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Efficacy of *Cassia nodosa* extracts in the management of cercospora leaf spot of sugar beet caused by *Cercospora beticola*

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Abstract *Cercospora beticola*, the causative agent of cercospora leaf spot (CLS) disease, is a serious problem that remarkably impact the yield of sugar beet worldwide. Our aim was to assess the antifungal effect of a natural and more safe extracts of Leaves, flowers and stem bark of *Cassia nodosa* and salicylic acid (SA) individually or in combination relative to the recommended treatment tetraconazole fungicide. The fungicidal effect was assessed *in vitro* by measuring the radial growth of *C. beticola* and *in vivo* by determining the alteration in CLS disease severity, sugar beet root weight and quality traits (sucrose, total soluble solids and purity %). Also, the relationship between disease severity % and root weight, sucrose % and leaf biochemical components was determined. *In vitro*, all the tested plant extracts at different concentration levels inhibited the growth of *C. beticola*. The results of all *in vitro* and *in vivo* experiments were revealed that the best effects were for *n*-butanol extract of stem bark, methanolic extract of flowers and ethyl acetate extract of stem bark either alone or in combination with SA. Stem bark *n*-butanol extract was the most effective one. Four salicylic acid-plant extract mixtures demonstrated a synergistic effect (SA with flowers methanolic, leaves ethyl acetate, and stem bark ethyl acetate and *n*-butanol extracts). A highly significant negative correlation was found between CLS severity % and all of root weight, sucrose % and biochemical components of sugar beet leaves.

Keywords *Beta vulgaris L., Cercospora beticola, Cassia nodosa* extracts, Salicylic acid, Root weight, Sucrose %, Phenols and Polyphenol oxidase activity

1. Introduction

Sugar beet (*Beta vulgaris* L.) is an important sugar crops, which is the source of about 30% of the world's sugar [1]**.** In Egypt, it is ranked the second crop for sugar production after sugar cane [2]. Cercospora leaf spot (CLS) disease is a worldwide challenge for growing sugar beet of good yield and quality [3, 4]. CLS is a destructive foliar disease of sugar beet, which is caused by *Cercospora beticola* Sacc. [4, 5-8]. Losses due to CLS have gone as high as a 42 % reduction in gross sugar and 32 % reduction in root weight [9]. CLS disease causes yield loss in susceptible varieties ranged from 10 -50 % in Australia and from 15 – 40 % in France [10].

The control of CLS is currently includes treatments with benzimidazoles, morpholine, strobilurins and dithiocarbamates [11-13]. However, using these compounds raises concerns of exposure risks, fungicide residues, diseases resistance and other health and environmental hazards. As a result, finding effective, safe and environmentally friendly fungicides against CLS is imperatively needed [14]**.** In this regard, one of the most

promising approaches is to test plant extracts with fungicidal activity, which has been shown to be eco-friendly and effective against many plant pathogens other than CLS [13, 15-18].

Cassia nodosa Buch.-Ham. ex Roxb. belongs to family Leguminosae [19]. It is known also as *Cassia javanica* L. var. indochinensis Gagnepain [20]. It is a fast-growing, medium sized perennial tree up to 15 m in height. It is a beautiful ornamental tree.

Cassia nodosa exhibited antifungal activity [21]. Therefore, the present study was designed to investigate the antifungal activity of different extracts of *Cassia nodosa* against CLS under laboratory and greenhouse conditions alone or in combinations with salicylic acid, and their effect on root weight and quality traits. Finally, determine the relationship between disease severity vs. root weight, sucrose and leaf biochemical components.

2. Materials and Methods

2.1. Materials

Experiments were conducted in 2013-2015 under Laboratory and greenhouse of Gemmeiza Agricultural Research Station (Agricultural Research Center). The *C. beticola* isolated from sugar beet plants was obtained as culture slant from the Department of Mycology and Plant Disease Survey, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt. Sugar beet (*Beta vulgaris* L.) seeds cultivar Pleno provided by Sugar Crops Research Institute, Agric. Res. Center (A.R.C), Giza, Egypt were used in the present study as host plant for cercospora leaf spot (CLS) disease. Leaves, flowers and stem bark of *Cassia nodosa* Buch.-Ham. ex Roxb. were obtained from trees cultivated in El-Dakahlia Governorate in Egypt.

The control fungicide used in this study was tetraconazole with a trade name of Eminent 12.5% EW produced by Kafr El-Zayat company for chemicals and pesticides, this fungicide recommended for control CLS disease in sugar beet in Egypt. The control inducer, salicylic acid (SA) was obtained from El-Gomhoria Company for chemicals, used as a chemical inducer alone or in mixture with plant extracts.

