



THE ACTIVITY OF GIBBERELIC ACID AND *CONOCARPUS ERECTUS* AGAINST POTATO SOFT ROT DISEASE CAUSED BY *ERWINIA CAROTOVORA* SUB SP. *CAROTOVORA*

Abdul jabbar S. Ahmed* and T.A. Kareem

Dept. of Plant Protection, Coll. of Agri. Engineering Sciences, University of Baghdad, Iraq.

Abstract

This study was carried out in order to test the efficacy of *C. erectus* alcoholic extract and gibberellic acid to control potato soft rot bacterial disease caused by the bacteria type *E. c. sub sp. carotovora* was investigated. The inhibition ability of the extract at 250, 500 and 1000 ppm concentrations was examined on nutrient agar (N.A) medium. Each concentration was mixed with the medium before solidification then inoculated with the bacterial pathogen. The highest inhibition percentage of *Conocarpus* was %86.1 at 1000 ppm concentration. In pots experiments, the lowest percentage of infection was %0.00 for tubers treated with 1000 ppm of conocarpus and 1.5g/L beltanol pesticide compared to control which was 41.14%. Whereas inhibition percentages were 6.77, 4.85 and 2.67 respectively, for all three concentration of conocarpus treated in compared to control which was %34.15, in the field experiment Potato grown in pots treated with 500 and 1000 ppm scored the highest tubers weight which were 175 and 154 gm/plant respectively compared with control which was 118.33 gm /plant. While for those grown in the field, tuber treated with beltanol pesticide scored 1197gm/plant highest weight compared to control 248 gm/plant. The highest number of tuber was 27.67 tuber/plant for gibberilin treatment compared to 9.5 tuber/plant for control plants grown in pot. In field experiments, the highest tuber number was 58.33 tuber/plant in potato plants treated with conocarpus at 1000 ppm concentration, compared with control which was 10 tuber/plant. Potato plants treated with 500 ppm conocarpus extract scored the highest branch number which was 6.37 branch/plant compared 2.67 branch/plant for control in pot experiment. While in field experiments, plants treated with conocarpus at 500 ppm scored 4.67 branch/plant compared to 1.33 branch/plant in control. The branch lengths were 18.33 cm and 19.67 cm for the plants treated with gibberilin compared to 12.88 and 8.16 cm. for control for pots and field experiments, respectively. The wet and dry weights of root system for potato plants in grown in pots and treated with 1000ppm conocarpus scored 74.2 and 34.48 gm/plant compared to control with 38.1 and 14.2 gm/plant. While in those grown in the field potato plants treated with 500 conocarpus, scored the highest wet and dry weights which were 297,1 and 140.42 gm/plant compared to control 49.1 and 24.87 gm /plant respectively.

Key words : *E. carotovora*, potato disease, *Conocarpus erectus*.

Introduction

Potato *Solanum tuberosum* L. is one of the important crops grown worldwide. It comes after wheat and rice crops in terms of production and consumption globally. In Iraq, potato is grown in spring and autumn seasons. Potatoes are infected with various pathogens, including the bacteria *Erwinia carotovora* sp. *Carotovora* that limit their production. This bacteria causes the potato soft rot disease that affects the potato in the field and the store as well. This disease reduces the productivity of potatoes in addition to the marketing value. The pathogen is transmitted by contaminated tubers or soils. It attacks

the cultivated tubers then transfers to the stem and the vegetative parts (Perombdon and Hyman1995). Infected plant exhibit are black stem, yellow upward rolling of leaf edge and plant decay. At the early stage of infection, the disease may cause plant death, rot and decomposition of the tuber (Perombelon *et al.*, 1987; Suliman2016). Due to the high disease impact on the potato crop, many methods of control have been used to minimize its spread including the use of plant extracts. Many extracts including, cloves, cinnamon, *Aldora*, neem, eucalyptus, lemon, garlic and ginger were used (Aljoburi 2010 and Simeon and Akpa, 2014 and Alkubaisi, 2014), but the disease is still a real threat to the production of potatoes in different regions in the world. In Iraq the disease was

*Author for correspondence : E-mail : j.salieh@yahoo.com

observed in potato fields of Yusufiya and Abu Ghraib during the growing season 2015-2016. This study aimed to examine the effect of *Conocarpus erectus* extraction and gibberilic acid 10% on the infection of potato tubers var. Burin under the green house condition and in the field and study of some growth parameters including the number of tubers, tuber weight, number and length of branches and wet and dry weight of the root system.

