6 *Egypt. J. Phytopathol.,* Vol. **46**, No. 2, pp. 107-129 (2018)
ISSN 1110-0230

Using Some Fungicide-Alternatives to Control Septoria Leaf Spot of Celery and Improve its Yield.

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Field experiments were carried out under natural infection of Septoria leaf spot in the Experimental Farm of Sids Agricultural Research Station, Agric. Res. Center, Beni-Sweif governorate in 2015/2016 and 2016/2017 to evaluate the efficacy of hot water, organic acids, natural oils and bioagents on Septoria leaf spot and productivity of celery plants. Generally, all treatments were effective in reduction of disease severity. Also, a significant increase in fresh and dry weights of the herb, total chlorophyll as well as essential oil percent and oil yield was observed at the end of experiment. The use of the tested materials individually as seed treatments and foliar spray were less effective in reducing the disease incidence and severity than combined treatments with hot water-treated seeds and foliar spray with any of the tested materials. The highest efficacy was obtained from plots received hot water-treated celery fruits combined with phycare + thyme oil as a foliar spray in addition to the fungicide Ridomil gold MZ 68 WG. Furthermore, all the treatments showed significant increases in the defence-related enzymes, peroxidase (POD) and polyphenoloxidase (PPO) as compared with the untreated control.

Keywords: Septoria leaf spot, hot water, organic acids, natural oils, bioagents, defence-related enzymes, peroxidase, polyphenoloxidase, productivity and celery.

Celery (*Apium graveolens* L., Family Apiaceae) is an economically important annual herb crop cultivated mainly for medicinal purposes, food flavoring as well as for exportation. Celery gives significant amount of vitamins A and C, calcium, sodium and contains very little carbohydrate or fat, making it used as a diet food (Lacy *et al.*, 1996).

Recently, a new serious disease affecting the cultivation of celery, Septoria blight, caused by *Septoria apiicola* Speg. was observed for the first time in Egypt in Beni-Sweif governorate in 2015. Since then, the disease has been quickly spread to all the Egyptian celery-growing areas, affecting yield, quality and causing losses of 50-90 % (Hilal and Ghebrial, 2015). Symptoms appear as irregularly shaped spots of necrotic tissue on leaves, petioles and seed coats, in which spore-containing pycnidia are immersed. The spots expand with time and coalesce leading to leaf death. The epidemiology of this disease depends on weather conditions, being favored by high moisture and temperature from 10 to 30^0C. Conidia within the pycnidia are surrounded by a mucilaginous matrix consists of proteins and sugars which swells when the mucilaginous matrix absorbs free water or is exposed to relative humidity higher than 90 % (Lacy *et al.*, 1996). Each pycnidium is capable of releasing 1500 to 5400 conidia. Germination of conidia was higher in the presence of free moisture and infection occurs when celery leaves remain wet for ≥12 h (Mathieu & Kushalappa, 1993 and Lacy, 1994). The pathogen is transmitted with seed and it also overwinters on plant debris. Seed-borne diseases are considered high problem for successful production at the level of yield and its quality where seed can carry the pathogen which represents an important threat to crop yield, causing a reduction in germination, seedling damages, primary infection onset and species spreading (Riccioni and Orzali, 2011). So, effective seed treatments are needed to eradicate or at least to reduce the seed-borne inoculum. Seed treatment by fungicides remains the most effective means in this respect but this is difficult for medicinal and exportation crops like celery, due to the risk of the presence of residues at harvest besides health hazards and environmental pollution. Several non-chemical methods of seed treatment include physical treatments, seed treatments using organic acid, natural essential oil and bio-control agents offer an effective way to replace the use of synthetic fungicides (Mancini and Romanazzi, 2014). The successful result of a seed treatment depends not only on the effectiveness of the compound applied but also on the degree of internal infection of the seed, the amount of inoculum in seeds and the specificity and phytotoxicity of the treatment (Du Toit, 2004). Physical treatments consist of heat treatments of seeds with hot water that consists of the immersion of seeds in agitated water at a predetermined temperature and time. Thermotherapy inactivates or kills the pathogen, while it leaves the host tissue viable (Schmitt *et al.*, 2009). Many essential oils have been found to contain natural antimicrobial compounds that varied in their toxicity against different fungal pathogens (Pina-Vaz *et al.*, 2004 and Soylu *et al.*, 2006). Biological control using antagonistic microorganisms investigated as a viable method in the protection of plants against *Septoria* sp. (Kashyap, 2013). However, the bio-control agents, *Trichoderma* spp. and *Pseudomonas* spp. can improve the growth of plants directly by increasing germination percentage, plant height, dry weight (Nzanza *et al.*, 2011 and Elekhtyar, 2015) and/or indirectly by control of diseases (Harman *et al*., 2001 and Lucy *et al.*, 2004). The essential oils and biocontrol agents can be used alone for seed disinfection or in combination with physical treatments (Begum *et al.*, 2010). Organic acid such as citric acid, ascorbic acid can be a potential control agent against some plant pathogenic fungi (Haggag and Abd El-Khair, 2007). Organic acids are a source of both carbon and energy for cells and are used in the respiratory cycle and some other biochemical pathways (Darandeh and Hadavi, 2012). Foliar application of citric acid, ascorbic acid stimulated the growth, increasing productivity from the fresh herb, seeds and increased the essential oil content of sweet basil, dill, and thyme by enhancing photosynthesis and more tolerance to stress. (Jafari and Hadavi, 2012 a,b.; Miri *et al.*, 2015 and Azoz *et al.*, 2016). Therefore, this study aimed to evaluate the effectiveness of hot water, natural oils, organic acids and bio-control agents as seed treatments and foliar spray suspensions on Septoria blight of celery and its productivity under field conditions.

Materials and Methods

A two-year field experiment was achieved in Sids Experimental Farm of Agricultural Research Station, Agric. Res. Center, Beni-Sweif governorate in 2015/2016 and 2016/2017 to evaluate the efficacy of some treatments, physical method (hot water), organic acids, natural oils and bio-agents against Septoria leaf spot and productivity of celery. The soil of the experimental field was clay in texture (16.5 % sand, 30.1 % silt, 53.4 % clay), pH of 8.1, EC 1.2 dSm^{-1}; 1.3 % organic matter and 26.2, 10.1 and 176 ppm available N, P, and K, respectively. Field experiments were conducted under natural infection in plots (3 x 3.5 m) in a randomized complete block design with three replicates for each treatment.

