treatment for controlling root rot diseases are faces some difficulties due

Caspian Journal of Environmental Sciences, Vol. 22 No. 1 pp. 129-136 Received: March 20, 2023 Revised: June 03, 2023 Accepted: Aug. 12, 2023
DOI: 10.22124/CJES.2024.7477

Publisher: University of Guilan,

to a diverse host spectrum and their persistency in the soil. In addition, the excessive application of fungicides causes environmental pollution and food contamination, leading to hazardous affects on human health (Rauf 2000). Hence, an alternative safe methods to control root rot diseases is an urgent. The positive impacts of humic acid on plant outgrowth and development include their effects on cell membranes which promotes minerals transmission, boosted photosynthesis, plant hormone-as activity, modified enzyme activities, amended protein synthesis in addition to lowering vigor degrees of toxic minerals, all of these can protect the plants from pathogen invasions (Nardi *et al.* 2002). Humic acid impact plant outgrowth and in combination with amino acids take into consideration that most of organic nitrogen in the soil boosts soil production (Schnitzer 2001). According to McDonnell *et al.* (2001), they are a crucial soil ingredient due to containing a stable proportion of carbon then boost water retaining capacity, pH buffering in addition to thermal insulation. Also, Khedr *et al.* (2003) stated that proline generates the expression of salt-stress responsive proteins and it may boost the plant response to salt stress, in addition, in the same way, it can be used for plant disease management. Some authors studied the fungicidal effects of humic acid, amino acids and proline against some fungal diseases such as faba bean root rot (Abd El-Hai & El-Saidy 2016) using humic acid and proline, faba bean chocolate spot (El-Ghamry *et al.* 2009) and also application of humic acid and amino acids. The present study aimed to examine the efficiency of humic acid (HA), amino acids (AA) and proline at different concentration for treating damping-off or root rot diseases compared to fungicide Rizolex-T 50 W.P.

MATERIALS AND METHODS

Soybean seeds

Seeds of soybean cv. Giza 35 were obtained from Legume Crop Research Department, Field Crop Research Institute, Agriculture Research Centre, Giza, Egypt.

Chemical source

Commercial humic acid product (Actosol) containing 80% humic acid + 6% K_2O and amino acids (Peptonic; containing 6% free amino acids + 12% organic nitrogen + 3.5% K_2O) were obtained from Egyptian Fertilizers Development Centre, Talkha, El-Mansoura, Egypt. L-proline was provided from Sigma Chemical Company. Moreover, Rizolex-T50® Wp fungicide (contains 20%Tolclophos-methyl and 30%Thiram) was provided from Al- Gomhoria Chemical Company, Egypt.

Causal pathogens isolation, purification and identification

Samples of soybean showing typically damping-off or root rot signs were gathered from the inherently-infected diverse fields in the summer 2022. The infected roots and minimum steam parts were properly cleaned with tap water then chop into little bits (one cm approximately). The small pieces were surface-sanitized with sodium hypochlorite (2%) for two min; then re-washed with sanitized water and dried between two sanitized filter papers, followed by positioning on plates (petri-dishes) containing potato dextrose agar (PDA) media enriched with streptomycin-sulphate (100 µg mL^{-1}) to avoid the bacterial outgrowth. The plates were incubated at 27℃ for five days. The cultivating fungi were refined employing the single spore technique as stated by Hawker (1960), then recognized as per taxonomic norms according to culture properties, morphological and microscopic characteristics as described by Sneh *et al*. (1992) for *Rhizoctonia solani*, Booth (1977) for *Fusarium solani* and Ellis (1976) for *Macrophomina phaseolina*.

Fungal inoculum preparation

The fungal artificial inoculum of *R. solani* (12 isolates), *F. solani* (8 isolates) and *M. phaseolina* (5 isolates) were prepared employing media with different proportion of sorghum: coarse sand: water (two: one: two v/v). The media contents were goodly blended, bottled then autoclaved for 20 min at 1.5 air pressure. The sanitized media were inoculated utilizing agar discs provided from the almost five-day-old culture colonies of each fungal isolate. The inoculated media were incubated at 27℃ for 15 days to use for soil infestation and examining the pathogenic capability of all fungal isolates.