2.2. Preparation of plant extracts

Air dried powdered flowers, leaves and stem bark of *Cassia nodosa* were extracted with 95% methanol till exhaustion. Cold maceration method was carried out. The combined methanolic extracts of each organ were concentrated at 50 °C under reduced pressure. Thereafter, the concentrated methanolic extract of each organ was separately dissolved in methanol-water mixture (1:1), then successively re-extracted with petroleum ether, methylene chloride, ethyl acetate then *n*-butanol to give different sub-fractions of each *Cassia nodosa* organ (Table 1).

Table 1: Different extracts of *Cassia nodosa* Buch.-Ham. ex Roxb.

Extract No.	Plant organ	Solvent used for extraction
1	Leaves	Methanol
2	Flower	Ethyl acetate
3	Stem-bark	n-butanol
4	Leaves	n-butanol
5	Stem-bark	Methanol
6	Flowers	Methanol
7	Leaves	Ethyl acetate
8	Stem bark	Ethyl acetate
9	Flowers	n-butanol

3. Screening of plant extracts against *C. beticola*

Plant extracts, salicylic acid and tetraconazole were tested for their *in vitro* as well as *in vivo* fungicidal activity against *C. beticola* in a completely randomized design.

3.1. The *in vitro* experiments

The tested treatments were evaluated alone or in mixture and the fungicidal activity was determined as percent of inhibition in the growth of selected *C. beticola* relative to the control treatment. Three concentrations (100, 500 and 1000 ppm) for each of the tested 9 plant extracts, as well as salicylic acid and five concentrations of tetraconazole (0.01, 0.1, 1.0, 10, and 100 ppm) were tested. Different treatments and the no-treatment control were prepared in autoclaved PDA medium (200 gm potato extract, 20 gm dextrose sugar, 20 gm agar and completed to final volume of 1000 ml distillated water and then it was autoclaved at 1.5 atm. for 20 minutes, then cooled to about 40°C). Three Petri-dishes, 9 cm in diameter were used as a replicates for each group, inoculated in the center with a disk (5mm diameter) bearing the mycelia growth from *C. beticola* culture (7 days old); thereafter, the dishes were incubated at 26ºc until the full growth of the control treatment. Radial growth was measured in cm by taking the average of two perpendicular diameters. The inhibition percentage (I %) of radial growth of *C. beticola* was calculated using the following formula [17]:

$$\mathrm{I}\,\% = \{(\mathrm{A} - \mathrm{B})/\mathrm{A}\}\mathrm{x}\,100$$

Where, A is the radial growth of the tested fungus in control, and B is the radial growth of the tested fungus in treatment.

Also, the synergistic effects of salicylic acid and plant extracts against *C. beticola* was evaluated *in vitro*. Mixtures of salicylic acid and each individual plant extract were tested against *C. beticola* by using the radial growth. Three concentrations (100, 500 and 1000 ppm) of 1:1 mixtures were used. The antifungal tests were evaluated as described above with three replicates.

The mean lethal concentration (observed IC_{50}), 95% confidence limits (FL_{95}), and slopes were estimated by probit analysis [22]. The expected median lethal concentration (Expected IC_{50}) of fungicide mixtures was calculated by the following equation [23]:

$$\boldsymbol{Expected\ IC50 = a + b/[a/IC50(a)\ + b/IC50(b)\ ]}$$

In this equation, the expected IC_{50} of the mixture, which is the harmonic mean of the IC_{50} observed for fungicide a and b acting separately, and a and b are the relative proportions of fungicide a and fungicide b in the mixture, respectively.

The synergism ratio (SR) of fungicide mixtures was calculated by dividing the (expected IC_{50} by the observed IC_{50} values, based on [24].

$$\boldsymbol{Synergism\ ratio\ (SR) = Expected\ IC50/Observed\ IC50}$$

The value of SR greater than 1 indicates a positive synergistic effect (synergism); while less than 1 indicates a negative synergistic effect (antagonism). SR equal to 1 indicates an additive effect [25].

3.2. The *in vivo* experiments

The experiment was also performed in a greenhouse under artificial infection with *C. beticola*. Seeds of sugar beet cultivar Pleno were sown in micro plots 3.2 m² and each micro plot contain two rows with 2 m long and 80 cm apart. The experiment was arranged as a randomized block design in three replications. The normal methods of sowing and agricultural practices were applied as recommendations.

