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AGRICULTURAL AND BIOLOGICAL TREATMENTS TO OVERCOME WILTING DISEASE OF CUMIN (*Cuminum cyminum* L.)

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Abstract: The investigation aimed to identify and control the causal agent of wilting disease that significantly decreases success of growing cumin, which is one of the most important medicinal, aromatic and spices plants, using safety agricultural and biological treatments. *Fusarium* spp. were isolated from roots of wilted cumin plants by Komada's selective medium. The pathogenic isolate of *Fiusarium* sp. was identified as *F. oxysporum* f. sp. *cumini*. Control experiment was carried out in new reclaimed sandy soil naturally infested by *F. oxysporum* f. sp. *cumini* at Agriculture Experimental Farm of Sohag University and fertilized organically for two successive seasons, 2006/2007 and 2007/2008. Three irrigation intervals of 2, 4 or 8 weeks and three biological treatments of cumin seeds before planting using chitosan, bion or *Streptomyces alni* were studied beside control (without seed treatment). The treatments were significantly effectives in the reduction of cumin wilting and increasing of growth and seeds yield. Irrigation of cumin plants every two weeks was found to be the most suitable irrigation interval in such sandy soil. All biological compounds significantly increased survival plants percentage up to 100% compared to the control. Chitosan was the best compound for cumin growth and seeds yield. Treatment of cumin seeds by chitosan before planting followed by irrigation of plants at two weeks irrigation intervals gave the highest survival plants percentage, growth and seeds yield of cumin. The obtained results showed the possibility of overcoming wilting disease and producing cumin organically using safety treatments. This may encourage growers to increase cumin production for medicinal uses and exportation.

Key words: cumin, medicinal, spices, wilting, Fusarium, biological control, irrigation, reclaimed, organic, exportation

Introduction

Cumin (*Cuminum cyminum*, L.) is an annual plant belongs to family Apiaceae. It is one of the most important aromatic and medicinal plants largely used as spice and medicinal plant since ancient Egypt

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for both local consumption and exportation.International production of cumin is centered in warm regions, principally in India, China and southern Egypt. Demand of cumin is increased while its production is limited (Abu-Nahoul and Ismail, 1995). One of the limiting factors affecting its success is the susceptibility to Fusarium wilt disease incited by the soil borne pathogen *Fusarium oxysporum* f. sp. *cumini* (Omar *et al*., 1997). This disease has been reported as a problem limiting cumin production worldwide including Egypt (Arafa, 1985) and India (Champawat and Pathak, 1990). The chlamidiospores of Fusarium may persist in soil for many years in the absence of a susceptible host (Agrios, 1991). Infested field may not be replanted with cumin for at least 10 years. Many approaches can be followed to overcome Fusarium wilt disease such as crop rotation, pre-sowing treatments of seeds with certain chemical fungicides and management of agricultural practices (Agrios, 1991). Fungicidal application is widely restricted for organic production of medicinal plants and environmental concerns. Biological control is one of recent developed approaches complying with sustainable environment issues (Larkin *et al*., 1996; Whipps, 1997). Numerous plant-associated fungi and bacteria are known for their antagonistic activity to soil-borne pathogens and could be utilized as biocontrol agents against wilt disease including Fusarium (Weller, 1988; Cook, 1993). Successful biocontrol application as pre-sowing treatments to control Fusarium wilt has been reported in different ornamental plants (Postma and Rattink, 1992; Hassan and Tawfik, 1996). Tawfik and Allam (2004a) concluded that efficient biocontrol agents might be developed via bioassay for microorganisms associated with local cumin cultures. The same authors studied the effect of cumin seed treatments with two fungal antagonists (*Trichoderma harzianum, and T. humatum*), one bacterial antagonist (*Bacillus subtilis),* 2-days water priming, combined water priming with *T. harzianum* and biocide 'PlantGuard' on two landraces of the 'Balady' cumin (Tawfik and Allam, 2004b). They recommend utilizing combined 2-days water priming with *T. harzianum* antagonist for pre-sowing seed treatment. Chitosan was widely reported as natural compound having an antifungal activity against several fungi and potential elicitor of many plant defense responses (El-Ghaouth *et al*., 1992 and 1994). Bion is also a potent of systemic acquired resistance (SAR) increasing crop resistance to diseases by activating the SAR signal transduction pathway in several plant species (Conrath *et al*., 2001; Hongxia *et al*., 2005). *Streptomyes* spp. are biocontrol agents used against some soil borne plant pathogens by production of antibiotics and Indole Acetic Acid

