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Reduction of Soybean Root and Stalk Rots by Growth Substances Under Salt Stress Conditions

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Abstract: The causal pathogens of root rot and stalk rot of soybean were isolated from infected plants. *Fusarium oxysporum, F. solani, Macrophomina phaseolina* and *Rhizoctonia solani* were the main causal pathogens of pre- and post-emergence damping-off. The isolated fungi especially *R. solani* caused anatomically deformation in the basal portion of infected stem including complete disruption in epidermal cells and degradation of primary cell wall in cortical cells. The growth substances; Ethrel, Indole Butyric acid (IBA) and Cycocel (CCC) were tested *in vitro* on the growth of the pathogenic fungi. Ethrel at 200 ppm was the most effective in reducing fungal growth. During two successive growing seasons (2008 and 2009) of soybean under different salinity stress (1000 to 3000 ppm), the application of growth substances led to significant reduction in pre- and post-emergence damping-off as well as root and stalk rot diseases. Ethrel, CCC and IBA increased significantly branches number plant^{-1}, photosynthetic pigments, total phenol, proline content, yield and seed quality (oil and protein %). Ethrel and CCC at 200 ppm were the best treatments. Soaking soybean seed in Ethrel, CCC or IBA at 200 ppm is recommended to be incorporated into the production program of soybean to decrease root and stalk rots and increase productivity and seed quality under salt stress conditions.

Key words: Growth substances, soybean, root rot, stalk rot, anatomically deformation, salt stress conditions

INTRODUCTION

Soybean (*Glycine max* L. Merrill) is one of the main fabaceous crops all over the world. It is an important crop providing the abundant proteins and vegetable oils for human nutrition. Its highly digestible oil contains unsaturated fatty acids and no cholesterol. Its protein content is superior in substantial levels of most essential amino acids. The carbohydrate content is about 30%, with total soluble sugars equal to about 10%. Also, soybean can be considered as a friendly crop to the environment related to its supply of the soil by nitrogen fixation. Additionally, it is a good dietary source of calcium and phosphorus that is why soybean is a remarkable plant (Singh *et al*., 1987; Nassiuma and Wasike, 2002; Akande *et al*., 2007).

Salinity and soil-borne fungal diseases are limiting factors of soybean growth and productivity. Soybean has been recognized as a salt-sensitive plant according to the relative growth ratio under high-salt condition (Greenway and Munnus, 1980). High levels of salinity significantly reduce germination, seedling growth, shoot and root growth, photosynthetic pigments, protein content and productivity for many plant species including soybean (Muthukumarasam and Panneerselvam, 1997; Wang *et al*., 2001), because of the high-salt environments cause molecular damages as hyperosmotic stress, homeostasis disruption and ionic toxicity to plant cell that become a resultant limiting factor for plant development and crop productivity (Aoki *et al*., 2005).

Root rot and charcoal rot diseases are among the most destructive diseases attacking soybean seeds, seedlings and roots as well as lower part of the stem causing serious damage (Hassanien, 1985; Singh *et al*., 1987; Sinclair and Backman, 1989). *Rhizocronia solani* Kuchn causes foot and root rots of young soybean plants. Signs of infection are reddish-brown decay of the outer cortical tissues and later sunken reddish cankers that girdle the stem at the soil line (Sinclair and Backman, 1989). Moreover, charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid causes damping-off of seedlings, which characterized by reddish-brown lesions girdling the stem near the soil. The fungus turns stellar tissues of the root to dark brown and as the infected plant approach senescence, numerous blackish microsclerotia of the fungus from below the epidermis of the underground

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parts, giving the affected portion a charcoal appearance (Oyekan and Niak, 1987). *Fusarium* species have been reported to cause wilt in soybean, which characterized by the browning of the vascular tissues of roots and stems of infected plants and yellowing and withering of the foliage (Sinclair and Backman, 1989).

Many authors studied the effect of growth substances on overcoming the depressing effect of salinity (Zaidi and Singh, 1995; Galley and Fletcher, 1997; Abd El-Hai, 2001) and on linear growth, sporulation and sclerotial formation of the pathogens and they reported that auxins (IAA, IBA and NAA) are potential antifungal (Michiewiez and Rozej, 1987; Khalifa, 2003; Metwally *et al*., 2006). Under such adverse conditions, plants are more susceptible and secreted low yield.

Therefore, reduction of soybean root and stalk rots under salt stress conditions by means of growth substances, i.e., Ethrel, Indole Butyric acid (IBA) and Cycocel (CCC) was the objective of the present investigation.

MATERIALS AND METHODS

Isolation, purification and identification of the causal pathogens: Samples of soybean plant showing typical symptoms of root rot and stalk rot diseases were collected from the farm of Tag El-Ezz Agricultural Research Station, Dakahlia, Egypt during the summer season of 2007. The infected stem lower parts and roots were washed with tap water and cut into pieces, surface sterilized by immersing them in sodium hypochlorite (2%) for 2 min. The sterilized pieces rewashed several times with sterilized water, dried between two sterilized filter paper, placed on Potato Dextrose Agar (PDA) plates supplemented with streptomycin sulfate (100 μg mL^{-1}) and incubated at 28°C for 5 days. The growing fungi were purified according to Hawker (1960). Identification of the isolated fungi was carried out based on taxonomic criteria as described by Ellis (1976) for *Macrophomina phaseolina*, Booth (1977) for *Fusarium solani* and *F. oxysporum* and Sneh *et al*. (1992) for *Rhizoctonia solani*.

Preparation of the fungal inoculum: The individual fungal inoculum of each fungus was prepared using sorghum: coarse sand: water (2:1:2 v/v) medium. The ingredients were mixed, bottled and autoclaved for 15 min at 1.5 air pressure. The sterilized medium was inoculated using agar discs obtained from the periphery of 6 days old colony of the isolated fungi. The inoculated media were incubated at 28°C for 15 days and were then used for soil infestation in the greenhouse experiment.

Greenhouse experiment

Pathogenicity test: Pathogenicity test of the isolated fungi was carried out under greenhouse. Each pathogen was added to the autoclaved clayey soil at a rate of 0.3% (w/w), then putted in sterilized pots (25 cm in diameter) filled up with 4 kg soil. The infested pots were watered daily for one week to enhance growth and distribution of the fungal inoculum. At the same time three pots containing autoclaved soil were prepared to serve as control. Seeds of soybean (Giza 22) were surface sterilized by immersing them in 0.1% sodium hypochlorite for two minutes then washed several times with sterilized water and sown at the rate of ten seeds pot^{-1}, three replicates were used in each particular treatment. Disease assessment was recorded as pre and post-emergence damping-off as well as healthy survival plants after 20, 40 and 80 days from sowing, respectively.