Each of the plant extracts, salicylic acid or tetraconazole were individually tested for their efficacy against *C. beticola* at the rate 0.1% as foliar spray. Mixtures were also tested at the rates of 1:1 (w/v) salicylic acid to plant extracts similar to what was performed *in vitro*. Sugar beet plants in control were sprayed with water only at the same intervals used in treatments application. The tested treatments were applied fourth times, the first at 15 days before inoculation, and three times were applied at 15, 30, and 45 days after inoculation. The sugar beet plants, 90 days of age, were dusted with dried and ground, infected sugar beet leaves collected in the previous season. Disease severity %, root weight (kg) and quality traits {sucrose % and total soluble solids % (T.S.S.)} were determined. Some biochemical changes were estimated in leaves of sugar beet.

In addition, CLS severity was assessed on 10 plants (five leaves from each plant) selected random in the center two rows of each micro plot [3].

CLS severity was recorded three times every 15 days beginning 2-week after the last spray as percentage on the average. A lower leaf was randomly selected and rated according to a spot – percentage scale and assigned to a category (1-10) based on disease severity. 1-5 spot per leaf = 0.10% (category 1); 6-12 spots = 0.35 % (category 2); 13-25 spots = 0.75 % (category 3); 26-50 spots = 1.5 % (category 4) and 51-75 spots = 2.5 % (category 5); at higher disease incidences, the average effected area per leaf was estimated from standard area diagrams and categories 6 through 10 represented 3, 6, 12, 25 and 50% severity, respectively [26]. The disease severity was calculated as:

$$\text{Disease severity \%} = \frac{\sum(\text{Each category x number of leaves in each category})}{\text{The total leaf number x the highest degree of category}} \times 100$$

The Efficacy of each treatment in reducing CLS severity % was calculated using the following formula [17]**:**

$$\text{Efficacy\%} = \{(\text{DSC} - \text{DST})/\text{DSC}\} \times 100$$

Where, DSC is the disease severity under control, and DST is the disease severity under treatment.

Also, representative leaves of each treatment were randomly collected from each micro plot (r =3) 15 days after the last spray to determine biochemical components as follow:

a) Estimation of phenols (total and *ortho*-dihydroxy (OD)) and total free amino acids. The samples were weighted and extracted in hot 80% ethanol and aqueous phase of this extract was used for analysis [27].

i. Total phenol was estimated by colorimetric method of folin- phenol reagent at 650 nm, as described by [28]**.** The results are expressed as milligram per gram fresh weight of plant sample (mg/g f w).

ii. *Ortho*-dihydoxy phenol (OD phenols), Arnow's method [29] was used for determination of OD phenols. The results are expressed as milligram per gram fresh weight of plant sample (mg/g f w).

iii. Total free amino acids were determined in the ethanolic extract using the colorimetric method of Ninhydrin at 570 nm, as described by [30]**.** The results are expressed as milligram per gram fresh weight of plant sample (mg/g f w).

b) Polyphenol oxidase activity was determined by the extraction of sugar beet leaves, as described by [31] using spectrophotometer procedure at 495 nm, as described by [32]**.** The enzyme activity was expressed as the change in the absorbance per minute per gram fresh weight (ΔA/min/g f w).

Finally, at harvest time, roots were harvested and ten roots were taken randomly for determination of root weight and quality traits (total soluble solids (T.S.S.), sucrose and purity %). Quality traits viz. total soluble solids (T.S.S.) % was measured in fresh roots using the hand refractometer according to Mc Ginnis [33]. Sucrose % was determined by using saccharometer according to Anonymous [34]. Purity % was calculated by using the formula that {(sucrose % / T.S.S %)} × 100.

Statistical analysis

The obtained data were subjected to analysis of variance [35]. Least significant differences (L.S.D) and Duncan's multiple range tests (DMRT) were applied to comparing means under study [36]. Values are expressed as mean ± standard error (SE). A simple correlation and regression analysis between two data sets was calculated in Excel Spread Sheet.

4. Results

4.1. Effect of the tested plant extracts and salicylic acid on radial growth of *C. beticola*:

Plant extracts and salicylic acid and tetraconazole were evaluated for their antifungal activity against *C. beticola*. Results are illustrated in Table 2.

Table 2: Antifungal effect of some plant extracts, salicylic acid and tetraconazole on *C. beticola*.

Treatments	Conc. (ppm)	Inhibition%±SE	IC_{50} (ppm)	Slope	Confidence limits
Ext. 1	100	11.28±0.66	1235.34	1.14	843.03-2415.30
	500	31.20±1.64			
	1000	47.39±1.61			
Ext. 2	100	16.13±2.58	1474.42	0.85	873.01-4689.84
	500	34.57±2.52			