(IAA) auxin (Tahvonen, 1988). Farrag (2005) reported that chitosan were more efficient than *Trichoderma harzianum* for controlling root-rot disease in chamomile. Hassanein and Dorion (2006) showed the favorable effect of irrigation on growth and quality of Pelargonium. Indeed, water or soil humidity and biological control could be considered the most important factors affecting wilting disease. This research aimed to study the effect of these two factors, irrigation intervals (agricultural practices) and seed biological treatments (biological control), and their interaction on wilting disease and production of cumin grown organically in new reclaimed soil. The final goal is to find an efficient environment friendly strategy for the management of Fusarium wilt disease in cumin used for medicinal uses and exportation.

Materials and Methods

Isolation, purification and pathogenicity of *Fusarium* spp.

Isolation method was applied for *Fusarium* isolation from diseased roots of cumin plants growing at Agriculture Experimental Farm of Sohag University. Samples were taken from plants showing typical wilt symptoms according to Arafa (1985). This study was conducted in the plant pathology laboratory, Faculty of Agriculture, South Valley University. Dissected roots of wilted plants were sterilized then plated on Komada's medium, specific for *Fusarium* isolation (Komada, 1975). Pure isolates were transferred to Potato-Dextrose Agar (PDA) medium containing sulphate streptomycin then identified based on morphological criteria proposed by Booth (1971). Isolated *Fusarium* spp. were used to examine their pathogenicity after growing on 500 ml glass bottles containing sterilized barley grains medium at 28 ±˚C for 15 days. The experiment was carried out using pots of 20 cm diameter filled by sterilized sandy soil under greenhouse conditions. Five cumin transplants were transplanted in each pot one week after soil infestation by Fusarium inoculums at 1.5 % (w/w), and then pots were directly irrigated. Three months later, the percentage of wilt disease incidence was recorded. Re-isolation from artificially infected plants was performed to meet Koch's postulate. The isolate with highly pathogenicity was forma specials determined.

Plant materials and treatments

Experiment was effectuated to study the possibility of overcoming cumin wilting disease, using safety treatments. The study was conducted in new reclaimed sandy soil naturally infested by *F. oxysporum* f. sp. *cumini* at Agriculture Experimental Farm of Sohag University during 2006/2007 and 2007/2008 seasons. Cumin seeds were planted in such sandy soil, mostly coarse in texture, in 14 October for both seasons. Physical and chemical properties of used soil are shown in Table 1. Cumin (Balady) seeds were obtained from growers of Sohag governorate in southern Egypt. Two factors were studied to investigate their effect on cumin wilting disease and consequently growth and yield of cumin. The first factor was irrigation intervals, applied after germination, at three levels of two, four and eight weeks. The second factor was biological control using three biological and safety chemical compounds beside control with no seed treatment. Safety chemical compounds were chitosan (β-1,4 – linkedglucosamine polymer, sigma chemical Co. St. Louis, USA) and bion (Benzo-thiadiazole-7-carbithioic acid S-methyle ester, Novartis Ltd. Basel, Switzerland). Biological treatment was *Streptomyces alni* provided from previous work (Ziedan *et al*., 2008). Chitosan solution was prepared by dissolving it in 0.05 N-HCL under continuous stirring and pH was adjusted to 5.0 using 2 N-NaOH. The resulted solution was diluted to 25.0 mg/L. Bion solution was prepared from 50% wettable granule formulation with sterile distilled water plus 0.05% Tween-80. *S. alni* suspension (1 x 10^8 spores/ml) was also prepared. Cumin seeds were soaked, just before planting, in one of these three solutions for 30 minutes while control seeds were soaked in sterile distilled water. After germination, irrigation treatments were applied where plants were irrigated at 2, 4 or 8 weeks intervals. Organic fertilization using farmyard manure at 6 tons per feddan was achieved similarly for all treatments as recommended by El-Keltawi *et al*., (2003). Standard cultural practices for cumin production were carried out.