Pathogenicity test

The pathogenicity testes on the isolated fungi were carried out in a greenhouse conditions. Sterilized pots (30 cm in diameter) filleting up with autoclaved clay soil were artificially infested with the beforehand prepared fungal inoculum at the rate of 2% weight per weight. The pots contents were blended fully with inoculum then watered and left for one week to enhance growth spread of the fungal inoculum. Three pots were employed as a replicate for every fungal isolate. At the same time three pots having autoclaved soil without fungal inoculum infection were prepared to act as a check. Soybean seeds cv. Giza 35 were surface-sanitized by submerging them in 0.1% sodium hypochlorite for 1 min, then cleaned with sanitized water, while dried then planted at an average of ten seeds per pot in the summer 2023. Three replicates were employed in every specific treatment. The pathogenic capability of each fungal isolate was set as the rate (%) of pre- or post-emergence damping-off and the health-existence plants after 20, 40 or 60 days after planting, respectively. The disease assessments (pre-, post-emergence and survival rate) were measured on the basis of total number of seeds sown in each pot. The most aggressive isolate of each fungal genera was chosen for additional researches (in the present inquiry; *R. solani* No 6, *F. solani* No 3 and *M. phaseolina* No 5).

Safe organic compounds *vis* pathogens linear growth (laboratory treatment)

The effect of safe organic compounds as compared with Rizolex-T50 fungicide on the linear outgrowth of the most aggressive fungal genera isolate were tested in *in vitro* conditions. Humic acid, amino acids at 1000, 2000 and 3000 ppm and proline at 100, 200 and 300 ppm as well as Rizolex-T50 W.P. at 3g L^{-1} were added to 10 mL sterilized PDA medium before solidification (1: 9 v/v), respectively and poured in sterilized petri-dishes. Three plates for every concentration were inoculated with fungal disc cut from the periphery of 5 days old culture of each the selected fungal isolate. The plates were incubated at 27 ± 1℃. The fungal linear growth of the most aggressive tested pathogenic fungi were measured when any treatments of a tested fungus showed full growth. A completely Randomized Design with three replicates was used in this experiment.

Greenhouse experiment

Root rot pathogens as affected by safe organic compounds

A mix of the three selected pathogenic fungi made up of the same quantity of every fungal genera inoculum was well blended with clay soil in pots (30 cm in diameter) at an average of 2% weight per weight under greenhouse circumstances. An antagonism tests among the tested pathogens were done to assure that there is not any antagonism each of them. The infested pots were irrigated before sowing then preserved for only week to assure even spread of the fungal inoculum. Soybean seeds cv. Giza 35 soaked with HA, AA at 1000, 2000 and 3000 ppm, proline at 100, 200 and 300 ppm and Rizolex-T50® Wp at 3g L^{-1} for 15 min before sowing. The moist seeds were left until air dried then planted at an average of 10 seeds pot^{-1}. The percent of pre or post emergence damping-off were registered at 20 also 40 days after planting, respectively. Moreover, root rot, wilt diseases and survival plants (%) were estimated after 80 days from planting following:

$$\text{Pre-emergence damping-off (\%)} = \frac{\text{Number of non-germinated seeds after 20 days}}{\text{Total number of sown seeds}} \times 100$$

$$\text{Post-emergence damping-off (\%)} = \frac{\text{Number of dead seedlings after 40 days}}{\text{Total number of sown seeds}} \times 100$$

$$\text{Root rot (\%)} = \frac{\text{Number of plants infected with root rot after 80 days}}{\text{Total number of sown seeds}} \times 100$$

$$\text{Wilt (\%)} = \frac{\text{Number of plants infected with wilt after 80 days}}{\text{Total number of sown seed}} \times 100$$

Number of survival plants after 80days

$$\text{Survived plants (\%)} = \frac{\quad}{\text{Total number of sown seeds}} \times 100$$

Statistical analysis

All the information gathered were evaluated utilizing CoStat software 4.6 version for comparing among means at 1% in laboratory experiment and at 5% in greenhouse experiment according to Tukey test and Duncan test, respectively.