During both experimental seasons, the following treatments were applied as seed treatment and foliar spray:

T_1: The commercial product Nimbecidine, containing neem oil 90.57 % (obtained from Gaara Establishment for Import & Export, Egypt) was applied as seed treatment and foliar spray at a rate of 0.6 cm/L water.

T_2: Thyme oil (obtained from National Research Center, Egypt), applied as seed treatment and foliar spray at a rate of 1 %.

T_3: The commercial product Phycare, containing phosphorus 28 % w/w, citric acid, lactic acid, palmitic acid 25 % w/w and ascorbic acid 0.625 % w/w (obtained from Gaara Establishment for Import & Export, Egypt), applied as seed treatment and foliar spray at a rate of 0.5 cm/L.

T_4: *Pseudomonas fluorescens* (1×10^8 cfu) was kindly obtained from Department of Microbiology, Soil and Water Res. Inst. and applied as seed treatment and foliar spray.

T_5: *Trichoderma harzianum* (1×10^6 conidia/ml) was kindly obtained from Department of Microbiology, Soil and Water Res. Inst., applied as seed treatment and foliar spray.

T_6: Hot water at 47 ^oC as a seed treatment.

T_7: Hot water as seed treatment and phycare + neem oil as a foliar spray.

T_8: Hot water as seed treatment and phycare + thyme oil as a foliar spray.

T_9: Hot water as seed treatment and phycare + *P. fluorescens* as a foliar spray.

T_{10}: Hot water as seed treatment and phycare + *T. harzianum* as a foliar spray.

T_{11}: The fungicide Ridomil gold MZ 68 WG (Metalaxyl-M 4 % + Mancozeb 64 %) was applied as seed treatment and foliar spray at the rate of 50 gm/100 L water.

T_{12}: Control (untreated).

Celery fruits were soaked individually in the suspension of the tested treatments with continuous stirring for 4 h. before sowing. A few drops of the emulsifier Tween 20 were added to the thyme oil to obtain an emulsion feature. The fruits were placed in emulsion (usually in 100 ml beaker), plugged and continue stirring for 4 h. *T.*

harzianum was grown in *Trichoderma*-selective medium broth (TSM) (Elad *et al*., 1981), amended with 300 mg/l streptomycin and 50 µg ml^{-1} rose Bengal in a conical flask, incubated at 25^{o}C for 15 days. *P. fluorescens* was multiplied in King B (KB) liquid medium for 48h at 28 ± 2°C on a shaker (King *et al.*, 1954). Celery fruits were soaked in the suspension of *Trichoderma* isolate ($1x10^6$ conidia/ml) and *P. fluorescens* ($1×10^8$ cfu) for 4 h before sowing. For hot water treatment, celery fruits were wrapped loosely in a woven cotton bag (such as cheesecloth) and pre-warmed in the water at 37^{o}C for ten minutes then placed in a water bath at 47^{o}C for 30 minutes (Miller and Lewis Ivey, 2005). After treatment, the fruits were cooled under cold tap water (10^{o}C) for 5 minutes to stop the heating action and then dried at room temperature (Lewis Ivey & Miller, 2005). Celery fruits were soaked in water served as control. Germination tests were performed according to ISTA rules (Anonymous, 2000) using seeds placed on moist filter paper as described by (Schmitt *et al.*, 2009). The percentage of germinated fruits was recorded after 21 days.

In each season, the soil was mechanically plowed and planked twice. During the preparation for cultivation, calcium super-phosphate (15.5 % P_2O_5) as a source of phosphorus was added at the rate of 200 kg/fed. The treated and untreated celery fruits were sown in the field at the rate of 6-8 kg/fed on October, 15th in the two experimental seasons. Weeds were removed by manual operations as needed and plants were irrigated regularly as necessary, throughout the growing season to maintain constant growth. Nitrogen was applied in the form of ammonium sulfate (20.6 % N), at the rate of 400 kg/fed. (recommended rate) as follow: the first one (100 kg/fed.) was added after 21 days from sowing fruits and the second amount (100 kg/fed.) was added after 15 days from the first application. The remainder amounts were added after each cut. Potassium sulfate (48 % K_2O) as a source of potassium at the rate of 100 kg/fed. was used two times. The first one was applied with the second amount of nitrogen application and the second one after the 1st plant cut. The plants were sprayed weekly, always performed early in the morning, with the tested compounds, 1% Tween 20 before the appearance of first symptoms until run off. Monitoring and scouting the plants weekly for the appearance of leaf spot and disease incidence and severity were estimated as follow:

Disease incidence:

Percentage of disease incidence was recorded and efficiency was calculated using the formula:

$$\text{Efficiency \%} = \frac{[C-T]}{C} \times 100$$

Whereas: C and T are the percentage of Septoria leaf spot incidence in control and treated plants, respectively.

Disease severity:

Disease severity was measured according to (Yan *et al.*, 2002). Percentage of disease severity was recorded according to the following equation:

$$\text{Disease severity \%} = \frac{[\sum (n \times c)]}{(N \times C)} \times 100$$

Whereas: n = Number of infected leaves, c = Category number, N = Total number of examined leaves and C = the highest category number of infection.

The plants were harvested three times in each growing season. The first cut was done on 1st January, the second and third ones were done every 45 days by cutting the vegetative parts of the plants 10 cm above the soil surface with three replications in each cut. Fresh and dry weights of herb yield (ton/fed.) were determined.

Total chlorophyll (mg/g fresh weight) was determined at harvesting herb of celery fresh leaves samples using the method by Moran (1982).

To determine the percentage of essential oil, the fresh herbs were collected from each treatment during the three cuts. They were dried by air and weighted (100g dry herb/treatment) representing each replicate then subjected to steam distillation and determined according to Guenther (1961) and oil yield (kg/ fed.) was determined.

Peroxidase activity was determined using the method described in the Worthington enzyme manual (Worthington, 1971). Polyphenoloxidase activity was measured following the method described by (Esterbaner *et al.*, 1977). Analysis of enzymes was carried out at the Central Lab. for Biotechnol., Plant Pathol. Res. Inst., ARC.

Data were statistically analyzed for computing L.S.D. test at 5 % probability according to the procedure outlined by Snedecor and Cochran (1989).