	1000	43.63±2.07			
Ext. 3	100	12.77±0.90	935.69	1.19	674.10-1562.94
	500	35.35±1.66			
	1000	53.01±0.99			
Ext. 4	100	10.88±1.57	6254.28	0.69	2117.52-34817.66
	500	20.69±1.11			
	1000	30.07±1.41			
Ext. 5	100	4.11±1.95	5024.03	1.01	2158.75-61618.27
	500	16.91±1.70			
	1000	23.3±2.05			
Ext. 6	100	7.47±3.01	3194.42	1.00	1635.82-16188.41
	500	19.18±0.77			
	1000	31.59±1.27			
Ext. 7	100	7.86±2.54	3073.69	0.96	1565.88-16120.38
	500	21.03±1.51			
	1000	32.72±1.27			
Ext. 8	100	17.68±1.40	904.32	1.04	626.50-1649.34
	500	32.32±1.40			
	1000	57.14±1.35			
Ext. 9	100	1.88±1.00	3075.07	1.38	1730.72-12438.88
	500	13.52±1.09			
	1000	25.19±0.74			
Salicylic acid	100	31.85±1.96	253.39	1.30	183.93-331.30
	500	58.15±1.96			
	1000	83.33±1.93			
Tetraconazole	0.01	15.78±1.19	0.26	0.77	0.17-0.40
	0.1	41.35±1.38			
	1.0	54.54±2.37			
	10	93.64±3.19			
	100	100.0±0.00			

All the tested treatment at different concentration levels inhibited the growth of *C. beticola* (Table 2). Salicylic acid was the most effective one against the fungus with IC_{50} of 253.39 ppm followed by Ext.8 and Ext.3 with IC_{50} values 904.32 and 935.69 ppm, respectively. On the other hand Ext. 4 (IC_{50}= 6254.28 ppm) and Ext.5 (IC_{50}= 5024.03 ppm) were the lowest effect on *C. beticola*. Other plant extracts fell in between. However, tertraconazole fungicide was still the most effective treatment in reducing radial growth of *C. beticola*. It was found that stem bark ethyl acetate (ext. 8) and *n*-butanol (ext. 3) fractions exhibited the best significant activity.

4.2. Synergistic effects of salicylic acid and plant extracts against *C. beticola:*

The synergy of salicylic acid with tested plant extracts at the rate 1:1 were listed in Table 3. four mixtures demonstrated a synergistic effect *i.e.* salicylic acid mixed with Ext.6, Ext.7, Ext.8 and Ext.3 with synergistic ratio (SR) values 2.14, 2.07, 1.05 and 1.03, respectively. But other combinations indicated an antagonistic effect hence in the case of these mixtures (Table 3) can be indicated that the observed values of IC_{50} for these mixtures were greater than those of expected one.

Table 3: Synergistic effects of salicylic acid and plant extracts against *C. beticola*

Treatments	Conc. (ppm)	Inhibition%±SE	Observed IC_{50} (ppm)	Slope	Confidence limits	Expected IC_{50} (ppm)	SR	Interaction
Ext. 1+SA	100	9.26±0.98	1213.04	1.20	842.90-2284.01	420.52	0.35	Antagonistic
	500	34.82±2.59						
	1000	43.71±2.43						
Ext. 2+SA	100	8.52±0.74	982.96	1.45	739.32-1497.80	432.46	0.44	Antagonistic
	500	27.04±2.25						
	1000	55.19±0.37						
Ext. 3+SA	100	19.26±2.25	386.14	1.42	298.22-496.94	398.79	1.03	Synergistic
	500	60.74±1.61						
	1000	68.89±1.92						
Ext. 4+SA	100	7.78±1.28	1573.35	1.16	1027.42-3561.77	487.05	0.31	Antagonistic
	500	30.00±1.11						
	1000	39.63±1.33						
Ext. 5+SA	100	12.96±0.74	839.99	1.35	630.23-1266.56	482.45	0.57	Antagonistic
	500	28.15±1.48						
	1000	61.48±1.33						
Ext. 6+SA	100	35.19±2.25	219.85	1.18	148.34-296.41	469.54	2.14	Synergistic
	500	63.70±3.23						
	1000	79.63±2.25						
Ext. 7+SA	100	41.85±3.24	225.93	0.72	108.48-358.54	468.18	2.07	Synergistic
	500	53.33±2.94						
	1000	72.59±2.25						
Ext. 8+SA	100	11.11±1.28	376.87	2.19	314.64-448.16	395.86	1.05	Synergistic
	500	57.78±1.70						
	1000	84.08±2.67						
Ext. 9+SA	100	8.89±1.69	2025.63	1.06	1210.73-5902.02	468.20	0.23	Antagonistic
	500	22.96±2.25						
	1000	39.26±1.33						

4.3. Effect of the tested treatments on controlling CLS disease and their effect on root weight and quality traits

The effect of plant extracts alone or in combination with salicylic acid relative to the fungicide tetetraconazole on infection with *C. beticola* evaluated in the greenhouse is shown in Table 4. The results showed that tetraconazole was the most effective in controlling CLS disease (5.40 % disease severity and 90.78 % efficacy), followed by Ext.3, Ext.6 + SA, SA, Ext.3+SA and Ext.7+SA with disease severity of 6.47, 7.53, 9.47, 10.73 and 11.27%, and efficacy % of 88.95, 87.15, 83.84, 81.69 and 80.77 %, respectively.