Effect of soil-borne fungi on the anatomical structure of soybean seedlings: Specimens of soybean seedlings were chosen after 30 days from sowing for determination the anatomical changes which occurred by the pathogenic fungi. Small parts, 5 mm in length, were taken from the stem base (infected region). Specimens were killed and fixed in formalin: alcohol: acetic acid mixture (1:18:1 v/v), washed and dehydrated in alcohol series and embedded in paraffin wax (52-54°C m.p.). Sections at 12-15 μ thick were prepared by a rotary microtome, stained in crystal violet and erythrosine, cleared in xylol and mounted in Canada balsam (Gerlach, 1977). Sections were examined microscopically.

Effect of growth substances on the linear growth of pathogenic fungi: Ethrel (2-chloroethyl phosphonic acid) obtained from BDH chemicals LTD, U.K, indole butyric acid 99% (Alderich chemical company, England) and Cicocel 40% (Alderich chemical company, England) at 100 and 200 ppm as well as Kocide 101 77% w.p. and rizolex-T50 w.p. at 3 g L^{-1} were tested in vitro on the linear growth of the pathogenic fungi. Different concentrations were added to 10 mL of sterilized PDA before solidification and then poured in sterile petri-dishes. After solidification the plates were inoculated with fungal disc (5 mm) in the center of the plate and incubated at 27±1°C. Three plates for each particular treatment for each fungus were used as replicates; three plates were prepared to serve as control for each fungus. Linear growth was observed daily and diameter of fungal colonies were recorded when plates of any treatment were filled with the fungal growth.

Field experiments: Field experiments were carried out during 2008 and 2009 Summer seasons at Tag El-Ezz Research Station, Dakhalia Governorate, Egypt. On the base of soil salinity by measuring the Electrical conductivity (EC), the farm soil was divided into three blocks (1000, 2000 and 3000 ppm, approximately). Soybean seeds were soaked for 20 min in each tested growth substance (100 or 200 ppm) while, both fungicides (Kocide 101 or Rizolex T-50) were used as seed coating at the rate of 3 g kg^{-1} seed. The wetted seeds were air dried before sowing. Treated soybean seeds were sown in the first week of May of both seasons. A split plot design with three replicates was used in this experiment. The main plots were occupied by the salinity levels while sub-plots were occupied by the growth substances and fungicides. The area of each sub-plot was 3.5×3 m, sown with 375 seeds. The germination percentage and pre-emergence damping-off were determined after 20 days from sowing while post-emergence damping-off was determined at the 40th day from sowing.

Morphological and yield characters: Samples were taken at the 65th day from planting to estimate plant height (cm) and No. of branches and leaves $plant^{-1}$. At harvesting, No. of pods $plant^{-1}$, plant yield and weight of 100 seed were recorded.

Physiological characters: At the 65th day from sowing, the blade of the third leaf from tip (terminal leaflet) was taken to determine photosynthetic pigments (chlorophyll a, b and carotenoids) which extracted with methanol after adding traces of sodium carbonate (Robinson and Britz, 2000) and determined according to Mackinney (1941).

Total phenolics were determined after 65 days from sowing in fresh shoot using the folin-ciocalteau reagent according to Singleton and Roosi (1965).

Proline content was determined in shoot-dried material taken at the 65th day from sowing by the modified ninhydrine method (Troll and Lindsley, 1955) omitting phosphoric acid to avoid interference with concentrated sugars (Magne and Larher, 1992). Two grams of shoot dry weight were placed into tube containing 10 mL of distilled water. The tubes were kept in boiling water bath for 30 min, then cooled at room temperature. An aliquot from corresponding water extract was added to 2 mL ninhydrine reagent and the mixture was maintained in boiling water bath for 20 min, then cooled in an ice water bath. The product formed was extracted by 3 mL toluene by vigorous shaking. Absorption was measured spectrophotometically at 520 nm, using L-proline as standard.

Disease assessment: The disease severity of root-rot in mature stage was determined according to the scale suggested by Kravea (1960) as follows:

0 = Healthy plant
1 = The lower part of stem is slight darkened
2 = The region between the knots is heavily darkened
3 = The lower part of the stem is heavily darkened while, the upper is whitened
4 = Damping-off plant

Stalk-rot disease severity was determined following the scale of Phillips (1971) in which:

0 = 0 cm (no discoloration under the first node)
1 = 5 cm (discoloration through the first node)
2 = 13 cm (discoloration through the second node)
3 = 17 cm (discoloration through the third node)
4 = 28 cm (discoloration through the fourth node)

The severity of root rot and stalk rot diseases were calculated from the following formula:

$$Sd = \frac{\sum (ab) \times 100}{AK}$$

Where:
sd = Severity of disease.
a = No. of diseased plants having similar degree of infection
b = Degree of infection
Σ = Sum of (ab)
A = No. of examined plants
K = Highest degree of infection (in this case = 4)

Sees quality: Soybean seeds were dried at 70°C for 48 h, grounded and analyzed for oil percentage (AOAC, 1970) and total nitrogen by semi-micro-Kjldahle (Pregl, 1945). The protein percentage was calculated by multiplying the N% by 6.25.

Statistical analysis: Data were analyzed with the statistical analysis software (CoStat, 2005). All multiple comparisons were first subjected to analysis of variance. Comparisons among means were made using Duncan's multiple range test at $p<0.05$ according to Gomez and Gomez (1984).

RESULTS

Isolation and pathogenicity test: *Macrophomina phaseolina*, *Fusarium solani*, *F. oxysporum* and

Rhizoctonia solani, the causal pathogens of soybean root rot and stalk rot (brown stem rot) were isolated from infected plants. After identification trials, the four pathogens were further investigated for pathogenicity, the percentage of pre and post-emergence damping-off as well as healthy plants under greenhouse conditions are presented in Fig. 1. The tested fungi were pathogenic and caused pre- and post-emergence damping-off. This result was confirmed by the absence of emergence damping-off under non-infested soil (control). *Rhizoctonia solani* gave highest percentage of pre-emergence damping-off followed by *M. phaseolina* then *F. solani*. On the other hand, *F. solani* was recorded as the most aggressive pathogen in post-emergence damping-off followed by *F. oxysporum* then *R. solani*. The highest percentage of survival plants occurred under soil infested with *F. oxysporum* (64%) followed by *F. solani* (57.34%) then *M. phaseloina* (52%). In contrast, *R. solani* gave the highest percentage of diseased plants.