Results

The field experimental data revealed that the tested treatments applied alone or in combination have the potential to reduce the incidence of the Septoria induced disease on celery (Table, 1). In the 1st growing season, T_{11} (Ridomil gold MZ 68 WG) was the most efficient in this regard which significantly reduced the number of leaf spots and disease incidence with efficiency 89.4, 83.7 and 85.4 %, respectively for the first, second and third plant cuts followed by T_8 (hot water-treated seeds combined with phycare + thyme oil as foliar spray) which statistically at par with the fungicide treatment. The efficiency of this treatment in reducing the disease was 87.0, 78.5 and 81.8 %, respectively. T_7 (hot water-treated seeds combined with phycare + nimbecidine as foliar spray) came in the second rank with efficiency reached 78.9, 69.7 and 74.1 % followed by T_9 (hot water-treated seeds combined with phycare + *P. fluorescens* as foliar spray). Meanwhile, T_{10} (hot water-treated seeds combined with phycare + *T. harzianum* as foliar spray) showed the lowest efficiency in the combined treatments. However, the use of the tested materials individually as seed treatments and foliar spray were less effective in reducing the disease incidence than combined treatments with hot water-treated seeds and foliar spray with any of the tested materials. In this respect, T_3

(the commercial product phycare) was the most effective in individual treatments which reduced disease incidence by 60.3, 49.1 and 53.4 % for the three plant cuts, respectively. The essential thyme oil as seed treatment and foliar spray (T_2) did not differ significantly with the phycare treatment (T_3) which was effective in reducing the numbers of leaf spots than nimbecidine (T_1). The efficiency of thyme oil treatment was 57.6, 45.9 and 50.5 %, respectively for the three plant cuts. Meanwhile, the application of *P. fluorescens* as seed treatment and foliar spray (T_4) was more effective than the bioagent *T. harzianum* treatment (T_5). T_6 (Hot water treatment) was the lowest efficient in controlling Septoria leaf blight. The same trend was observed in the 2nd growing season.

Table 1: Efficiency percentage of different treatments against Septoria leaf spot through the three celery plant cuts during 2015/2016 and 2016/2017 successive growing seasons.

Treatments	Efficiency (%) of different treatments on disease incidence					
	1st Season (2015/2016)			2nd Season (2016/2017)		
	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
T_1	52.4	38.7	45.0	54.0	37.1	46.8
T_2	57.6	45.9	50.5	60.3	47.3	51.4
T_3	60.3	49.1	53.4	64.6	50.8	55.6
T_4	46.9	30.3	37.0	46.2	33.6	39.3
T_5	34.5	22.2	27.9	37.6	24.6	27.5
T_6	32.9	17.0	22.6	33.0	20.5	25.3
T_7	78.9	69.7	74.1	81.0	68.4	76.4
T_8	87.0	78.5	81.8	89.4	79.3	84.2
T_9	71.7	58.3	64.5	73.2	60.5	66.4
T_{10}	65.0	52.4	57.8	65.9	51.7	58.0
T_{11}	89.4	83.7	85.4	92.0	82.6	87.1
T_{12}	0.0	0.0	0.0	0.0	0.0	0.0
L.S.D $_{0.05}$	5.1	5.6	4.9	5.7	3.4	4.5

Results in Figures, 1 and 2 indicate that there were significant differences in severity of Septoria leaf blight among the treatments tested compared with the untreated control. In the 1st growing season, disease severity was higher in plots received the treatments individually than plots received combined treatments. The most effective treatments were T_{11} (8.0, 17.8 and 12.4 %, respectively for the three plant cuts) followed by T_8 (10.9, 20.2 and 15.0 %, respectively) without any significant differences between them. Moderate effects were shown with T_9 followed by T_{10} treatment. From individual treatments, T_3 significantly reduced disease severity to an important level followed by T_2 with no significant differences between them. The corresponding mean values were 25.6, 35.7 & 31.8 and 27.6, 38.6 & 33.9 %, respectively. The bioagent *P. fluorescens* (T_4) was significantly more effective in reducing severity and number of leaf spots than *T. harzianum* treatment (T_5). Meanwhile, treated celery seeds with hot water (T_6) only showed the least effective in this concern. The same trend was observed in the 2nd growing season.

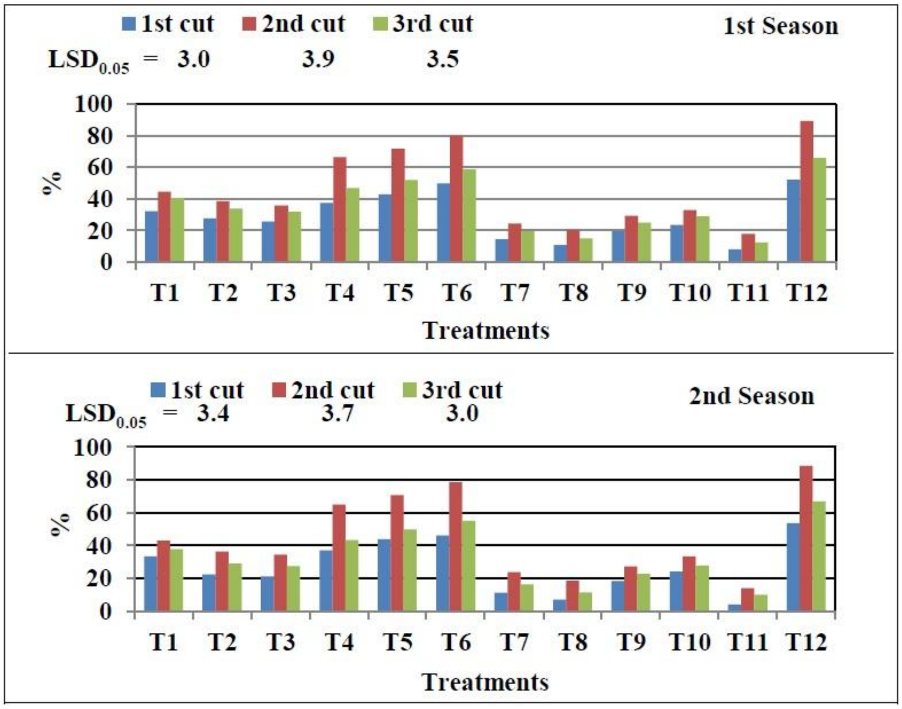

Figure 1: Effect of different treatments on severity of Septoria leaf spot through the three celery plant cuts during two successive growing seasons (2015/2016 and 2016/2017).

All the tested treatments had no negative effect on the germination of celery seeds (Table, 2). No significant differences among the means of various treatments, control, essential oils, the commercial product phycare, bio-agents, and hot water were detected. Nimbecidine, thyme oil, phycare, *T. harzianum*, and *P. fluorescens*-treated seeds showed the same germination level (100 %) as control seeds in the two seasons. Seeds-treated with hot water at 47^{o}C for 30 min. had no significant effect on germination (98 and 97 %) compared to the control (100 %) in the two seasons, respectively.