Table 4: The effect of the tested treatments in controlling cercospora leaf spot, and the resulted improvement in root weight and quality traits

Treatments	Disease severity %	Efficacy %	Root weight/ plant (kg)	T.S.S %	Sucrose %	Purity %
Ext. 1	25.47 ± 1.16^{cd}	56.54 ± 3.89^{jk}	1.03 ± 0.04^{fgh}	$17.80\pm0.40^{e-f}$	13.04 ± 0.32^{fgh}	73.26 ± 0.26^{fg}
Ext.2	12.00 ± 2.23^{ijk}	$79.52\pm3.08^{b-e}$	1.26 ± 0.04^{bc}	18.87 ± 0.47^{cde}	14.80 ± 0.51^{cde}	78.43 ± 0.88^{cd}
Ext. 3	6.47 ± 0.82^{lm}	88.95 ± 1.17^{ab}	1.35 ± 0.03^{b}	$19.93\pm0.47^{a-d}$	16.50 ± 0.35^{ab}	82.79 ± 1.16^{a}
Ext.4	20.07 ± 1.84^{efg}	$65.75\pm4.87^{g-j}$	1.00 ± 0.03^{fgh}	$18.27\pm0.88^{d-h}$	13.75 ± 0.92^{efg}	75.26 ± 1.64^{ef}
Ext.5	13.33 ± 1.18^{ij}	77.25 ± 0.99^{de}	1.17 ± 0.03^{cde}	$18.80\pm0.35^{c-f}$	14.91 ± 0.28^{cde}	79.31 ± 0.37^{bc}
Ext.6	24.20 ± 1.68^{cde}	58.70 ± 4.90^{ijk}	1.03 ± 0.04^{fgh}	$17.73\pm0.64^{e-h}$	12.95 ± 0.45^{fgh}	73.04 ± 0.09^{fgh}
Ext.7	14.67 ± 0.88^{hi}	74.97 ± 0.32^{def}	1.11 ± 0.04^{def}	18.67 ± 0.35^{def}	14.68 ± 0.39^{cde}	78.63 ± 0.57^{cd}
Ext.8	18.93 ± 2.45^{fgh}	$67.70\pm5.73^{f-i}$	$1.06\pm0.04^{d-g}$	$18.13\pm0.24^{e-h}$	$13.82\pm0.24^{d-g}$	76.23 ± 1.07^{de}
Ext.9	36.73 ± 1.79^{b}	37.32 ± 6.13^{l}	0.93 ± 0.04^{h}	16.80 ± 0.99^{ghi}	12.10 ± 0.76^{hi}	72.02 ± 0.31^{gh}

Ext.1+SA	21.93 ± 1.83^{def}	62.58±3.00^{h-k}	1.03±0.02^{fgh}	17.73±0.75^{e-h}	13.12±0.64^{fgh}	74.00±0.73^{efg}
Ext.2+ SA	18.53 ± 3.12^{fgh}	68.38±5.95^{fgh}	1.06±0.04^{d-g}	18.20±0.70^{e-h}	13.84±0.63^{d-g}	76.04±1.65^{de}
Ext.3+ SA	10.73 ± 1.37^{i-l}	81.69±1.58^{a-e}	1.38±0.04^{b}	19.33±0.88^{b-e}	15.61±0.84^{bc}	80.76±0.65^{abc}
Ext.4+ SA	33.13 ± 2.02^{b}	43.46±6.28^{l}	1.01±0.01^{fgh}	16.67±0.85^{hi}	12.03±0.68^{hi}	72.17±0.61^{gh}
Ext.5+ SA	12.67 ±1.50^{ij}	78.38±2.50^{cde}	1.18±0.02^{cd}	19.33±0.57^{b-e}	15.30±0.51^{bcd}	79.15±0.36^{bc}
Ext.6+ SA	7.53 ± 1.04^{klm}	87.15±1.63^{abc}	1.34±0.03^{b}	20.67±0.71^{ab}	16.80±0.64^{ab}	81.28±0.35^{ab}
Ext.7+ SA	11.27 ± 1.44^{i-l}	80.77±3.35^{b-e}	1.30±0.08^{b}	19.40±0.50^{b-e}	15.33±0.42^{bcd}	79.02±0.67^{bc}
Ext.8+ SA	15.40 ± 1.72^{ghi}	73.72±3.34^{efg}	1.10±0.04^{def}	18.47±0.24^{d-g}	14.10±0.15^{c-f}	76.34±0.39^{de}
Ext.9+ SA	27.13 ± 1.58^{c}	53.70±4.61^{k}	0.97±0.04^{gh}	17.13±0.44^{f-i}	12.47±0.44^{ghi}	72.80±0.72^{fgh}
Salicylic acid	9.47 ± 0.48^{j-m}	83.84±0.78^{a-d}	1.29±0.04^{bc}	20.40±0.31^{abc}	16.57±0.41^{ab}	81.23±0.84^{ab}
Tetraconazole	5.40 ± 0.53^{m}	90.78±0.61^{a}	1.51±0.07^{a}	21.40±0.40^{a}	17.77±0.30^{a}	83.04±1.10^{a}
Control	58.60 ± 2.80^{a}	0.00±0.00^{m}	0.92±0.03^{h}	15.73±0.48^{i}	11.14±0.59^{i}	70.82±1.75^{h}