RESULTS

Samples collection, isolation in addition to identification of soybean root rot causal pathogens

Soybean plant samples appearing damping-off or root rot signs were gathered from naturally infected in some fields in 2022 summer season. Twenty five isolates demonstrated three species of three fungal genera. The obtained outcomes in Table 1 show that, the highest frequency of isolated fungi was *R. solani* (12 isolates; 48%) from the total isolates. Moreover, *F. oxysporum* came second order (8 isolates; 32%) while, *M. phaseolina* imply the least pathogen isolates (5 isolates; 20 %).

Table 1. Frequency of soybean root rot isolated fungi.

Fungi	Isolated number	Frequency (%)
R. solani	12	48
F. solani	8	32
M. phaseolina	5	20
Total	25	100

Pathogenicity test

Under greenhouse conditions, the investigated fungal isolates varied in their capacity for triggering pre or post emergence damping-off in addition to survived plants. Table 2 indicated that, all tested isolates of three fungal genera have the capability to destroy soybean cv. Giza 35 plants causing damping-off symptoms. Generally, the highest mean values of pre-emergence damping-off happened under soil infested with *R. solani* which recorded 31.42% followed by *M. phaseolina* (24.6%) and *F. solani* (23.04%). On the opposite side, the soil artificially infested with *F. solani* causing the greatest mean of post-emergence damping-off recording 34.21 % followed by *M. phaseolina* giving 29.87% then *R. solani* (25.91%). *R. solani* isolate No 6 exhibited the highest percentage of pre-emergence damping-off recorded 48.67%. while *F. solani* isolate No 3 caused the highest percentage of post-emergence damping-off giving 35.33% infection. On the base of healthy survival plant, *R. solani* isolate No 6 was the most aggressive isolate causing the lowest percentage of healthy survival plants (19.33) followed by *F. solani* isolate No 3 (33.34%) then *M. phseolina* isolate No 5 (41.33%). Therefore, these isolates were selected for further studies.

Save organic compounds *vis.*, pathogens linear growth

The possible inhibitory effects of the tested safe organic compounds against linear growth of the most aggressive isolate of three pathogenic fungal genera in parallel with fungicide Rizolex-T50 were evaluated *in vitro*. Data presented in Table 3 shows that Rizolex-T50 W.P. fully suppressed the linear outgrowth of the three examined pathogenic fungi, while AA at 1000 ppm promoted the fungal outgrowth of all examined fungi, hence, showed a full cultivation of all examined fungi and reached to plate edge from inoculated under incubation at 27 ± 1 °C. From Table 3, it can be observed that *M. phaseolina* was the foremost sensitive for organic compounds followed by *R. solani* and *F. solani*. After fungicide, the highly reduction in the fungal linear growth was recorded with proline at concentrate of 300 ppm followed by HA at 3000 ppm. The lowering percent of the three fungal genera was increased by raising the concentration of safe organic compounds. In the other words, a negative association was found between organic compounds concentration and fungal linear outgrowth.

Disease assessment under artificially infested soil in greenhouse:

The effect of safe organic compounds i.e. HA, AA at 1000, 2000 and 3000 ppm and proline at 100, 200 and 300 ppm as well as Rizolex-T50® Wp on disease assessment i.e. pre, post-emergence damping-off, root rot or wilt diseases percentage in addition to hygienic soybean plants had assessed in infested soil with a mix from an equal amount of pathogenic fungi under greenhouse circumstances. Results in Table 4 appeared that Rizolex-T50 W.P. demonstrates the greatest lowering in pre or post-emergence damping-off and the percentage of root rot in addition to wilt diseases. Consequently, it exhibited the highest increase in healthy survival plants (83.33 %). In contrast, the low level of AA (1000 ppm) led to an opposite effect, hence it increased the infection of all tested disease assessment as compared to chick (mixture fungi). On the other side, regardless of low AA concentrate, other treatments reduced disease assessment parameters. The maximum reduction was recorded under the implementation of high proline level (300 ppm) followed by HA at high level (3000 ppm) then moderate level of proline (200 ppm). From the aforementioned results, it was be observed that the best protection against the reasoned pathogens of damping-off, root rot or wilt diseases occurred under the application of proline at 300 ppm and/ or HA at 3000 ppm as seed treatment.