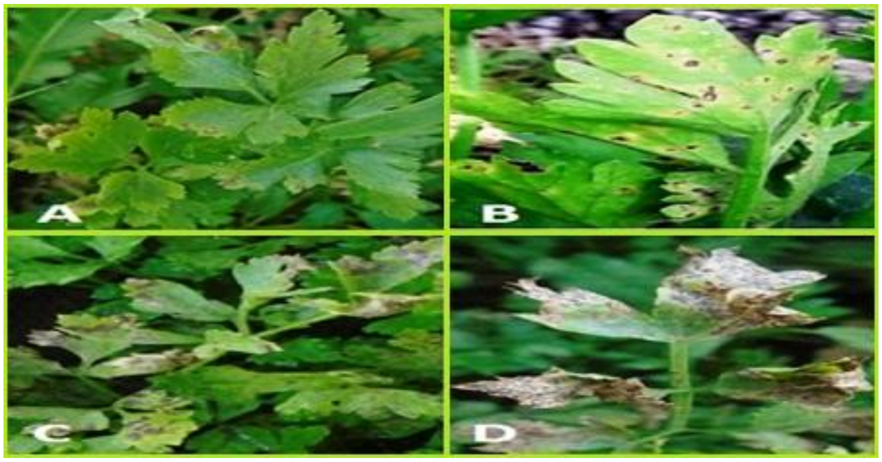

Figure 2: Effect of hot water-treated seeds combined with phycare + thyme oil as foliar spray treatment (A); Phycare (B) and hot water treatment (C) on severity of septoria leaf spot of celery compared with the untreated control (D).

Table 2: Effect of different treatments on celery seed germination

Treatments	Germination percent	
	1^{st} Season	2^{nd} Season
Nimbecidine	100	100
Thyme oil	100	100
Phycare	100	100
P. fluorescens	100	100
T. harzianum	100	100
Hot water	98	97
Ridomil gold MZ 68 WG	100	100
Control	100	100
L.S.D $_{0.05}$	ns	ns

All treatments increased the proportion of healthy celery plants and did not show any signs of phytotoxicity. As seen in Figures, 3 and 4 there were significant differences among the treatments in terms of the fresh and dry weights of celery plants compared with the untreated control. The highest weights were observed with T_{11} (Ridomil gold MZ 68 WG) treatment, being 3.96, 5.64 & 4.90 ton/fed., respectively, for the three plant cuts in fresh weight and dry weight, being 0.75, 1.07 & 0.93 ton/fed., respectively, followed by T_8 with no significant differences between them which gave 3.85, 5.47 & 4.87 ton/fed., and 0.73, 1.03 & 0.95 ton/fed., respectively for the three plant cuts during the 1st season. T_7 gave also best results followed by T_9 treatment. A single treatment of phycare (T_3) followed by thyme oil treatment (T_2) showed a moderate increase. The lowest fresh and dry weights were recorded with T_6. The same trend was observed in the ^{2nd} growing season.

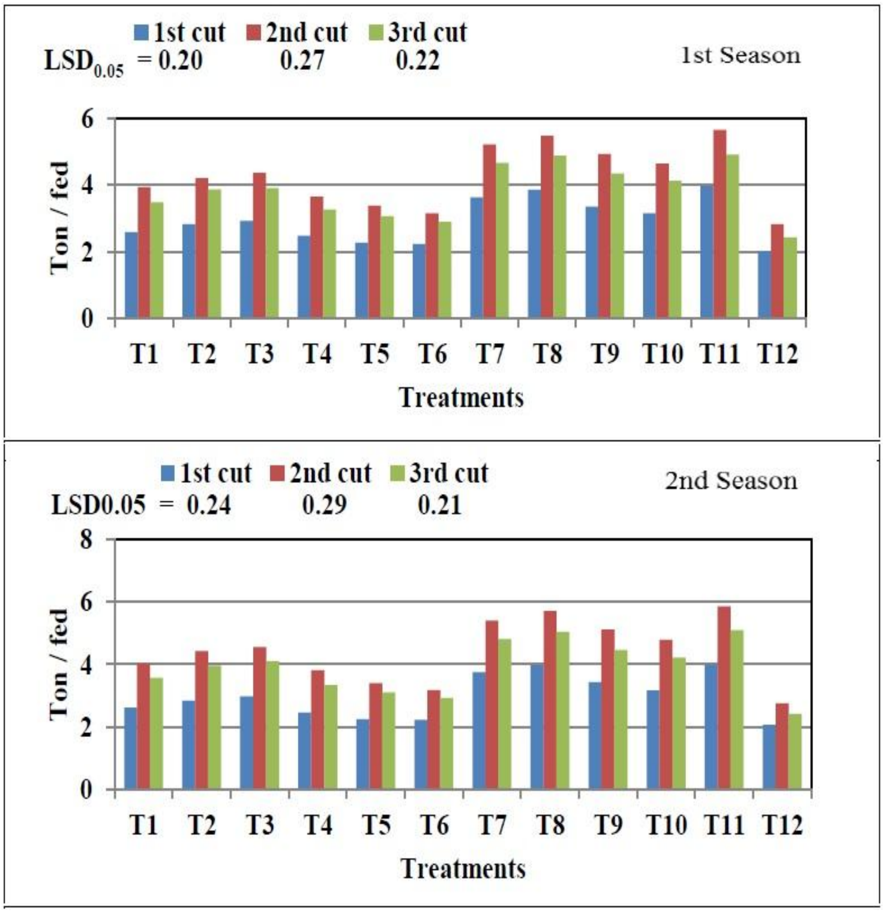

Figure 3: Effect of different treatments on fresh weight (ton/fed.) through the three celery plant cuts during two successive growing seasons (2015/2016 and 2016/2017).