Materials and Methods

Pathogen isolation and diagnosis

Samples of potato plants exhibiting black stem symptoms were collected from fields in Abu Ghraib and Yusufiya - Baghdad province. Samples were cut to small pieces (0.5 cm), sterilized by soaking for 3 minutes in a 1% sodium hypochlorite solution (1% free chlorine) at a concentration of 10%, rinsed with sterilized distilled water several times and dried on sterilized filter papers. Plant pieces were transferred into a glass petri dish and a small amount of distilled water was added then crushed well. Small quantity of the suspension was spread on Petri dishes including nutrient agar (NA) medium, previously autoclaved at 121 °C and 1.5 kg / cm³, by a loop sterilized with the flame., which was. Cultures were incubated at 25 + 2 °C for 48 hours. Single bacterial colonies were selected separately from the center of the plate and picked by syringe needle and streaked on NA medium in Petri dishes. The inoculation was repeated to obtain pure isolates from the pathogen and a group of isolates was selected for diagnosis (Goszezynska and Serfontein, 1998). Colonies for the selected isolates were placed in a sterile distilled water droplet on a glass slide, spread on the slide as a membrane, dried and fixed by an incandescent lamp flame, stained with gram staining (Al-Delaimy, 1988) and examined by the oil immersion microscopy.

Test of pathogenicity on potato slices

The experiment was carried out in the Laboratory of Plant pathology Research, Department of Plant Protection, Coll. of Agri. Engineering Sciences, University of Baghdad. A healthy potato tuber was sliced into 8mm thickness slices, sterilized by (10%) sodium hypochlorite solution for 3 min, washed by sterilized distilled water then dried by using dried paper. The slices were placed in a petri dish (4 slice / dish). About 100 ml of liquid bacterial culture at 10⁶ colony/ ml concentration was added to each dish then incubated for 7 days at 25 + 2 °C according to the method described by Kelement and Radolph (1990).

Tobacco leaf hypersensitivity reaction test

Tobacco plants at 4 true leaves age, provided from

Virus research were used.. Five bacterial isolates were inoculated by injecting 1 ml from a liquid bacterial suspension with 10⁶ colony formation unit (cfu) / ml in the secondary veins of the tobacco leaf using a 1 ml needle. The inoculated leaves were marked by using plastic tapes of different colors. After 7 days of inoculation the results were calculated as described by Kelement and Lovrekovich (1964).

C. erectus alcoholic extraction

Dried leaves were grinded and 100 g of powder were added to 500 mL flask. About 200 ml of 80% ethyl alcohol were added and the flask was sealed with a lid and homogenized for 24 hours in medium-speed electric shaker. The extract was filtered through the filter paper Typeman No.1 in the Buchner funnel with a vacuum. The sample was re-extracted as mentioned above. The final filtrate was concentrated with rotary evaporator at 40°C to obtain a high-density liquid. The extract was stored at -20°C until use (Harborne, 1973).

Effectiveness of the Conocarpus extract against the growth of pathogenic bacteria on the culture medium

The poisoned food medium method was used to test the efficacy of alcoholic extract against pathogenic bacteria. Three concentrations of leaf extract (250, 500, 1000ppm) were prepared in 100 ml of Nutrient Agar medium at 45°C and pre-sterilized in autoclave at 121°C and 1.5 kg / 3 kg to obtain the required concentration. In addition, Beltanol and gebriline were added at 1500 ppm and 10% concentrations, respectively as recommended. Each poisonous medium was mixed in a conical flask using a rotary motion to ensure uniformity of the material within the culture medium. The medium poured in 9 cm petri dishes then left to solidify. A 0.5 cm hole was made using a sterilize cork borer and 20 µl of 48-hour liquid bacterial culture at 4 × 10⁶ cfu concentrations were added to each hole. The experiment was carried out with three replicates for each treatment and three of pathogenic bacteria without treatment were inoculated as a control. The dishes were incubated at 25 + 2°C for 24 h. Results were recorded and the following equation was used to calculate the percentage of inhibition adopted by Ahmed and Alani (2013).

% of inhibition =

$$\frac{\text{Rate of bacteria in comparison} - \text{rate of bacterial growth in treatment}}{\text{The growth rate of bacteria in comparison}} \times 100$$

Results were statistically analyzed using CRD.