Table 2. Pathogenicity test of pre or post-emergence damping-off in addition to survival of soybean plants infected with isolated fungi.

Fungus	Isolate Number	Pre-emergence	Post-emergence	Survival
Check	**………………..**	**1.00 ^{g}**	**0.00 ^{g}**	**99.00 ^{a}**
	1	28.67 ^{c}	26.33 ^{e}	45.00 ^{bc}
	2	30.33 ^{bc}	25.67 ^{ef}	44.00 ^{c}
	3	28.33 ^{c}	23.67 ^{f}	48.00 ^{b}
	4	29.00 ^{c}	26.33 ^{e}	44.67 ^{c}
	5	31.00 ^{b}	24.00 ^{f}	45.00 ^{bc}
R. solani	6	48.67 ^{a}	32.00 ^{c}	19.33 ^{g}
	7	28.67 ^{c}	24.67 ^{ef}	46.66 ^{bc}
	8	30.33 ^{bc}	26.00 ^{e}	43.67 ^{d}
	9	31.00 ^{b}	25.00 ^{ef}	44.00 ^{c}
	10	29.67 ^{c}	27.67 ^{de}	42.66 ^{e}
	11	30.33 ^{bc}	24.33 ^{f}	45.34 ^{bc}
	12	31.00 ^{b}	25.33 ^{ef}	43.67 ^{d}
Mean	………..	31.42	25.91	42.67
	1	22.00 ^{ef}	34.67 ^{a}	43.33 ^{d}
	2	23.33 ^{e}	33.33 ^{ab}	43.34 ^{d}
	3	31.33 ^{b}	35.33 ^{a}	33.33 ^{f}
F. solani	4	21.33 ^{f}	33.00 ^{b}	45.67 ^{bc}
	5	23.00 ^{e}	34.00 ^{a}	43.00 ^{d}
	6	20.33 ^{f}	35.00 ^{a}	44.67 ^{c}
	7	22.67 ^{e}	34.00 ^{a}	43.33 ^{d}
	8	20.67 ^{f}	34.33 ^{a}	45.00 ^{bc}
Mean	…………	23.0^{4}	34.21	42.75
	1	24.33 ^{d}	30.00 ^{c}	45.67 ^{bc}
	2	22.67 ^{e}	29.33 ^{cd}	48.00 ^{b}
M. phaseolina	3	25.00 ^{d}	30.33 ^{c}	44.67 ^{c}
	4	23.00 ^{e}	29.00 ^{d}	48.00 ^{b}
	5	28.00 ^{c}	30.67 ^{c}	41.33 ^{e}
Mean	………..	24.60	29.87	42.53

Means within every column accompanied by same letter non-significantly vary at 5%.

Table 3. The linear outgrowth (cm) of soybean root rot pathogens as influenced by safe organic compounds.

Treatments	***R. solani* No. 6**	***F. solani* No. 3**	***M. phaseolina* No. 5**
Check	7.20 ^{b}	8.50 ^{a}	7.00 ^{b}
HA 1000 ppm	6.00 ^{c}	6.30 ^{c}	5.20 ^{c}
HA 2000 ppm	5.20 ^{d}	5.60 ^{d}	4.40 ^{d}
HA 3000 ppm	3.00 ^{f}	3.40 ^{e}	2.70 ^{f}
AA 1000 ppm	9.00 ^{a}	9.00 ^{a}	9.00 ^{a}
AA 2000 ppm	7.00 ^{b}	8.00 ^{b}	7.00 ^{b}
AA 3000 ppm	6.00 ^{c}	6.30 ^{c}	5.80 ^{c}
Proline 100 ppm	5.10 ^{d}	5.50 ^{d}	4.70 ^{d}
Proline 200 ppm	3.40 ^{e}	3.70 ^{e}	3.10 ^{e}
Proline 300 ppm	2.30 ^{g}	2.40 ^{f}	2.10 ^{g}
Rizolex-T50® Wp	0.00 ^{h}	0.00 ^{g}	0.00 ^{h}

Means within every column accompanied by same letter non-significantly vary at 1%.