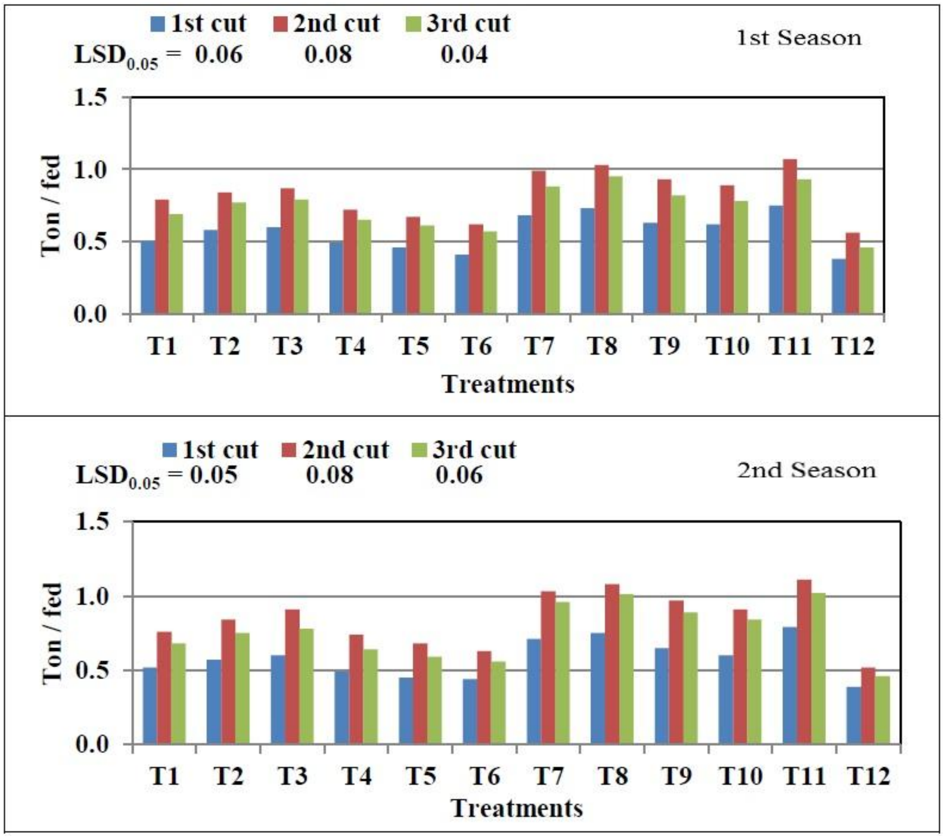

Figure 4: Effect of different treatments on dry weight (ton/fed.) through the three celery plant cuts during two successive growing seasons (2015/2016 and 2016/2017).

It seems that all the treatments significantly increased the chlorophyll content compared with the untreated control (Figure, 5). In the 1^{st} growing season, T_8 treatment had a profound effect on the total chlorophyll by collapsing the spots on the leaf surface which gave the highest chlorophyll content (10.19, 9.54 and 9.46 mg/ g fresh weight), respectively for the three plant cuts followed by T_7 treatment which was statistically at bar with the T_{11} treatment. In general, the combined treatments showed more chlorophyll content than the single treatments. The least total chlorophyll content (8.61, 8.30 and 8.20 mg/ g F.W.) was observed with T_6 which statistically showed no significant differences with control treatment (8.36, 8.00 and 7.69 mg/ g F.W.), respectively for the three plant cuts. The same trend was observed in the 2nd growing season.

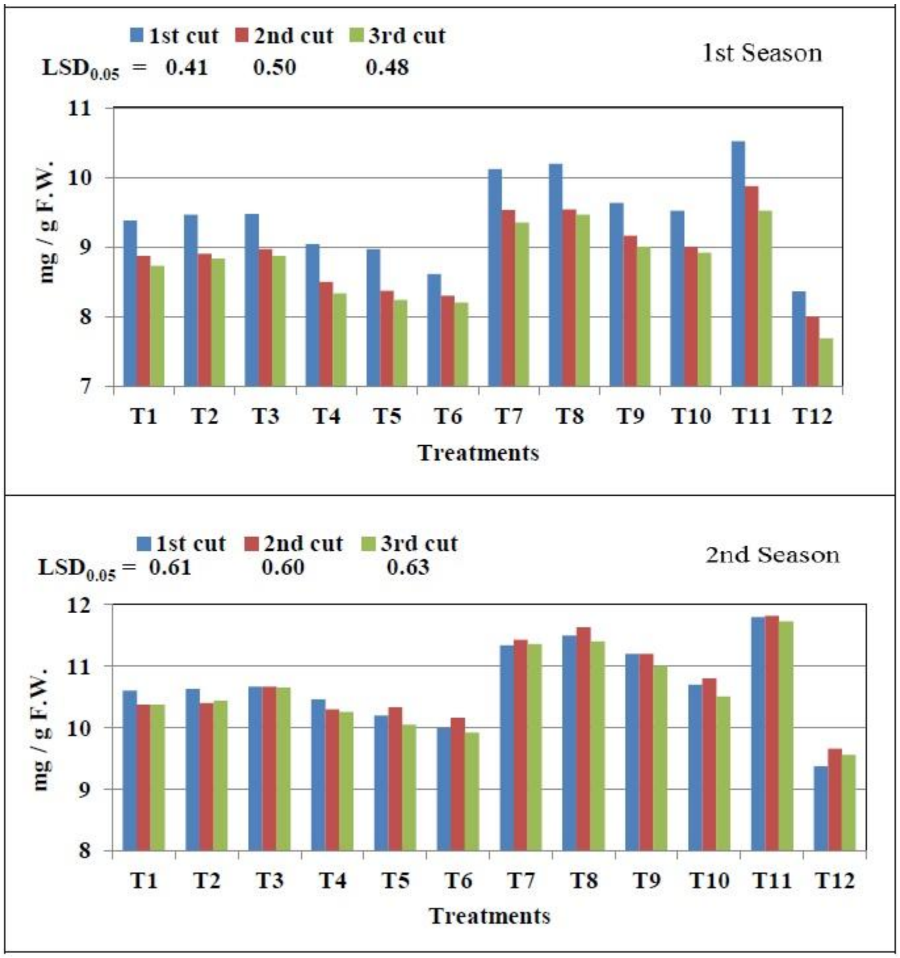

Figure 5: Effect of different treatments on total chlorophyll (mg/g fresh weight) through the three celery plant cuts during two successive growing seasons (2015/2016 and 2016/2017).

Data presented in Figures, 6 and 7 clearly show that all the treatments tested in single or in combination induced a significant increase in oil percent and oil yield over the untreated control. The maximum significant increase in oil % was detected with T_8, being 0.63, 0.65 & 0.67 % and 4.60, 6.69 & 6.36 kg/fed. in oil yield, respectively for the three plant cuts which showed no significant differences with T_{11}. T_7 came in the second rank followed by T_9. In general, the combined treatments were more effective in this respect than single treatments. At the same time, the use of hot water only as seed treatment (T_6) showed no significant effect with the untreated plants in this respect which gave the lowest oil % and yield compared with the other treatments.