Experimental design and measured parameters

Treatments were arranged in the field as a split-plot in randomized complete-blocks (RCB) design with three replicates for both seasons**.** The main plots were assigned for the three irrigation intervals while each main plot consisted of four sub-plots represented the three biological treatments and control. Each plot had an area of 6 m^2 and contained three ridges of 3 m in length and 50 cm apart planted, on its southern side, by 12 hills of 2 plants per each ridge. Plots were completely harvested at the 29^{th} of March in both seasons then ten plants were randomizely selected from each plot to record the growth and yield parameters. Number of plants per plot was recorded for each plot then divided by 72 (the standard total number of plants per plot) and multiplied by 100 to record survival plants percentage. The following parameters were measured on the ten plants of each plot: stem height (cm), root length (cm), plant height (the sum of the last two parameters), stem diameter (mm), number of branches and number of umbels per plant. After drying, plant dry weight (g) was recorded for each plant and seeds

were collected for the ten plants to record seeds yield per 10 plants (g). The last parameter was divided by 10 and multiplied by number of plants per plot to record seeds yield per plot (g). The last parameter was divided by 6 m^2, plot area including the borders, and multiplied by 4200 m^2, feddan area, to record seeds yield per feddan (g). All collected data were subjected to statistical analysis.

Statistical analysis

The original data were used for analysis of variance (ANOVA) of all measured parameters separately for each year (2006/2007 or 2007/2008). Analysis of variance (ANOVA) was carried out to determine significant differences followed by Tukey's test for the comparison of means using (S-Plus 6.0, Professional Release 1; 1988–2001). Least Significant Difference (LSD) was calculated for each parameter at significant level of 5% for both factors and their interaction.

Results

Identification and pathogenicity of isolated *Fusarium* spp.

Sixteen colonies obtained on PDA medium were identified as *Fusarium* spp. based on their morphology. Few colonies showed that microconidia were formed on long branched phialides and clamydospores were formed intercalary on the hyphae. These colonies were identified as *F. solani*. Other colonies showed that microconidia were formed on short phialides, these colonies were identified as *F. oxysporum. F oxysporum* isolate revealed to be pathogenic to cumin. Vascular wilt was observed then identified as *F. oxysporum* f. sp. *cumini*. The fungus was re-isolated confirming Koch's postulates.

Cumin plants survival

Data illustrated in Table 2 show effects of irrigation intervals, seed biological treatments and their interaction on the number and percentage of survival cumin plants. Irrigation intervals of 2 or 4 weeks increased significantly the number and percentage of survival plants compared to 8 weeks irrigation interval for both seasons regardless seed treatments. All seed biological treatments showed significantly higher number and percentage of survival plants compared to control for both seasons regardless irrigation intervals. Seed treatment by *Streptomyces* or chitosan gave better results than bion for both seasons. The highest percentage of survival plants all over years was obtained when seeds were treated by *Streptomyces* or chitosan before planting and cumin plants were irrigated every two weeks (100%). The lowest results were found with non treated seeds (control) whatever irrigation interval where only 51-69% of plants were survived for both seasons.