Anatomical structure of the infected soybean stem: The stem cross sections of healthy and infected plants by *R. solani*, *M. phaseolina*, *F. solani* and *F. oxysporum* showed obvious differences in anatomical structure (Fig. 2a-e). As depicted, the basal portion of the infected

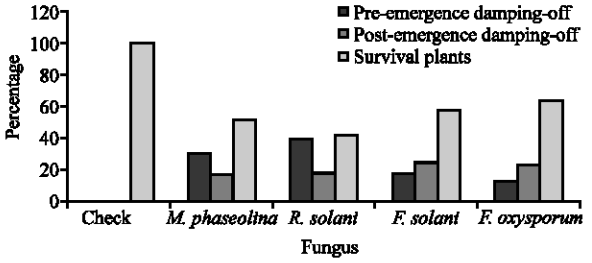

Fig. 1: Pathogenicity test of pre- and post-emergence damping-off and survival soybean plants infected with isolated rot fungi under greenhouse conditions

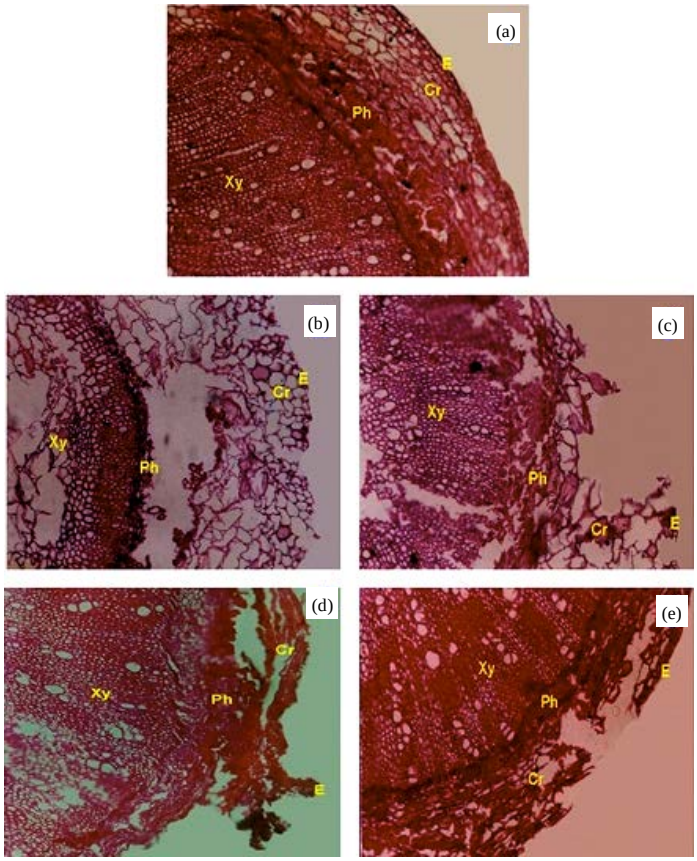

Fig. 2: Cross sections of soybean stem basal portion in (a) healthy plant, (b) infected plant with *R. solani*, (c) infected plant with *M. phaseolina*, (d) infected plant with *F. solani* and (e) Infected plant with *F. oxysporum*. E: Epidermis; Cr: Cortex; Ph: Phloem; Xy: Xylem

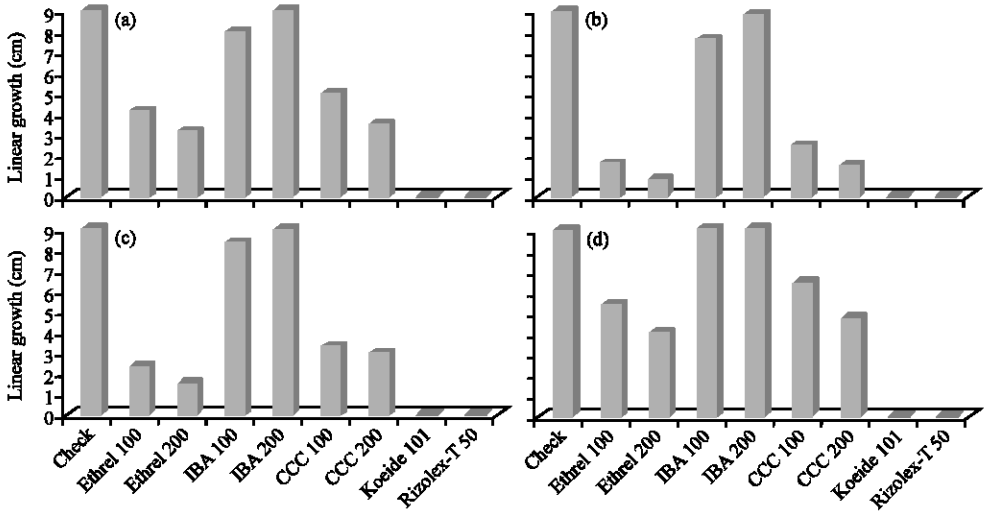

Fig. 3: Effect of the tested growth substances and fungicides on the linear growth (cm) of the tested pathogenic fungi. (a) *M. phaseolina*, (b) *R. Solani* (c) *F. solani* and (d) *F. oxysporum*

stem showed deformation in the anatomical structure occurred mainly in epidermis, cortex and pith compared with healthy plants. Complete disruption was observed in the epidermal cells and sever plasmolysis in the cortical cells with destruction of the outer cortical cells; *R. solani* was the most aggressive fungus. The disruption of cortical cells takes place in the deeper part followed by decay and degradation of primary cell wall. Some areas of cortex tissue showed a breakdown of cell wall components. The hyphae of fungal growth could be clearly seen in the inter- and intera-cellular epidermis, cortex and pith tissues. In severely infected stem by *R. solani*, the extensive damage in both cortex and pith tissues leading eventually to cell separation in some areas that leading to cell death. Generally, *R. solani* followed by *M. phaseolina* led to the injurious effects on basal stem structure more than *F. solani* and *F. oxysporum*.

Growth substances vis linear growth of the isolated pathogens: The effect of both levels of growth substances (Ethrel, IBA and CCC) and fungicides (Kocide 101 and Rizolex-T50) on the linear growth of the tested fungal pathogens are presented in Fig. 3a-d. Both fungicides completely inhibited the growth of all tested pathogenic fungi. On the other side, there is inversely relationship between increasing the concentration of growth substances and growth of the tested pathogenic fungi except IBA. The minimum growth of all pathogenic fungi occurred under the application of Ethrel (200 ppm) followed by CCC at 200 ppm then Ethrel 100 ppm as compared with the check. On contrast, IBA had no significant effect on fungal growth. There were differences in the sensitivity of the pathogens to the growth substances; *R. solani* being the most sensitive fungus, *F. solani* came next while, *F. oxysporum* came late.