Values followed by the same letters in the same column are not significantly different (P< 0.05).

Values are means of three replications ± SE.

Tetraconazole, Ext.3+SA, Ext.3, Ext.6+SA, Ext.7+SA and SA gave the highest root weight. T.S.S, sucrose contents were the highest in plants root sprayed with tetraconazole, Ext.6+SA, SA and Ext.3. High negative correlation between disease severity % and both of root weight (r^2 = 0.640**) and sucrose % (r^2 = 0.794**) as shown in Figs. 1 and 2.

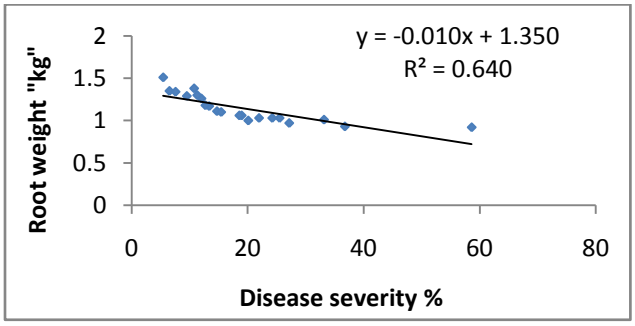

Figure 1: Relationship between CLS severity % and root weight of sugar beet as affected by different treatments

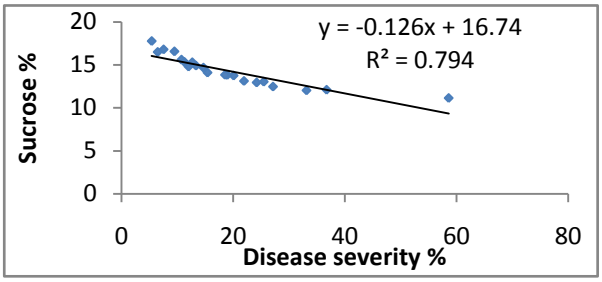

Figure 2: Relationship between CLS severity % and sucrose % of sugar beet as affected by tested treatments

4.4. Effect of tested treatments on biochemical components of sugar beet leaves

Data in Table 5 show that spray treatments with SA exhibited the highest amounts of total and *ortho*-dihydroxy phenols (2.72 and 1.27 mg/g fresh weight) followed by Ext.6+SA, Ext.3 and tetraconazole. While Ext.9 gave the lowest amount of phenolic compounds (0.81 and 0.52 mg/g fresh weight for total and *ortho*-dihydroxy phenols, respectively) compared with other treatments. Other treatments varied in between. The correlation coefficient between disease severity % and total phenols was highly significant (r^2 = 0.766**) as shown in Fig. (3). Disease severity % of CLS and *ortho*-dihydroxy phenols had a high negative correlation (r^2 =0.713**) as shown in Fig. (4).

Table 5: Impact of different treatments in increasing biochemical components on sugar beet leaves