Cumin seed yield

Effects of irrigation intervals and seed biological treatments as well as their interaction on seed yield of cumin plants grown organically are shown in Table 2. Irrigation of cumin plants every 2 weeks revealed significantly higher number of umbels, seeds yield per 10 plants, seeds yield per plot and per feddan compared to other irrigation intervals for both seasons. Treatment of cumin seeds by chitosan was found to be the best treatment for seeds yield per plot and per feddan compared to control and other treatments for both seasons. Moreover, plants resulted from chitosan treated seeds also gave the highest seeds yield per plant compared to other biological treatments for both seasons. However, control plants gave similar seeds yield per plant and higher number of umbels. Indeed, it was noticed that high number of umbels with no or little seeds number in control treatment. The highest seeds yield per plot and per feddan was obtained when seeds were treated by chitosan and plants were irrigated at two weeks irrigation intervals for both years. However, control plants irrigated at 2 weeks intervals gave better number of umbels and seeds yield per plant.

Plant vegetative growth

Vegetative growth parameters obtained from cumin plants grown organically in sandy new reclaimed soil under several biological seed treatments and irrigation intervals are illustrated in Table 3. Concerning irrigation factor, irrigation of cumin plants every two weeks was found to be the best irrigation intervals compared to other tested ones for all vegetative growth parameters in both seasons. For biological treatments factor, it was noticed that chitosan and control treatments gave the best results in all vegetative growth parameters for both seasons. The overall results revealed that the best interaction treatment for plant vegetative growth parameters is the irrigation of cumin plants every two weeks and treatment of seeds by chitosan or without treatment.

Discussion

The aim of this study was to control cumin wilting disease, which affects dramatically its production, using safe treatments enabling the producer to export it as medicinal crop. Biological control and irrigation are safe treatments especially when studied under organic production conditions. Chitosan and bion are safety compounds used for increasing crop resistance to disease in several plant species (El Ghaouth *et al*., 1992; Conrath *et al*., 2001). These two compounds were studied in comparison with *Streptomyces alni* and control treatments. In our study, irrigation intervals of two weeks was found to be more suitable for cumin production compared to 4 or 8 weeks irrigation intervals in such sandy new reclaimed soil. It is well

known that cumin doesn't need more irrigation, usually once per month, but this result can be explained by loss of water by percolation in such sandy soil (Table 1) and evaporation in such warm region of southern Egypt. The vital role of water in plant biological system especially under sandy soil and warm conditions is well known. Treatment of seeds before planting by studied biological compounds showed favorable effects on survival plants percentage and seed yield compared to control. A significant increment in plant growth and development has been noticed to be associated with utilizing biocontrol agents (Linderman, 1994; Ousley *et al*. 1994). In this study, chitosan was found to be the most effective one for plant survival, growth and seed yield. It was reported that chitosan treatment suppresses root-rot caused by Fusarium spp. in many hosts (Benhamou *et al*., 1994; Lafontaine and Benhamou, 1996). It is also found to be more effective than *Trichoderma harzianum* in controlling pathogenic fungi of chamomile (Farrag, 2005). The beneficial effect of chitosan may relate to its direct fungitoxic effect and defense biological reactions including phytoalexins, synthesis β 1-3 glucanase, chitinase, chitosanase and lignin (El-Ghaouth *et al*., 1994). *Streptomyces* also give similar good results as previously reported on melon by Etebarian (2006). Despite the favorable effect of bion on plant vegetative growth parameters, it was the least effective one on survival plants percentage and seeds yield. Its enhancement of growth can be related to the lower plant density in this treatment. Indeed, bion has different mechanism of plant protection where it has been developed as a potent of systemic acquired resistance (ASR) which doesn't have antimicropial properties but increases crop resistance to disease by activating the ASR signal transduction pathways in several plant species (Conrath *et al*., 2001; Hongxia *et al*., 2005). Control treatment showed good results for vegetative growth parameters only which can be related to the low density of control plants, resulted from Fusarium infection, provided more available nutrients per plant. The high number of umbels accompanied with little seed yield in this treatment may be related to Fusarium infection of control plants at late stage. The best interaction treatment is the treatment of seeds before planting by chitosan and irrigation of cumin plants every two weeks. This result is in according to that obtained by Tawfik and Allam (2004b) who stated that 2-days water priming associated with biocontrol combine complementary mechanisms of action against the pathogen enabling a consistent control of the disease. Advanced research is needed to examine these studied factors in various soil types.