Green house experiment

This experiment was carried out in the green house

belongs to the Department of Plant Protection - Coll. of Agri. Engineering Sciences - University of Baghdad. The experiment was carried out on potato variety the IPR Burren (class A) produced by IPR. Tubers with the similar size, intact and free of infection were selected. Plastic pots of 35 × 21 × 15 cm size were filled with mixed soil. Completely randomized design (CRD) was performed using three replicates for each treatment. Two tubers were planted in each pot. The experiment was performed during the autumn growing season of 2015. The treatments were divided into two sections: the first was left untreated without any addition as a comparison. Tubers in the second section were soaked for 20 minutes in a liquid culture of the bacteria *Erwinia carotovora* subsp. *carotovora* at 10⁶ colony / 1 ml concentration at age of 48 hours and then left on drying paper until dry. For Gibberellic acid treatment, tubers were soaked in 10% gibberellin solution for 20 minutes and those treated with a three-concentration alcoholic extract of 250, 500 and 1000 ppm for 20 min for *conocarpus* treatment. For pesticide treatment, tubers were dipped in the recommended concentration of 1.5 ml / l for 20 min. The comparison between 10% gibberellin and *conocarpus* alcoholic leaf extraction 1000 ppm were carried out in the absence of bacteria was applied. The percentage of infection, the number of tubers produced, the weight of the tubers, the number of branches, the length of the branches and the wet and dry weight of the root mass were calculated 60 days after germination.

Field experiment

The experiment was carried out in the research station of the Plant Protection Department Coll. of Agri. Engineering Sciences / University of Baghdad in Abi Gharib region. The experimental field was prepared through soil plowing, smoothing and dividing into three sectors. Each sector contained (9) experimental units. Two meters distance between each sector and 25 cm between each plant were left. Each experimental unit included five potato tubers. Field experiment was carried out in the spring season, 2016 using the same treatments in pot experiments and randomized complete block design (RCBD) in three replicates for each treatment, was used for the above mentioned field experiment, Infection percent, number of branches, the weight and the number of tubers, and the wet and dry weight of the root system at the end of the season.

Results and discussion

Isolation and diagnosis of the pathogen

Five isolates of the pathogen were obtained, showing the morphological characters of the developing colonies

on the Nutrient Agar medium. Inoculated with suspension from parts of potato plants showing the symptoms of mild rot. These colonies have a circular shape, soft, convex, yellow creamy in color with regular or irregular edges phenotypically characteristics to *Erwinia* (Schaad, 1980). Whereas, the microscopic examination of the pathogen showed short-circuiting endocardial cells, most of them were single and some were double, negative to Gram staining. These characters were typical to *Erwinia carotovora* described by Saettler and Schaad (1989). Biochemical tests confirmed that the bacteria belonged to the subspecies *carotovora* according to Schaad (1980) and Helias (2000).

Pathogenicity test on potato slices

The results showed all isolates obtained from the potato slices were able to cause the disease. The symptoms of mild rot on potato slices in the dishes with foul odor after three days of inoculation were observed. On the seventh day turned the edges of the slices became brown (Lelliott and Stead 1987). Based on pathogenicity test, isolate number 3 which showed the highest ability to damage potato slices, was selected for subsequent experiments.

Hypersensitivity reaction on tobacco leaves

Yellow areas on the leaves around the inoculation position were shown 48 hours of injection of bacterial inoculum in the inner spaces boarded by veins of the leaves. Yellowing continued to increase after 72 hours and turned to a brown color (Ahmed (2011) which confirmed the 3rd isolate pathogenicity.

Test of the Effect of *conocarpus* on the Growth of Pathogenic Bacteria on the culture medium

It was found that there was an anti-bacterial activity of the investigated *conocarpus* leaf extract against the pathogenic bacteria under study at all concentrations (Table 1). All treatments showed a significant inhibition of pathogenic bacteria compared to the only pathogenic bacteria control treatment. *Conocarpus* treatment at 1000ppm concentration scored the highest 86.1% inhibition rate compared to other treatments at 250, 500ppm concentrations and Beltanol and gibberellin, with an inhibition rate of 64.1, 77.8, 74.1 and 53.7%, respectively. The antibacterial activity of the *conocarpus* extract may be attributed to the high content of this plant to many substances known to have inhibition effects on microorganisms (Abdel Hameed *et al.*, 2012).

Green house experiment

All the treatments applied resulted in a significant decrease in the percentage of *E. carotovora* infection

Table 1: Shows Effect of conocarpus on the Growth of Pathogenic Bacteria on the culture medium.