Field evaluation of the tested growth substances

Germination percentage and damping-off: The effect of seed treatment with growth substances and fungicides on soybean germination and damping-off under salt stress conditions are presented in Table 1. Under natural infection, the germination of soybean seeds decreased significantly with increasing salinity level from 1000 to 3000 ppm during the two growing seasons. In contrast, pre- and post-emergence damping-off increased significantly with the salinity levels taking in consideration that post-emergence damping-off was less than pre-emergence damping-off.

Data also, show that all treatments at any dose used increased significantly germination percentage, at the same time decreased significantly damping-off. In this respect, rizolex-T was more effective followed by IBA at 200 ppm then kocide 101. Whereas, ethrel (200 ppm) did not cause any significant effect.

As for the interaction treatments data indicate that all growth substances counteracted the harmful effects of salinity on germination and both damping-off, IBA gave the highest effect followed by CCC, the high concentration (200 ppm) was more effective than the low concentration (100 ppm).

Morphological characters: The effect of growth substances on soybean plant height, No. of branches

Table 1: Effect of the tested growth substances and fungicides on germination percentage and damping-off disease of soybean plants grown under different salt stress conditions

	2008			2009		
Treatments	Germination (%)	Pre-emergence	Post-emergence	Germination (%)	Pre-emergence	Post-emergence
1000 ppm						
Check	80.33f*	19.67j	9.33gh	77.92e	20.48j	9.82fg
Ethrel 100	83.33c-d	16.67k-m	7.33ij	80.83cd	17.36k-m	7.72h-j
Ethrel 200	81.33d-f	18.67j-l	8.33hi	78.89de	19.44j-l	8.77g-i
IBA 100	91.33b	8.67o	7.00jk	88.59b	9.03o	7.37i-k
IBA 200	93.33ab	6.67op	4.67no	90.53ab	6.94op	4.91m-o
CCC 100	84.00cd	16.00mn	6.33j-l	81.48cd	16.67mn	6.67jkl
CCC 200	86.00c	14.00n	6.00k-m	83.42c	14.58n	6.32j-m
Kocide 101	93.00ab	7.00op	4.33op	90.21b	7.29op	4.56no
Rizolex-T 50	95.00a	5.00p	3.33p	92.15a	5.21p	3.51o
2000 ppm						
Check	71.00j-k	29.00e	14.67cd	68.87i-k	30.21e	15.43c
Ethrel 100	74.33g-i	25.67gh	12.33e	72.10f-h	26.73gh	12.98d
Ethrel 200	71.67i-k	28.33ef	14.00d	69.52h-j	29.52ef	14.74c
IBA 100	82.00d-f	18.00j-m	8.67h	79.54de	18.75j-m	9.12gh
IBA 200	84.00cd	16.00mn	6.67j-l	81.48cd	16.67mn	7.02jk
CCC 100	77.00g	23.00i	11.67e	74.69f	23.96i	12.28de
CCC 200	81.00ef	19.00jk	8.33hi	78.57de	19.79jk	8.77g-i
Kocide 101	83.67c-d	16.33l-n	5.67l-n	81.16cd	17.01l-n	5.97k-n
Rizolex-T 50	86.00c	14.00n	5.00m-o	83.42c	14.58n	5.26l-n
3000 ppm						
Check	59.67o	40.33a	18.67a	57.88n	42.01a	19.65a
Ethrel 100	63.67mn	37.00bc	16.33b	61.76lm	38.54bc	17.19b
Ethrel 200	61.33no	38.67ab	17.67a	59.49mn	40.28ab	18.60a
IBA 100	70.00kl	30.00de	11.33ef	67.90jk	31.25de	11.93de
IBA 200	73.67h-j	26.33fg	9.00h	71.46g-i	27.43fg	9.47fg
CCC 100	65.00m	35.00c	15.33bc	63.05l	36.45c	16.14bc
CCC 200	68.33l	31.67d	10.33fg	66.28k	32.98d	10.88ef
Kocide 101	73.67h-j	26.33fg	8.33hi	71.46g-i	27.43fg	8.77g-i
Rizolex-T 50	76.33gh	23.67hi	7.33ij	74.04fg	24.65hi	7.72h-j

*Mean within a column followed by the same letter(s) is not significantly different according to Duncan's multiple range test at $p<0.05$

and No. of leaves under salt stress conditions during the two seasons of 2008 and 2009 are presented in Table 2. It can be easily noticed the negative correlation between the salinity and the tested morphological characters. The greatest reduction in morphological characters occurred at 3000 ppm. Data also, indicate that both levels of IBA (100 and 200 ppm) at any level of salinity increased significantly plant height, IBA at 200 ppm was more effective. On contrast, an additive effects of salinity on decreasing plant height was recorded due to both levels of ethrel (100 and 200 ppm) and CCC (200 ppm) during the two seasons. While, CCC at 100 ppm and kocide 101 had no significant effect in this respect.

Any level of either ethrel or CCC increased significantly No. of branches plant^{-1} under salt stress. Ethrel (200 ppm) followed by (CCC 200 pmm) at 1000 ppm salt stress were the most effective. Meanwhile, other treatments had no significant effect on this parameter. All growth substances excreted positive significant effect on No. of leaves/plant at the different levels of salt stress.

Disease incidence: Concerning the effects of salt stress on root rot and stalk rot disease, results of Table 3 show that the severity of root and stalk rots disease increased significantly with increasing salt stress from 1000 to 3000 ppm, the maximum increment occurred under the highest level (3000 ppm).

All tested materials clearly reduced the severity of root and stem rots compared to check during the two growing seasons. The maximum reduction in root and stalk rots were recorded with both fungicides. Ethrel (200 ppm) was the best growth substance followed by CCC (200 ppm) then ethrel (100 ppm). Taking in consideration that no significant differences between fungicides and high level of CCC or ethrel (200 ppm). The presence of growth substances at any level of salt stress decreased significantly the severity of root and stalk rots as compared with check. Ethrel (200 ppm) among growth substances gave the highest reduction in root-rot disease severity followed by CCC (200 ppm).