Table 4. Impact of organic compounds on damping-off, root rot, wilt in addition to survival plants under artificially infested soil with a mix. fungal pathogens.

Treatments	Pre-emergence	Post-emergence	Root rot	Wilt	Survival
Check	24.00^{a}	20.67^{a}	34.00^{a}	12.67^{a}	8.67^{i}
HA 1000 ppm	17.00^{d}	13.67^{cd}	20.33^{d}	9.00^{c}	40.00^{f}
HA 2000 ppm	14.33^{e}	12.67^{d}	18.00^{e}	8.67^{cd}	46.33^{e}
HA 3000 ppm	8.67^{g}	7.00^{f}	13.33^{g}	6.00^{de}	65.00^{c}
AA 1000 ppm	25.33^{a}	21.00^{a}	35.33^{a}	13.67^{a}	4.67^{i}
AA 2000 ppm	20.67^{b}	17.67^{b}	23.33^{c}	10.67^{b}	28.33^{h}
AA 3000 ppm	18.33^{c}	14.67^{c}	21.67^{cd}	9.33^{bc}	36.00^{g}
Proline 100 ppm	11.33^{f}	10.00^{e}	16.00^{f}	7.67^{de}	62.00^{d}
Proline 200 ppm	9.33^{g}	8.67^{f}	14.67^{fg}	7.33^{e}	67.00^{c}
Proline 300 ppm	7.00^{h}	5.33^{g}	10.67^{h}	4.33^{f}	72.67^{b}
Rizolex-T50® Wp	3.67^{i}	2.33^{h}	8.67^{i}	2.00^{g}	83.33^{a}

Means within every column accompanied by same letter non-significantly differ at 5%.