% inhibition	Growth rate after 48 h. (cm) Bacteria	Treatment
0	9.00	Pathogen Bac. Only (control)
64.1	3.23	<i>C. erectus</i> extract at 250 ppm + Bac.
77.8	2.00	<i>C. erectus</i> extract at 500 ppm + Bac
86.1	1.26	<i>C. erectus</i> extract at 1000 ppm + Ba.
74.1	2.33	beltanol pesticide + Bac.
53.7	4.16	Gibberilin + Bac.
7.96	0.72	L.S.D. _{0.05}

Each number in table represent mean of three Replicates.

(Table 2). The percentage of pathogenic bacteria in the potato plants at all three tested concentrations of the Conocarpus extract (250, 500, 1000 ppm) scored 5.95, 3.40, 0.00% infection percent, respectively. It was observed that the conocarpus leaf extract at 1000 ppm did not show a significant different with the chemical pesticide treatment. While the treatment of the growth regulator Gebriline could reduce the percentage of pathogenic bacteria infection to 14.14% he compared to the pathogen only treatment. There were significant differences in some of the growth parameters that were measured. Conocarpus treatments at 500 and 1000 ppm, were significantly higher in tuber weight when scored 154 and 175 g, respectively compared to pathogenic bacteria (118 g / plant), chemical pesticide (133 g / plant) and gibberellin (144 g). The number of tubers from the plants treated with the Conocarpus extract was of a good quality and free from infection. The gibberellin treatment was the highest in the number of tubers (27 tuber / plant) compared to pathogenic bacteria only treatment. It is known that growth regulators have a role in increasing the number and productivity of potato tubers. The

standard number of branches / plant of the Conocarpus treatment was 6, 6.37, 5 branches / plants respectively compared to pathogenic bacteria only (2.67 branch / plant). While no significant differences were observed in the length of the branches between Conocarpus extract and the pathogen only treatments. While, it was noted the gibberellin scored 17.67 cm highest branch numbers compared to other treatments with significant differences The wet and dry weights of the total root system of the potato plants for the Conocarpus leaf extract at

1000 ppm were 74.2 and 34.48 g / plant, respectively which were significantly higher than that of pathogenic bacteria only with 38.1 and 14.20 g / plant, and chemical pesticide treatments with 56.5 and 18.50 g / plant, respectively.

Field experiment

Field experiment showed significant differences in the percentage of *E. carotovora* infection in potato plants (Table 3). Infection percentage decreased in all conocarpus leaf extract treatments, when scored to 6.77, 4.85 and 2.65% for 250, 500 and 1000 ppm, respectively. compared to 34.5% for pathogenic bacteria only treatment. Morphological differences in growth parameters were shown in the weight of tubers / plant. Tuber weight scored 745, 1008 and 1003 g / plant for the three conocarpus extract concentrations, respectively, compared to treatment of pathogenic bacteria with 248 g / plant and gibberellin with 955 g / plant treatments. The total number of tubers in the treatment of conocarpus extracts was significantly higher with 53, 57.33 and 58.33 tuber / plants, respectively, than the pathogen only treatment with 10 tuber / plant, the chemical pesticide

Table 2: Shows Effect *C. erectus* alcoholic extract and Gibberilin against *E.carotovora sp. carotovora* pathogen Bacteria in pots.

Dry Weights	Wet Weights	Branch Lengths cm	Number of Branch/ plant	Number of tuber/ plant	Tubers Weight gm/plant	Infection %tuber	Treatment
29.60	58.6	7.66	2.67	8.67	143.33	0.00	1-tuber healthy (control)
14.20	38.1	8.61	2.67	9.50	118.33	41.14	2- tuber + Pathogen bacteria only (control)
20.81	44.07	17.61	4.00	27.00	144.00	14.14	3-tuber+ bac.+ gibberilin (10%)
15.20	31.70	7.16	6.00	10	118.33	5.95	4-tuber+bac. + <i>C.erectus</i> extra. (250ppm)
17.77	37.20	7.46	6.37	10	154.33	3.40	5—tuber+bac. + <i>C.erectus</i> extra. (500ppm)
34.48	74.24	7.83	5.00	11.33	175.00	0.00	6-tuber+bac. + <i>C.erectus</i> extra. (1000ppm)
17.78	43.10	8.83	3.33	15.83	133.00	0.00	7-tuber+bac+1.5g/L beltanol pesticide
18.50	56.47	18.33	4.00	27.67	142.00	0.00	8-tuber + gibberilin (10%) only
33.20	75.00	8.16	5.33	10.67	172.00	0.00	9-tuber + <i>C.erectus</i> extra. (1000ppm) only
2.77	8.71	1.58	1.27	0.93	6.04	0.91	L.S.D. _{0.05}

Each number in table represent mean of three Replicates and each replicate represent (2) plant .