Physiological characters

Photosynthetic pigments: Results in Table 4 show significant decrease in chlorophyll a and b with the increasing of salt stress level from 1000 to 3000 ppm, while, an induction response of salinity was recorded in carotenoids content. Concerning the effects of growth substances data show that all photosynthetic pigments

Table 2: Effect of some growth substances and fungicides on soybean morphological characters grown under different salt stress conditions

	2008			2009		
Treatments	Plant height (cm)	No. of branches	No. of leaves	Plant height (cm)	No. of branches	No. of leaves
1000 ppm						
Check	62.33g-j*	6.33de	55.33k-m	60.00g-i	7.33c-e	61.33j-l
Ethrel 100	58.67k-m	8.33b	72.67cd	56.67i-k	10.00b	80.67cd
Ethrel 200	53.00o-r	10.00a	84.00a	51.00m-p	12.00a	93.33a
IBA 100	85.33b	6.00d-f	69.67ef	82.33b	7.00d-f	77.67de
IBA 200	94.67a	5.33f-h	75.00bc	90.67a	6.33e-h	83.33bc
CCC 100	63.00g-i	7.33c	72.00de	60.33g-i	8.33c	80.00cd
CCC 200	59.67i-l	9.33a	76.67b	57.67h-j	11.33a	85.00b
Kocide 101	65.00fg	6.67cd	60.67hi	62.00fg	7.67cd	67.67gh
Rizolex-T 50	68.00ef	6.33de	56.67j-l	65.00ef	7.33c-e	62.67i-k
2000 ppm						
Check	56.00m-o	4.67h-j	48.67n	54.00k-m	5.67g-i	54.00m
Ethrel 100	49.33s	6.67cd	63.67g	47.33q	7.67cd	71.00fg
Ethrel 200	44.00t	7.33c	67.00f	42.00r	8.33c	74.33ef
IBA 100	73.33d	4.67h-j	54.00lm	70.33d	5.67g-i	60.00kl
IBA 200	77.00c	4.33ij	58.00i-k	74.00c	5.33h-j	64.33h-j
CCC 100	57.00l-n	5.67e-g	59.00ij	55.00j-l	6.67d-g	65.33hi
CCC 200	52.00p-s	6.67cd	62.67gh	50.00n-q	7.67cd	69.67g
Kocide 101	59.33j-m	4.67h-j	52.67m	57.33h-k	5.67g-i	58.67l
Rizolex-T 50	61.33h-k	4.33ij	49.33n	59.00g-i	5.33h-j	55.00m
3000 ppm						
Check	50.00rs	3.00l	34.67q	48.00pq	4.00k	38.67q
Ethrel 100	43.00t	5.33f-h	43.67o	41.00r	6.33e-h	48.67n
Ethrel 200	41.00t	6.00d-f	48.67n	39.00r	7.00d-f	54.00m
IBA 100	63.67gh	3.33kl	35.00q	60.67gh	4.33jk	39.00q
IBA 200	68.67e	3.00l	36.00q	65.67e	4.00k	40.00pq
CCC 100	53.67n-q	4.00jk	39.00p	51.67l-o	5.00i-k	43.33op
CCC 200	43.00t	5.00g-i	41.67op	41.00r	6.00f-i	46.67no
Kocide 101	51.33q-s	3.33kl	36.00q	49.33o-q	4.33jk	40.00pq
Rizolex-T 50	55.00n-p	3.00l	34.33q	53.00l-n	4.00k	38.33q

*Mean within a column followed by the same letter(s) is not significantly different according to Duncan's multiple range test at $p<0.05$

Table 3: Effect of the tested growth substances and fungicides on disease incidence of soybean root rot and stalk rot under different salt stress conditions

	2008				2009			
Treatments	Root rot (sd)	Reduction (%)	Stalk rot (sd)	Reduction (%)	Root rot (sd)	Reduction (%)	Stalk rot (sd)	Reduction (%)
1000 ppm								
Check	26.67d*	0.00	32.33cd	0.00	33.67c	0.00	30.33c	0.00
Ethrel 100	9.67m	63.75	23.67g-j	26.80	12.33l	63.38	22.67g-i	25.26
Ethrel 200	8.00no	70.00	20.33kl	37.11	10.00mn	70.30	19.33jk	36.27
IBA 100	15.33i	42.50	24.67f-h	23.71	19.33i	42.59	23.67fg	21.96
IBA 200	11.67l	56.25	27.67e	14.43	14.67k	56.43	26.67de	12.07
CCC 100	12.00l	55.00	21.67i-l	32.99	15.00k	55.45	20.67h-k	31.85
CCC 200	9.33mn	65.00	19.33lm	40.21	11.67lm	65.34	18.33kl	39.56
Kocide 101	7.67o	71.25	15.00n	53.61	9.67mn	71.28	14.00m	53.84
Rizolex-T 50	7.00o	73.75	17.00mn	47.42	9.00n	73.27	16.00lm	47.25
2000 ppm								
Check	29.00b	0.00	36.00b	0.00	36.00b	0.00	34.00b	0.00
Ethrel 100	15.00ij	48.28	25.00fg	30.56	18.67i	48.14	24.00fg	29.41
Ethrel 200	13.67jk	52.87	22.67g-k	37.04	17.33ij	51.86	21.67g-j	36.26
IBA 100	20.33g	29.89	27.67e	23.15	25.33f	29.64	26.67de	21.56
IBA 200	17.33h	40.23	32.33cd	10.19	21.67gh	39.81	30.33c	10.79
CCC 100	15.67i	45.98	24.00g-i	33.33	19.67hi	45.36	23.00f-i	32.35
CCC 200	14.33ij	50.58	21.33j-l	40.74	18.33i	49.08	20.33i-k	40.21
Kocide 101	12.67kl	56.32	17.67m	50.93	16.00jk	55.56	16.67l	50.97
Rizolex-T 50	12.00l	58.62	20.33kl	43.52	15.00k	58.33	19.33jk	43.15
3000 ppm								
Check	33.33a	0.00	39.33a	0.00	42.00a	0.00	37.33a	0.00
Ethrel 100	23.33e	30.00	26.67ef	32.20	29.33d	30.17	25.67ef	31.23
Ethrel 200	21.33fg	36.00	24.00g-i	38.98	26.67ef	36.50	23.00f-i	38.39
IBA 100	28.67bc	14.00	30.33d	22.88	35.67bc	15.07	29.00cd	22.31
IBA 200	27.33cd	18.00	33.00c	16.10	34.33bc	18.26	31.00c	16.96
CCC 100	24.00e	28.00	23.67g-j	39.83	30.00d	28.57	22.67g-i	39.27
CCC 200	22.67ef	32.00	22.33h-k	43.22	28.67de	31.74	21.33g-j	42.86
Kocide 101	20.00g	40.00	24.33f-h	38.14	25.00f	40.48	23.33f-h	37.50
Rizolex-T 50	18.00h	46.00	27.67e	29.66	22.67g	46.02	26.67de	28.56