Treatments	Total phenol (mg/g f w)	*Ortho*-dihydroxy phenol (mg/g f w)	Polyphenol oxidase activity (ΔA/min/g f w)	Amino acids (mg/g f w)
Ext. 1	1.32±0.12^{ij}	0.65±0.18^{hi}	0.49±0.01^{hi}	5.29 ±0.22^{hi}
Ext.2	1.71±0.07^{gh}	0.88±0.05^{ef}	0.79±0.01^{bcd}	7.07±0.20^{d}
Ext. 3	2.44±0.07^{bc}	1.23±0.05^{ab}	0.83±0.03^{abc}	9.62±0.28^{a}
Ext.4	1.74±0.11^{fgh}	0.67±0.03^{hi}	0.56±0.03^{fg}	6.10±0.17^{efg}
Ext.5	2.03±0.09^{de}	0.86±0.08^{ef}	0.78±0.02^{bcd}	7.73±0.18^{cd}
Ext.6	1.30±0.12^{ij}	0.72±0.02^{gh}	0.52±0.02^{gh}	5.66±0.21^{ghi}
Ext.7	1.91±0.07^{efg}	0.86±0.03^{ef}	0.77±0.02^{cd}	7.27±0.12^{cd}
Ext.8	1.76±0.05^{fgh}	0.73±0.04^{gh}	0.68±0.01^{e}	6.87±0.25^{de}
Ext.9	0.81±0.07^{l}	0.52±0.02^{jk}	0.37±0.02^{j}	4.04±0.24^{j}
Ext.1+SA	1.52±0.13^{hi}	0.73±0.03^{gh}	0.53±0.02^{gh}	6.02±0.11^{fgh}
Ext.2+ SA	1.74±0.06^{fgh}	0.80±0.01^{fg}	0.61±0.03^{f}	6.95±0.17^{d}
Ext.3+ SA	1.93±0.09^{d-g}	1.05±0.02^{cd}	0.82±0.01^{abc}	8.61±0.46^{b}
Ext.4+ SA	1.02±0.08^{kl}	0.59±0.03^{ij}	0.44±0.03^{i}	5.41±0.70^{ghi}
Ext.5+ SA	1.72±0.08^{fgh}	0.85±0.04^{ef}	0.78±0.02^{bcd}	6.79±0.21^{def}
Ext.6+ SA	2.50±0.08^{ab}	1.15±0.04^{bc}	0.84±0.04^{ab}	9.53±0.21^{a}
Ext.7+ SA	1.98±0.11^{def}	0.94±0.02^{de}	0.79±0.02^{bcd}	7.96±0.21^{bc}
Ext.8+ SA	1.78±0.07^{efg}	0.87±0.02^{ef}	0.75±0.03^{d}	7.11±0.25^{d}
Ext.9+ SA	1.17±0.03^{jk}	0.68±0.09^{ghi}	0.50±0.01^{ghi}	5.05±0.33^{i}
Salicylic acid (SA)	2.72±0.14^{a}	1.27±0.03^{a}	0.89±0.02^{a}	9.94±0.21^{a}
Tetraconazole	2.18±0.06^{cd}	1.05±0.04^{cd}	0.83±0.02^{abc}	8.24±0.21^{b}
Control	0.77±0.05^{l}	0.47±0.04^{k}	0.35±0.01^{j}	3.92±0.08^{j}

Values followed by the same letters in the same column are not significantly different (P< 0.05) according to Duncan's multiple range lest. Values are means of three replications ± SE.

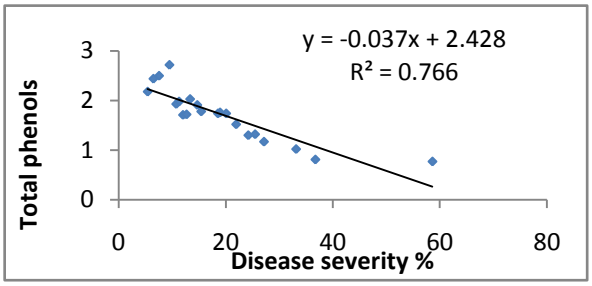

Figure 3: Relationship between CLS disease severity % and total phenols of sugar beet as affected by different treatments

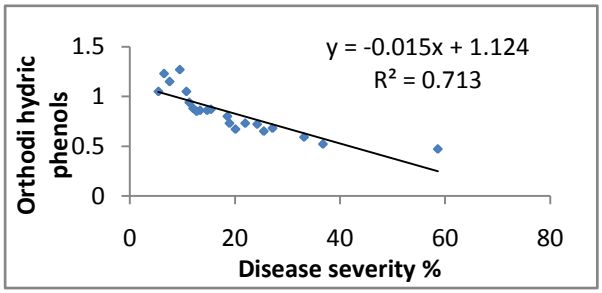

Figure 4: Relationship between CLS disease severity % and ortho-dihydroxy phenol of sugar beet as affected by different treatments

Polyphenol oxidase activity of sugar beet leaves after treatment are listed in Table 5. A significant increase in polyphenol oxidase activity in the plants treated with any of the tested treatments compared with the control. SA, Ext.6+SA, Ext.3, tetraconazole and Ext.3+SA treatments recorded the highest level of enzyme activity in sugar beet leaves. While Ext.9 was the lowest in this respect. A highly significant negative correlation ($r^2 = 0.818^{**}$) was found between CLS severity and polyphenol oxidase activity (Fig.5).