Conclusion

Treatment of cumin seeds by chitosan, bion or *Streptomyces alni* were found to be very effective to overcome wilting disease. All biological treatments significantly increased plants survival percentage up to 100% compared to control. Chitosan was found to be the most efficient of the three tested compounds for plant survival and also for growth and seeds yield per feddan. Irrigation intervals of two weeks gave the best results compared to 4 or 8 weeks. Two weeks treatment seems to be more suitable in such new reclaimed sandy soil than one month which is usually followed by the producers. Treatment of cumin seeds by chitosan before planting followed by irrigation of plants in such sandy soil every two weeks was found to be the best treatment to overcome cumin wilting disease and improve growth and yield of cumin. Control plants showed some enhancement in vegetative growth parameters which related to the low density remained after some plants death caused by Fusarium. The results published in this paper may encourage growers to produce such economically important cumin plant under safe conditions in the new reclaimed soils for medicinal uses and exportation.

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معاملات زراعية وحيوية للتغلب على ذبول الكمون النامى عضويا بأراضى مستصلحة حديثا

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هدف البحث الى التعرف ثم التغلب على العامل المسبب للذبول الذى يقلل معنويا نجاح زراعة الكمون، وهو أحد أهم النباتات الطبية والعطرية والتوابل، وذلك باستخدام طرق زراعية وطرق مقاومة بيولوجية امنة. تم أولا عزل الفيوزاريوم المسبب للذبول من جذور نباتات الكمون المريضة وتبين بعد تعريفه أنه *Fusarium oxysporum* f. sp. *cumini*. أجريت التجربة فى أرض رملية مستصلحة حديثا موبوءة طبيعيا بالفيوزاريوم بمزرعة كلية الزراعة بجامعة سوهاج وقد تم تسميدها عضويا خلال موسمين متتاليين 2006/2007 ، 2007/2008م. تم دراسة تأثير ثلاثة فترات للرى ، أسبوعين أو 4 أسابيع أو 8 أسابيع، وثلاثة معاملات حيوية للبذور قبل الزراعة باستخدام ، كيتوزان أو استربتوميسيس أو بيون، بجانب الكنترول (بدون معاملة حيوية للبذور). وجد أن العوامل المدروسة فعالة معنويا فى خفض ذبول الكمون وذيادة نموه وانتاجيته من البذور. وجد أيضا أن رى الكمون كل أسبوعين هى المعاملة الاكثر ملائمة فى تلك الاراضى الرملية المستصلحة حديثا تحت ظروف المناخ الحار جنوبا. جميع المركبات الحيوية المستخدمة زادت معنويا من نسبة النباتات الناجية من الذبول حتى 100% مقارنة بالكنترول. كان الكيتوزان أفضل المركبات المستخدمة بالنسبة لنمو الكمون وانتاجيته من البذور. وقد أدت معاملة بذور الكمون بالكيتوزان قبل الزراعة مع رى النباتات كل أسبوعين الى أعلى نسبة نجاة وأفضل نمو للنباتات مع أعلى انتاجيه من البذور. النتائج المتحصل عليها خلال البحث أوضحت امكانية التغلب على مرض ذبول الكمون وامكانية انتاجه عضويا فى أراضى مستصلحة حديثا باستخدام معاملات امنة. هذه النتيجة قد تشجع المنتجين على زيادة المساحة المنزرعة من الكمون فى تلك الأراضى لاستخدامه طبيا ولغرض التصدير.