*Mean within a column followed by the same letter(s) is not significantly different according to Duncan's multiple range test at $p<0.05$

Table 4: Effect of the tested growth substances and fungicides on the photosynthetic pigments contents of soybean plant grown under different salt stress conditions

	2008			2009		
Treatments	Chlorophyll A	Chlorophyll B	Caroteinoids	Chlorophyll A	Chlorophyll B	Caroteinoids
	(mg g^{-1} fresh weight)			(mg g^{-1} fresh weight)		
1000 ppm						
Check	1.20gh*	0.70de	0.21n	1.30g	0.82ef	0.25m
Ethrel 100	1.62a	0.94a	0.38a	1.76a	1.12a	0.47a
Ethrel 200	1.48c	0.90a	0.35b	1.57c	1.06b	0.42b
IBA 100	1.27f	0.80b	0.28d-g	1.46e	0.90d	0.35f-i
IBA 200	1.30ef	0.82b	0.31cd	1.50d	0.94d	0.37c-g
CCC 100	1.58b	0.91a	0.35b	1.67b	1.09b	0.45a
CCC 200	1.33e	0.83b	0.32c	1.52d	1.01c	0.39cd
Kocide 101	1.21g	0.70de	0.21mn	1.29g	0.84e	0.25m
Rizolex-T 50	1.21gh	0.70de	0.21mn	1.31g	0.82ef	0.26m
2000 ppm						
Check	0.92m	0.53hi	0.23k-n	1.18jk	0.65kl	0.30l
Ethrel 100	1.46c	0.75c	0.30c-e	1.45e	0.91d	0.39c
Ethrel 200	1.32e	0.72cd	0.27e-i	1.40f	0.79f	0.36e-g
IBA 100	1.18hi	0.58fg	0.24k-m	1.28g	0.72hi	0.34h-k
IBA 200	1.21g	0.60f	0.25h-l	1.24h	0.75gh	0.35g-j
CCC 100	1.39d	0.73cd	0.28d-g	1.42f	0.83e	0.38c-e
CCC 200	1.27f	0.67e	0.25h-l	1.31g	0.76g	0.35f-i
Kocide 101	0.92m	0.55gh	0.23l-n	1.17kl	0.66kl	0.31l
Rizolex-T 50	0.91m	0.52hi	0.24k-m	1.21ij	0.67jk	0.31l
3000 ppm						
Check	0.84n	0.28l	0.25i-l	1.02o	0.41o	0.32kl
Ethrel 100	1.16i	0.52hi	0.29d-f	1.30g	0.70ij	0.37d-g
Ethrel 200	1.09j	0.46j	0.26g-k	1.18jk	0.63l	0.35f-i
IBA 100	0.96l	0.39k	0.25h-l	1.07n	0.53n	0.34h-k
IBA 200	0.98l	0.41k	0.28e-h	1.10m	0.56m	0.35f-h
CCC 100	1.12j	0.50i	0.27f-j	1.21hi	0.66kl	0.37c-f
CCC 200	1.02k	0.42k	0.26g-k	1.15l	0.59m	0.35f-h
Kocide 101	0.84n	0.29l	0.24j-l	1.01o	0.42o	0.33i-l
Rizolex-T 50	0.87n	0.29l	0.25i-l	1.02o	0.42o	0.32j-l

*Mean within a column followed by the same letter(s) is not significantly different according to Duncan's multiple range test at $p<0.05$

(chlorophyll a, b and carotenoids) increased by using both levels of growth substances. The highly increase occurred under low level of ethrel followed by low level of CCC. It is worthy to mention that the low level of either ethrel or CCC was more effective than the high level. On the other hand, all photosynthetic pigments were not affected significantly under both of fungicides used.

The interaction between salinity level and growth substances significantly increased chlorophyll a and b as well as carotenoids content in both seasons. Generally, growth substances alleviated the injuries effects of salinity on chlorophyll content compared with fungicides.

Total phenols and proline content: Total phenols and proline content in soybean shoots increased significantly with increasing salinity levels from 1000 to 3000 ppm (Table 5), the high level of ethrel was more effective in this respect followed by the high level of CCC then IBA. However, both fungicides had not any significant effect on phenol and proline content in soybean shoots. Cycocel (200 ppm) followed by IBA (200 ppm) then ethrel (200 ppm) gave the highest values of total phenols and proline content under low salinity level, whereas, ethrel (200 ppm) followed by CCC (200 ppm) were the most effective under moderate and high salinity levels.

Yield and its components: The No. of pods $plant^{-1}$, plant yield (g) and weight of 100 seeds decreased significantly with increasing salinity level (Table 6). On contrast, all concentrations of growth substances increased significantly these parameters compared with the check. The highest values of No. of pods $plant^{-1}$ and plant yield occurred under application of ethrel (200 ppm) followed by CCC at 200 ppm while, no significant effect was recorded by using both fungicides. In both seasons, IBA at 200 ppm led to the maximum increase in weight of 100 seeds followed by ethrel at 200 ppm then IBA at 100 ppm. The application of growth substances counteracted the harmful effects of soil salinity on No. of pods $plant^{-1}$, plant yield and weight of 100 seeds. Ethrel was the most effective treatment followed by CCC then IBA.

Seed quality: It is clear from Table 7 that oil and protein percentage in soybean seeds decreased significantly with increasing salinity level. The great reduction occurred under the high salinity level (3000 ppm). On the other

Table 5: Effect of the tested growth substances and fungicides on the total phenol and proline content of soybean plant grown under different salt stress conditions