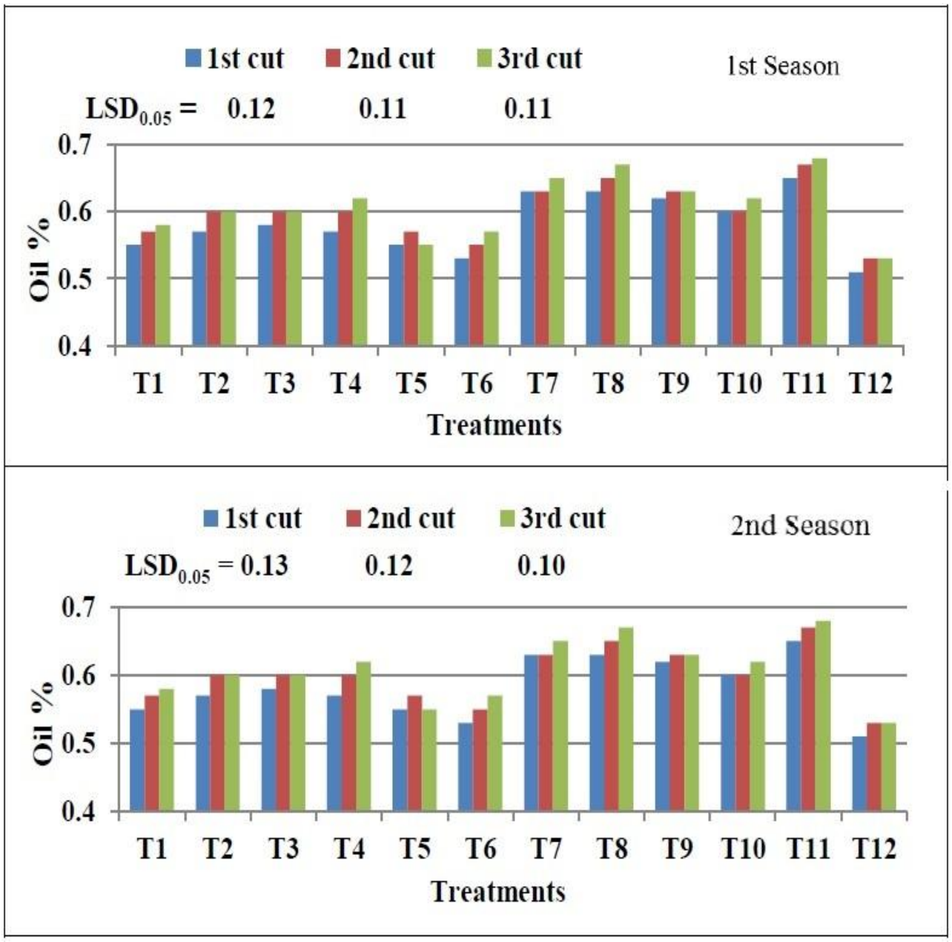

Figure 6: Effect of different treatments on oil percentage in the three celery plant cuts during two successive growing seasons (2015/2016 and 2016/2017).

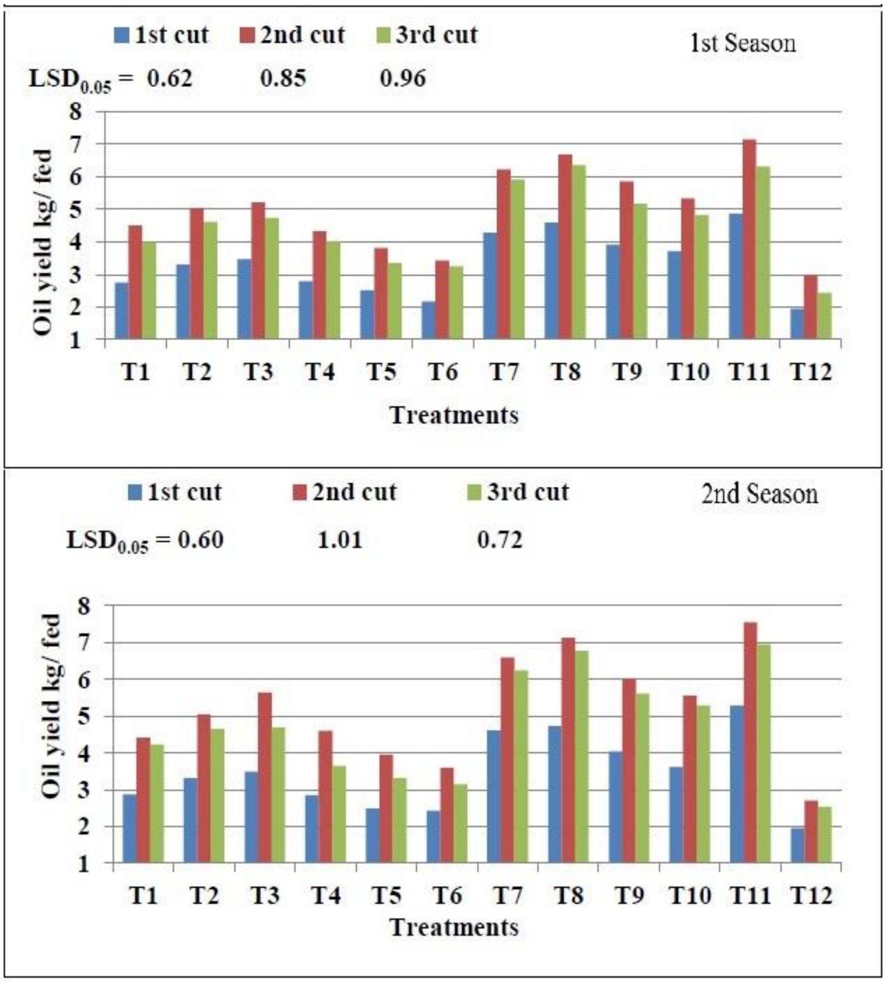

Figure 7. Effect of different treatments on oil yield (kg/fed.) through the three celery plant cuts during two successive growing seasons (2015/2016 and 2016/2017).

Results in Figure, 8 demonstrate that all treatments increased the peroxidase and polyphenoloxidase activities in celery plants. The highest activity was obtained with T_8 and T_7 which achieved 0.979 and 0.947 Umg^{-1} in peroxidase enzyme and 0.458 and 0.424 Umg^{-1} in polyphenoloxidase enzyme, respectively. Meanwhile, single treatment of hot water (T_6) was less effective.