DISCUSSION

Soybean (*Glycine max* L. Merrill) is a remarkable plant. This is because it contains a high proportion of protein with superior in terms of major amounts of the most critical amino acids and oil with contains unsaturated fatty acids. In addition, it generates root nodules which act as soil nitrogen replenishment, preserving soil fertility also lowering the utility for artificial fertilizers (Akande *et al.* 2007). Sufficient crop production is considered from the major important problem which facing the most countries everywhere in the globe. The plant diseases are among of the most important obstacles to crop production (Rouhi *et al.* 2011). Therefore, any factor causes a decrease in plant diseases, will lead to an elevation in the productivity. Soil-borne fungal diseases are considered from one of the foremost crucial factors that restrict cultivation and productivity of soybean (Prapagdee *et al.* 2007). Damping-off or root rot are the foremost devastating illnesses harming soybean seeds, seedlings in addition to roots causing many damages (Sinclair & Backman 1989; Ghaleb 2021). Soil-borne fungal diseases control focuses mainly on fungicidal treatment which causes many hazards to human health in addition to increasing the environmental pollution. So, the current inquiry was planted to study the potential roles of humic acid, amino acids in addition to proline on minimizing the negative consequences of root rot pathogens in soybean plants. In the current inquiry, soybean samples were gathered from naturally infected fields and the pathogens liable for damping-off or root rot were isolated and recognized as *M. phaseolina, F. solani* as well as *R. solani*. The latter (*R. solani*) recorded the highest frequency isolates followed by *F. solani* and *M. phaseolina*. These differences are possibly as a result of the difference in environmental conditions which suitable for the pathogens from one to each other and wide host range. In the pathogenicity tests, the results demonstrated the pathogenic nature of the isolated fungi as responsible factors of damping-off and root rot. *R. solani* caused the greatest mean values of pre-emergence damping-off, while *F. solani* was the most aggressive as post-emergence damping-off pathogen. From all fungal isolates, *R. solani* No 6, *F. solani* No 3 and *M. phaseolina* No 5 were the foremost hostile isolates in triggering damping-off or root rot of soybean plants. The differences among isolates in pathogenic ability may be attributed to the genetic variances involved in triggering infection among them. Root rot fungal infection causes damping-off because of mortality of seedlings either before emergence or seedlings shortly collapse after emergence and root rot (Hussain *et al.* 1989). In addition, fusaric acid produced by the genus fusarium inhibited seeds germination, and caused rotten lesions of seed cotyledons, as well as plumule soft rot (Gally *et al.* 1998). Root rot pathogenic fungi also caused breakdown in the lateral roots due to total disorder in epidermis cells in addition to deterioration of cortical primary cell wall, which in turn decrease the surface absorption of water leading to damping-off (Abd El-Hai *et al.* 2010). Also, root rot fungal diseases produce some enzymes which cause seed cotyledons rot followed by seed rot and plumule soft rot, then pre- or post-emergence damping-off (Mahmoud *et al.* 2013). In the present inquiry, all safe organic compounds proved to be the antifungal effects against the linear outgrowth of three examined pathogenic fungi. There is a negative association between concentrations and the linear outgrowth except the low level of AA (1000 ppm) which encouraged the fungal linear growth. The stimulated mycelia growth of pathogenic fungi under low AA level may be due to the use of AA in building the fungal protein, which is involved in building the protoplasm of fungal cells. This study showed also clearly that Rizolex-T50® Wp fungicide and all levels of organic compounds lowered pre, post-emergence damping-off, root rot or wilt under the artificially infested soil with a mixture of pathogenic fungi in the greenhouse, except AA at low level which led to inverse this. The application of Rizolex-T50® Wp was more efficacious and fast therapy for damping-off or root rot illnesses. This could be since the seeds need fungicide treatment for protect it from fungal pathogens until to make sure the field has a sufficient plant stand. Abd-El-Kareem *et al.* (2004) and Ali *et al.* (2009) were obtained similar findings. The beneficial impacts of proline on minimizing the negative consequences of soil-borne fungal pathogens might be due to that proline is considered as one of the amino acids which forms in plants to resist abiotic and biotic stresses. Ashraf *et al.* (2008) found that, proline conserves the photosynthetic system and exhibits an antioxidant stimulation, in turn leads to the elevated plant tolerant against any biotic stress. Therefore, Proline is frequently thought to be implicated in pathways for stress resistance. Proline is not only used as a preserving compound for energy and the lowered carbon demands, but also obviously plays an integral role in osmosis and radical detoxification (Taie *et al.* 2013). Abd El-Hai & El-Saidy (2016) reported that proline increases total phenols which contribute significantly to plant defence. Humic acid enhances minerals transport, hormones activity, modified enzyme activities and lowering the vigour levels of toxic minerals through its influence on cell membranes (Hamideh *et al.* 2013). Some hormone-like activities such as auxin-like substances increase total phenol, calcium content and enhances catechol oxidase activity which defend plants from pathogen invasion (Chowdhury 2003). Moreover, El-Galad *et al.* (2013) stated that HA application leads to elevation in the nutrient uptake, i.e., P, K, Mg, Na, Cu and Zn. These nutrients play an essential role in plant healthy, therefore enhance the plant resistance to diseases. Three plant enzymes demand Zn as an ingredient, i.e., carbonic anhydrase, alcohol dehydrogenase and superoxide dismutase (SOD). These enzymes play an antioxidant role which perform a crucial function in avoiding the expand of plant diseases. On the reverse side, amino acids perform an aspect in plant resistance due to their chelating effects on microelements absorption and transportation inside the plant through cell membrane. These microelements serve a function in plant resistance by organizing auxin levels. Auxin leads to elevating many materials such as phenol, catechol oxidase and calcium which protect plant against biotic stress (Chowdhury 2003). It could be concluding from the previous results and discussion that the implementation of proline at 300 ppm or Humic Acid at 3000 ppm is considered as a secure recommendation for controlling soybean root rot pathogens.

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Bibliographic information of this paper for citing:

Elsayed, SA 2024, Controlling soybean root rot pathogens using some safe organic compounds. Caspian Journal of Environmental Sciences, 22: 129-136.