	2008		2009	
Treatments	Total phenol mg/100 g fresh weight	Proline (µmole g^{-1} dry weight)	Total phenol mg/100 g fresh weight	Proline (µmole g^{-1} dry weight)
1000 ppm				
Check	64.68w*	23.03o	62.07z	24.24o
Ethrel 100	97.07m	25.00lm	93.15p	26.32lm
Ethrel 200	101.75j	25.51j	97.64k	27.90j
IBA 100	93.13p	24.35mn	89.37s	25.63mn
IBA 200	105.65g	25.63j-l	101.38h	26.98j-l
CCC 100	98.07l	25.45kl	94.11o	26.79kl
CCC 200	107.96f	26.18jk	103.60f	27.56jk
Kocide 101	81.48t	23.50no	78.19x	24.74no
Rizolex-T 50	88.32r	23.65no	84.75u	24.90no
2000 ppm				
Check	66.93v	27.73i	64.23z	29.19i
Ethrel 100	100.45k	30.14h	96.39n	31.72h
Ethrel 200	105.29h	33.37g	101.04i	35.12g
IBA 100	96.37n	28.34i	92.48q	29.83i
IBA 200	109.32e	29.71h	104.90e	31.27h
CCC 100	101.48j	29.92h	97.38l	31.50h
CCC 200	111.72c	32.62g	107.21c	34.34g
Kocide 101	84.31s	27.98i	80.90w	29.45i
Rizolex-T 50	91.39q	28.03i	87.70t	29.51i
3000 ppm				
Check	69.87u	34.94f	67.05y	36.78f
Ethrel 100	104.85i	36.92d	100.61j	38.86d
Ethrel 200	109.91d	42.22a	105.47d	44.44a
IBA 100	100.60k	35.40ef	96.54m	37.26ef
IBA 200	114.12b	38.34c	109.51b	40.36c
CCC 100	105.93g	36.25de	101.65g	38.16de
CCC 200	116.61a	40.94b	111.90a	43.09b
Kocide 101	88.01r	34.71f	84.45v	36.54f
Rizolex-T 50	95.40o	34.64f	91.55r	36.46f

*Mean within a column followed by the same letter(s) is not significantly different according to Duncan's multiple range test at $p<0.05$

Table 6: Effect of the tested growth substances and fungicides on yield components at harvest stage of soybean grown under different salt stress conditions

	2008			2009		
Treatments	No. of pods $plant^{-1}$	Plant yield (g)	Weight of 100-seeds	No. of pods $plant^{-1}$	Plant yield (g)	Weight of 100-seeds
1000 ppm						
Check	95.33ef*	33.89fg	17.43i	90.57ef	34.94fg	19.59i
Ethrel 100	154.67a	48.41d	18.63e	146.93a	49.90d	20.94e
Ethrel 200	146.33ab	67.35a	19.95b	139.20ab	69.43a	22.42b
IBA 100	111.67c-e	37.20f	19.60c	106.08c-e	38.35f	22.02c
IBA 200	127.00c	55.46c	20.42a	120.56bc	57.18c	22.94a
CCC 100	118.33cd	42.23e	17.96h	112.42cd	43.53e	20.17h
CCC 200	128.00bc	63.82b	19.09d	121.60bc	65.79b	21.45d
Kocide 101	102.00de	33.55gh	17.91h	96.90d-f	34.59fg	20.12h
Rizolex-T 50	96.00ef	35.74fg	17.60i	91.20ef	36.85f	19.78i
2000 ppm						
Check	45.00k-o	15.27m-o	11.90q	42.75j-l	15.74m-o	13.37q
Ethrel 100	52.67i-l	22.43jk	17.14j	50.03h-k	23.13ij	19.26j
Ethrel 200	82.00fg	30.32hi	18.18g	77.90fg	31.26gh	20.43g
IBA 100	63.00h-k	24.21j	17.92h	59.85g-j	24.96i	20.13h
IBA 200	68.00g-j	29.15i	18.43f	64.60g-i	30.05h	20.71f
CCC 100	49.67j-l	19.44kl	16.96j	47.18i-l	20.04j-l	19.06j
CCC 200	69.00g-i	25.20j	17.86h	65.55g-i	25.97i	20.07h
Kocide 101	48.00k-m	16.81lm	15.46l	45.60i-l	17.33k-n	17.37l
Rizolex-T 50	47.00k-n	15.80mn	14.24o	44.65i-l	16.28l-n	16.00o
3000 ppm						
Check	28.33no	11.40p	10.64r	26.92l	11.76o	11.96r
Ethrel 100	47.67k-m	15.21m-o	14.16o	45.28i-l	15.68m-o	15.91o
Ethrel 200	73.00gh	21.85jk	15.14m	69.35gh	22.52ij	17.01m
IBA 100	37.67l-o	13.16n-p	14.20o	35.78kl	13.57no	15.96o
IBA 200	53.00i-l	17.30lm	15.81k	50.35h-k	17.84k-m	17.76k
CCC 100	42.00l-o	13.82m-p	12.81p	39.90j-l	14.25m-o	14.39p
CCC 200	67.33g-j	19.75kl	14.66n	63.97g-i	20.36jk	16.74n
Kocide 101	29.33m-o	11.83op	12.00q	27.87l	12.20o	13.48q
Rizolex-T 50	28.00o	11.61p	11.94q	26.60l	11.20o	13.41q

*Mean within a column followed by the same letter(s) is not significantly different according to Duncan's multiple range test at $p<0.05$

Table 7: Effect of the tested growth substances and fungicides on the protein and oil content of soybean plant grown under different salt stress conditions

	2008		2009	
Treatments	Protein (%)	Oil (%)	Protein (%)	Oil (%)
1000 ppm				
Check	41.67f-h*	20.33hi	41.33g	20.00fg
Ethrel 100	47.33b	22.33fg	45.00cd	22.00d
Ethrel 200	50.33a	23.67c-e	48.67a	23.67bc
IBA 100	42.33ef	24.67a-c	43.00ef	24.33b
IBA 200	45.33b-d	25.67a	45.67bc	26.00a
CCC 100	44.67c-e	21.00h	44.00de	20.67ef
CCC 200	46.00bc	23.33d-f	46.33b	22.67cd
Kocide 101	41.67f-h	20.33hi	41.00g	20.33fg
Rizolex-T 50	42.00fg	20.67h	41.00g	20.33fg
2000 ppm				
Check	36.67j-l	18.33j-l	36.67ij	17.67hi
Ethrel 100	39.00h-k	21.33gh	41.00g	21.67de
Ethrel 200	43.00d-f	22.67ef	44.67cd	22.33d
IBA 100	37.00j-l	24.33b-d	38.00h	22.67cd
IBA 200	39.33g-j	25.00ab	40.67g	24.67b
CCC 100	38.67i-k	20.33hi	41.00g	19.33g
CCC 200	41.00f-i	21.33gh	42.00fg	20.67ef
Kocide 101	37.00j-l	18.67j-l	36.67ij	18.00h
Rizolex-T 50	36.67j-l	18.67j-l	36.67ij	18.00h
3000 ppm				
Check	28.67o	17.67l	31.00n	16.33j
Ethrel 100	36.33kl	19.33ij	36.00jk	20.33fg
Ethrel 200	39.33g-j	21.00h	38.67h	22.00d
IBA 100	34.67lm	22.33fg	33.00m	22.67cd
IBA 200	36.33kl	24.00b-d	35.33kl	23.67bc
CC 100	35.67l	19.00jk	34.33l	19.33g
CCC 200	38.67i-k	20.67h	37.67hi	20.33fg
Kocide 101	32.00n	17.67l	31.00n	16.67ij
Rizolex-T 50	32.33mn	18.00kl	31.33n	16.33j

*Mean within a column followed by the same letter(s) is not significantly different according to Duncan's multiple range test at $p<0.05$

hand, all concentrations of growth substances increased significantly soybean seed oil and protein percentage. The high level of ethrel followed by high CCC level recorded maximum protein content. Irrespective to the salinity level, the high seed oil content occurred under the application of IBA. On the other side, fungicides had no effect on seed oil and protein % in both seasons. Generally, the growth substances used counteracted the harmful effect of salinity on soybean seed oil and protein content.