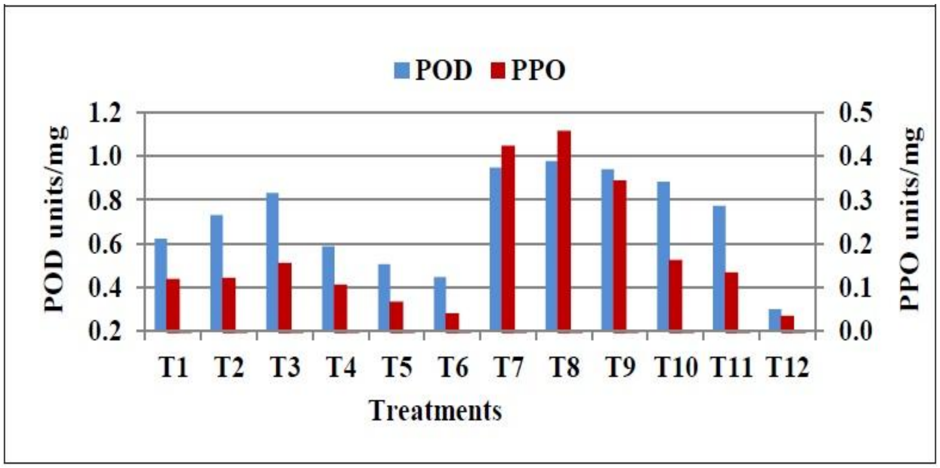

Figure 8. Enzyme activities in celery plants as affected by different treatments.

POD = Peroxidase; PPO = Polyphenoloxidase

Discussion

Organic acids, natural oils, bio-control agents and hot water treatment can be applied successfully in plant production by enhancing natural resistance against plant diseases and as a plant growth stimulant (Amein *et al.*, 2011). In the present study, it is worthy to note that all the tested treatments applied as seed treatment and foliar spray significantly reduced losses caused by Septoria leaf spot on celery plants and increased its productivity. The use of combined treatments increased the efficiency of the treatments and enhanced the suppressive effect against septoria blight. Hot water-treated seeds combined with phycare + thyme oil as foliar spray was the most effective treatment. It reduced disease incidence and severity to an acceptable level, increased fresh and dry weight, oil percent, oil yield and chlorophyll content as well as peroxidase, polyphenoloxidase activities of celery plants. Thyme oil contains p-cymene (36.5%), thymol (33.0%) and 1.8 cineole (11.3%) as a main components (Segvić Klarić *et al.*, 2007) which possess a wide range spectrum of fungicidal activity against seed-borne and air-borne fungi when used as seed treatments and/or foliar spray (Schmitt *et al.*, 2009 and Amein *et al.*, 2011) and significantly increased crop yield under field conditions (El-Mohamedy and Abd-El-latif, 2015). El-Mougy (2009) demonstrated that the application of thyme essential oil as foliar spray proved to be highly effective treatments inducing increases of potato tuber yield by improving plant health. Thymol affects the surface electrostatics of the cell membrane and membrane integrity (Sánchez *et al.*, 2004), as well as, damaging the cell wall, cell membrane and cellular organelles (Rasooli and Owlia, 2005). Neem plants contain several compounds of terpenoids that present in all parts of the plant from which Azadirachtin is the most active compound as antifungal, hormonal and increase nutrient efficiency by which the crop yield is enhanced when used as seed treatment and manorial application (Lokanadhan *et al.*, 2012). Nahak and Sahu (2015) reported that spray of neem extract on tomato plants effectively control early blight and leaf spot and increased fruits of tomato plant in comparison to controls under field condition. Neem oil provided control of many fungal diseases through metabolic changes in plants including induction of phenol, antioxidant defensive enzymes and phenol accumulation (Abbasi *et al.*, 2003). However, the promotive effect could be due to triterpene which acts by delaying the transformation of ammonium nitrogen into nitrate nitrogen (Akhtar, 1999). The volatile phases of the essential oils like thyme oil were found to be more toxic than the contact phase against plant pathogenic fungi (Inouye *et al.*, 2000). They suggested that the antifungal activity resulted from a direct effect of essential oil vapors on fungal mycelium and concluded that the lipophilic nature of oil components was as possible for them being absorbed by fungal mycelia and aided in the ability of the oil to penetrate the plasma membrane.

Treatment of celery fruits with hot water at 47°C for 30 min. can reduce contamination of seed by Septoria with the little negative effect on germination. This may be because the fungus was not found in seed embryos or endosperm but its mycelium was present in pericarps and testas (Sheridan, 1966). This result is closely in agreement with that reported by Muniz (2001) who reported that seed treatment with hot water reduced seed-borne pathogens such as fungi, bacteria even viruses of tomato. The possible postulated of decreasing the seed-borne pathogen may be due to hot water treatment, firstly the temperature acted upon the fungal contaminants and with increasing the temperature it penetrated within the seed and killed pathogen embedded in the seeds. Farahani and Chaichi (2012) showed that hot water treatment increased germination speed regardless of germination percentage; it can help earlier germination than weed. They added that to reduce the negative effect of hot water on germination, it is useful to induce water stress in the field to promote germination after hot water treatment. Mtui *et al.* (2010) reported that seeds of tomato treated with hot water gave higher fruit yields and lower severity of sunscald than plants from Ridomil treated seeds which were similar to control. This may be due to the higher vigor of plants from hot water treated seeds, which led to a denser canopy thereby providing better fruit protection from the sun.