Table 3: Shows Effect *C. erectus* alcoholic extract and Gibberilin against *E.carotovora sp. carotovora* pathogen Bacteria in Field.

Dry Weights	Wet Weights	Branch Lengths cm	Number of Branch/plant	Number of tuber/plant	Tubers Weight gm/plant	Infection %tuber	Treatment
47.19	100.4	17.33	2.67	36.33	1067	0.00	1-tuber healthy (control)
24.87	49.1	12.88	1.33	10.00	248	34.15	2- tuber + Pathogen bacteria only (control)
73.22	127.8	19.67	4.00	31.33	958	19.52	3-tuber+ bac.+ gibberilin (10%)
86.88	197.3	19.00	3.33	53.00	745	6.77	4-tuber+bac. + C.erectus extra. (250ppm)
140.42	297.1	19.00	4.00	57.33	1008	4.85	5—tuber+bac. + C.erectus extra. (500ppm)
139.81	292.2	19.33	4.00	58.33	1003	2.65	6-tuber+bac. + C.erectus extra. (1000ppm)
40.52	96.9	16.33	3.33	45.00	1197	0.00	7-tuber+bac+1.5g/L beltanol pesticide
60.37	120	19.33	4.67	37.67	968	0.00	8-tuber + gibberilin (10%) only
140.50	290.2	19.00	4.00	52.67	1010	0.00	9-tuber + C.erectus extra. (1000ppm) only
4.48	24.85	0.57	1.09	6.37	303.2	0.77	L.S.D. _{0.05}

Each number in table represent mean of three Replicates and each replicate represent (5) plant.

with 45 tuber / plant gibberellin with 31.33 tuber / plant) treatments. The number of branches / plant in the treatment of gibberellin and the three conocarpus extract concentrations were 3.67, 3.33, 4.67 and 4 branches / plant respectively compared to pathogenic bacteria treatment (1.33 branches / plants). While no significantly different in potato branch numbers per plant was scored for chemical pesticide treatment. conocarpus leaf extract was significantly higher in branch length when scored 19.67, 19 and 19.33 cm than in the pathogenic bacteria (12.88 cm) and the chemical pesticide (16.33 cm) treatments. The root wet weight of plants treated with conocarpus leaf extract was the highest when scored 127.8, 197.3, 297.1 and 292.2 g / plant for 250, 500 and 1000 ppm, respectively compared to 49.1 g / plant and treatment of the chemical pesticide (96.9 g / plant) for pathogen only and pesticide treatments, respectively.. It was also observed that the treatment of gibberellin and the three tested concentrations of conocarpus extract scored the highest dry weight which was 73.22, 86.88, 140.42 and 139.81 g / plant compared with pathogenic bacteria (24.87 g / plant) and chemical pesticide (40.52 g / plant) treatments.

The antibacterial effect of the alcoholic conocarpus leaf extract may be due to the presence of alkaloids, coumarins, flavonoids, saponins, tannins, triterpenes, a Antioxidants gallic acids, rutin, quercetin and apigenin which have the ability to inhibit many types of microorganisms (Naserallah, (2012), Abdel Hameed and others (2013, Bashir, and others, 2015). This is consistent with Adonizio *et al.*, (2008), Abdel- Hameed (2012) And Abdulrahman and Nehad (2013), who referred to the effect of conocarbase extracts in inhibiting the growth of many bacteria including *Bacillus subtilis*, *Staphylococcus aureus*, *Mycobacterium phlie*

Escherichia coli, *Pseudomonas aeruginosa*, *Kelbsiella pneumonia* and *Salmonella typhimurium* and some fungi namely, *Aspergillus niger*, *Penicillium chrysogenum*, *Saccharomyces cerevisiae* and *Alternaria solani*.

It was also observed in this study that the ability of gibberellin 10% to reduce the incidence of *E. carotovora* to protect plants from infection and may be due to the fact that the gibberellin ability to stimulate the division and elongation of plant cells, allowing the escape of plants from infection (AL-Khafaji, 2014).

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