DISCUSSION

The pathogenicity tests proved the pathogenic nature of the isolated fungi as causative agents of damping off. *Rhizoctonia solani* was the most aggressive as pre-emergence damping-off pathogen while, *F. solani* caused the highest rate of post-emergence damping-off. These results confirm the aggressive pathogenicity of the isolated fungi. The high rate of damping-off by such fungi may be occurred due to seed rot (Hussain *et al*., 1989) and inhibition of seed germination by fusaric acid, especially by *Fusarium* species (Matsui *et al*., 1988). Moreover, Gally *et al*. (1998) stated that *Fusarium* cause rotten lesions on seed cotyledons and plumule soft rot followed by pre-emergence damping-off. In addition, Oyekan and Naik (1987) found that *M. phaseolina* causes damping-off of seedlings. Also, the infection with these fungi leads to breakdown of the root system, in turn reducing the absorption surface and uptake of essential nutrients and water (Porter *et al*., 1990). In the present investigation, all tested pathogenic fungi cause heavy damage in seedlings anatomy structure (Fig. 2) which in turn causes post-emergence damping-off.

The harmful effects of salinity on germination, growth, yield and seed quality of soybean may be explained as; the salinity increased osmotic potential of the solution around the seeds and decreased water uptake by the seeds (Wang *et al*., 2001). Also, the depressing effect of chloride on enzymes activity such as hydrolytic enzymes e.g., protease and lipase (Younis *et al*., 1987; Munns, 2002) which is usually, followed by decrease in seed germination. Moreover, El-Nabarawy (1994) and El-Saht (1994) stated that the major effect of salinity on seed germination might be due to hormone imbalance in the seeds. The decrease in growth due to salinity may be attributed to the increase in respiration rate resulting from higher energy requirement (Schwarz and Gale, 1981), ionic imbalance (Abo-Hamid, 1994; Ashraf and Foolad, 2007; Sharifi *et al*., 2007) and decrease in meristematic activity and cell enlargement (Khadr *et al*., 1994). Kord and Khalil (1995) added that salinity reduced hormone delivery from root to leaves throughout the seedling, which inhibits plant growth. Van Hoorn *et al*. (2001) noted a decrease in rhizobium growth at increasing salinities and decrease of the No.of nodules. Moreover, salinity causes a disturbance of the nitrogen uptake (Jeuffroy and Sebillotte, 1997). In this study salinity decreased chlorophyll content in soybean leaves (Table 4) in turn, decrease carbohydrate content which followed by decrease oil and protein content in the seeds (Table 7). These results are in agreement with Smith *et al*. (1989) and Yang *et al*. (1990).

The possible mechanisms by which growth substances overcome the depressive effects of salinity are; enhancing water absorption by germinated seeds (Maske *et al*., 1997), cytokinins synthesis that affect plant-water balance (Mac Robbie, 1981), mineral composition (Abo-Hamid, 1994), physiological and hormonal levels within the plants (El-Saht, 1994), decreasing root resistance to water flow and Cl^- uptake (El-Banna, 1985). Etherl decreased chloride uptake and this in turn may increase plant salt tolerance (Kasele *et al*., 1995). In this investigation, the effects of growth substances on alleviation the harmful effects of salinity

may be due to the increase in total chlorophyll content (Table 4), phenol and proline (Table 5) which used as indicators in the osmoregulation of plant. The increase in branches number by etherl application may be due to the liberation of ethylene that considered as natural growth regulators which causes inhibition of terminal bud growth and stimulation of lateral shoot development (Helaly *et al*., 1984). Cycocel also increased No. of branches due to the imbalance in the endogenous hormone levels i.e., gibberellins, cytokinins and auxins, it also, lead to decrease in auxins level (Heather and Jones, 1983).

Indole butyric acid and CCC were the most effective in reducing soybean infections with damping-off. This is might be due to the inhibitory effect of growth substances on fungal growth (Fig. 3), sporulation and sclerotial formations (Khalifa, 2003). These results are in agreement with Marei (2000), Khalil (2002), Chowdhury (2003) and Metwally *et al*. (2006) on peanut root rot diseases. The results also indicate that etherl followed by CCC then IBA decreased significantly soybean infection with root rot and stalk rot disease. This may be due to the positive effect of these substances on chlorophyll, total phenol and proline content (Table 4 and 5). Phenolics are well known as antifungal, antibacterial and antiviral compounds occurring in plants. Moreover, proline is used as a storage compound for energy consequently, reduced carbon needs. Phenolics and proline are used as indicator for any stress conditions that occur to plants. The first step of the defense mechanism in plants involves a rapid accumulation of phenols at the infection site, which restricts or slows the growth of the pathogen (Matern and Kneusal, 1988; Gogoi *et al*., 2001). Moreover, Chowdhury (2003) stated that treated plants with growth regulators showed an increase in total phenol, calcium content and the activity of chatechol oxidase, which protect plants against pathogen stress. In this investigation, there was a positive relationship between chlorophyll and phenol content from one side and oil and protein percentage in soybean seeds from other side. This is in agreement with Smith *et al*. (1989), they reported that there is a relationship between photosynthesis and oil and protein content, hence induced sucrose translocated from leaf to the seeds, which metabolized to precursors for protein and oil. Therefore, any factor lead to increase in chlorophyll content will be expected to stimulate yield as well as oil and protein content in seeds.

There is no doubt that chlorophyll content is a good parameter reflecting the health condition of any plant. Photosynthesis or carbon assimilation consists in the synthesis of certain carbohydrates from CO_2 and H_2O by green cells in the presence of light. Carbohydrates are used as a source of energy. All living organisms require energy not only for growth and reproduction but also for maintenance of life itself. The light energy used is taken up by chlorophyll which present in chloroplast.

It could be concluded that the application of etherl, IBA and CCC at 200 ppm as seed soaking is recommended for reducing root and stalk rots in soybean plants as well as improving growth, yield and seed quality under salt stress conditions.

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