The application of organic acids had the potential to protect plants against pathogens by inducing the plant to acquire systemic resistance to pathogens and induced favorable changes in anatomical structures of vegetative organs and consequently promotes vegetative growth and productivity. Khan *et al.* (2011) concluded that ascorbic acid enhances the tolerance of plant and regulate response to pathogenic stress as a result of a complex sequence of biochemical reactions such as activation or suppression of key enzymatic reactions, induction of stress-responsive proteins synthesis, and the production of various chemical defense compounds. Azoz *et al.* (2016) showed that foliar application of ascorbic acid at 300 ppm increased stem diameter of basil plants by increasing thickness of the cortex, fiber strands, phloem tissue, and xylem tissue and decreased pith diameter as well as resulted in thicker leaves due to increase induced in thickness of both midvein and lamina. The thicker lamina induced by ascorbic acid treatment was mainly due to the prominent increase observed in the thickness of both palisade and spongy tissues. Wassel *et al*. (2007) assumed that the effect of ascorbic acid on the plant growth might be due to the auxinic action of ascorbic acid as well as, its improved role in many metabolic and physiological processes and enhancing the synthesis of carbohydrates. Omer (1999) reported that the increments of leaf area, leaf fresh and dry weight are due to ascorbic acid treatment which may be attributed to its effect on cell division and cell elongation. Maksoud *et al.* (2009) mentioned that increasing leaf dry weight might be due to the accumulation of dry matter production in the canopy, which can be assumed proportional to the solar radiation intercepted by foliage resulting in more efficiency of the photosynthesis process. Besides, El-Sayed (2013) reported that soaked tomato seeds in ascorbic acid at 50 ppm before planting leads to significant increase in the contents of chlorophyll a, b and in carotenoids as compared with the control plants. Increasing plant pigments chlorophyll % may be due to the role of ascorbic acid in increasing the rates of photochemical reduction. Ismaeil and Bakry (2005) noticed that treating papaya plants with citric acid at 2 g/l increased the thickness of epidermis, cortex, phloem zone and xylem zone in petiole flower. These findings refer to the possible increase of plants' resistance to fungal infection by spraying of certain organic acids such as ascorbic and citric acid through increasing the epidermis thickness as a mechanical barrier. Citric acid is one of the mobile forms of iron in plants, thus it plays an important role in iron transport (Darandeh and Hadavi, 2012). Jafari and Hadavi (2012b) assumed that citric acid alters the hydrocarbon partitioning towards pathways that are more related to resistance to pathogens, unfavorable conditions and secondary metabolism. They speculated that the application of citric acid as foliar spray increased proton pumping capabilities of roots, improving their organic acid efflux. This had, in turn, improved the overall ion absorption (especially P and N) by plants and this finally led to increased performances and yields. The enhancement effect of citric acid, ascorbic acid on vegetative growth, essential oils was also recorded on other medicinal and aromatic plants (Reda *et al.*, 2007, Khalil *et al.*, 2010 and Soltani *et al.*, 2014). Polymers of L-lactic acid increased plant biomass through increase chlorophyll accumulation and root growth. Promotion of chlorophyll accumulation and biomass may be due to increased ability to assimilate nutrients (Kinnersley, *et al.*, 1990). Ascorbic acid plays multiple roles in plant growth, functioning in cell division, cell wall expansion, and other developmental processes (Conklin, 2001).

Seed treatment and foliar spray with *T. harzianum* and *P. fluorescens* can effectively control Septoria blight of celery and increased seedling emergence, fresh and dry weight, oil percentage, oil yield and total chlorophyll. This result is in agreement with the study by Amein *et al.* (2011) who worked with broccoli seeds and cabbage seeds.

Pseudomonas spp. affect plant growth directly by producing and releasing secondary metabolites (plant growth regulators, phytohormones and biologically active substances), facilitating the availability and uptake of certain nutrients from the root environment and indirectly by inhibiting plant pathogenic organisms as a result of production of inhibitory substances or increasing the natural resistance of the host (Kaushal *et al*., 2013). However, Amutharaj *et al*. (2013) found that the favorable effect of *P. fluorescens* might be due to its stimulating effect for rapid growth or early seedling growth resulted in more cell division and elongation due to a secretion of indole acetic acid (IAA), abscisic acid (ABA) by bacteria leading to longest shoot because of the capability of bacteria for fixing nitrogen from air and enhanced metabolism process resulted in more energy and growth improvement. *Pseudomonades* produce cytokinins and gibberellins (gibberellic acid). *Trichoderma*, in association with plant roots, can trigger systemic resistance and improve plant nutrient uptake. Several strains of *Trichoderma* effectively affect the seed-borne phase of Fusarium head blight (FHB) or Scab in wheat when used as seed treatments (Xue *et al.*, 2017). However, Yadav *et al.* (2015) showed that foliar application and seed coating of *Trichoderma harzianum* significantly reduced the incidence and severity of the spot blotch disease (*Bipolaris sorokiniana*) and leaf rust (*Puccinia triticina*) (El-Sharkawy *et al.*, 2015). Furthermore, Contreras-Cornejo *et al.* (2016) reported that *Trichoderma* induces root branching and increases shoot biomass as a consequence of cell division, expansion, and differentiation by the presence of fungal auxin-like compounds. This plant growth promotion due to its role in plant hormone production, vitamin production or conversion of materials to a form useful to the plant, nutrient release from soil or organic matter, increased uptake and translocation of minerals in addition inhibited the pathogen through parasitism, predator, antibiosis, competition for space and nutrition as well as by inducing the resistant in plants against pathogens (Suarez *et al.*, 2005).

Conclusions

These treatments may have a place in the integrated management of Septoria leaf spot on celery, increased its productivity and consequently, reduced the fungicides used. These treatments may not result in the development of resistance in the pathogens.

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(Received 01/08/2018;
in revised form 12/09/2018)

استخدام بعض بدائل المبيدات لمكافحة التبقع السبتورى لأوراق الكرفس وتحسين انتاجيته

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تم اجراء تجارب فى الحقل تحت ظروف العدوى الطبيعية بمرض التبقع السبتورى فى الكرفس خلال موسمين ٢٠١٥/ ٢٠١٦ , ٢٠١٦ / ٢٠١٧ م فى المزرعة البحثية بمحطة البحوث الزراعية بسدس-مركز البحوث الزراعية- محافظة بنى سويف من اجل تقييم فعالية بعض الطرق الفيزيائيه (الماء الساخن) والأحماض العضوية والزيوت الطبيعية والمقاومة الحيوية على وبائية مرض التبقع السبتورى وانتاجية نباتات الكرفس. عموما، كل المعاملات كان لها تأثير ايجابى على الحد من شدة المرض مع زيادة معنوية فى الوزن الطازج والجاف للعشب ونسبة ومحصول الزيت فى نهاية التجربة. علاوه على ذلك، وجد أن استخدام المعاملات مفرده كمعاملة بذرة ورش كانت أقل فعالية فى تقليل شدة الأصابة عن المعاملات المشتركة مع الماء الساخن كمعاملة بذره ورش المجموع الخضرى بأى من المعاملات المختبرة. لوحظ أعلى تأثير فى القطعة التى تم زراعتها ببذور الكرفس المعاملة بالماء الساخن مع رش المجموع الحضرى بمركب فى كير وزيت الزعتر بالأضافة الى المبيد الفطري ريدوميل جولد ام زد ٦٨. علاوة على ذلك ، اظهرت كل المعاملات زيادة معنوية فى انزيمات المقاومة مثل انزيمات بيروكسيديز وبولى فينول أوكسيديز بالمقارنة مع نباتات الكرفس الغير معاملة.