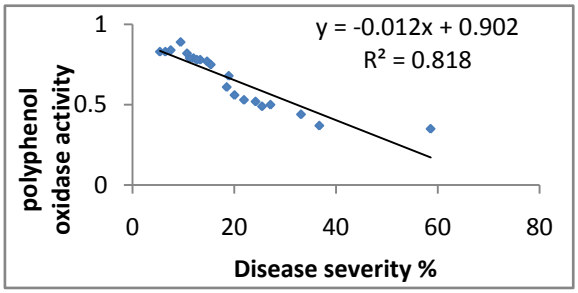

Figure 5: Relationship between CLS severity% and polyphenol oxidase activity of sugar beet as affected by different treatments

Total amino acids of sugar beet leaves are presented in Table 5. The result showed that salicylic acid, Ext.3 and Ext.6+SA recorded the highest total free amino acid (9.94, 9.62 and 9.53mg / g fresh weight, respectively) as compared to other treatments and control. While spray treatment with Ext.9 produced the lowest amino acids when compared with other treatments. There was highly significant negative relationship between total free amino acids and CLS severity ($r^2 = 0.746^{**}$) in sugar beet leaves (Fig.6).

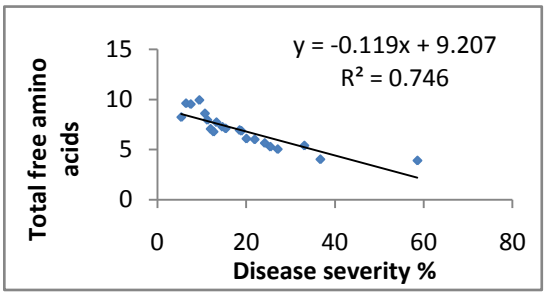

Figure 6: Relationship between CLS severity % and total free amino acids of sugar beet as affected by different treatments

5. Discussion

The assessment of *in vitro* antifungal effect showed that all the tested plant extracts at different concentration levels cause inhibition of *C. beticola* growth. It was shown that salicylic acid alone or in combination with *n*-butanol extract of stem bark, methanolic extract of flowers and ethyl acetate extracts of leaves and stem bark were the most effective against CLS.

In vivo experiments informed us that *n*-butanol extract of stem bark, methanolic extract of flowers and ethyl acetate extract of leaves either alone or was combined with salicylic acid give the best effects in improving roots weight and quality traits.

A highly significant negative correlation was found between CLS severity and all of root weight, sucrose % and biochemical components of sugar beet leaves. The previous research work [7] recorded reduction of root yield and sugar content reached to 30 and 50%, respectively. Similar results were obtained by Kaiser & Varrelmann [10] who stated that yield loss due to CLS disease in susceptible varieties ranged from 10 to 50% in Australia.

Spray treatments with salicylic acid, *n*-butanol extract of stem bark and methanolic extract of flowers exhibited the highest amounts of phenols (total and *ortho*-dihydroxy), polyphenol oxidase activity and total free amino acids.

Total and *ortho*-dihydroxy phenols are two components that induce resistance in plants against pathogens, phenols is oxidized to highly toxic *ortho*-dihydroxy phenols by enzymatic action (polyphenol oxidase) and its concentration is highly correlated with low disease susceptibility to *Gloesporium ampelophagum* of grape [37]; *Peronospora*

plantaginis of isabgol [27] *C. beticola* of sugar beet [38]. A decreasing phenol content in mustard was correlated with its increased susceptibility to white rust [39]. According to Matern and Kneusal [40], the first step of the defense mechanism in plants involves a rapid accumulation of phenols at the infection site, which act as mobilized defense system can be translocated by plants and enzymatically converted into defensive substance at the site of attack. Several results have been reported on the role of oxidative enzymes during plant infection by fungal pathogens. Polyphenol oxidase, involved in formation of melanin compounds in the necrosed tissues [41]**.**

There was highly significant negative relationship between total free amino acids and CLS severity ($r^2 = 0.746^{**}$) in sugar beet leaves (Fig.6). Plant amino acid biosynthetic pathways lead to the production of various secondary product that function as growth regulators, in defense pathogens and other environmental stresses, and as structural components [38, 42-46]. The decrease of amino acids content may be either due to the utilization by the pathogen enzymatic degradation or have been utilized by the host plant for the defense mechanism [47]**.**

The different extracts of *Cassia nodosa* were tested to evaluate the anti-fungal activity as it was expected for this plant. The chemical components of different plant organ extracts may contribute to its antifungal activity. It was reported that stem bark of *Cassia nodosa* contains anthraquinones (rhein and chrysophanol) [48]; saponins [49]; flavonoids (kaempferol and quercetin) [48] and fatty acids [50].

Cassia nodosa flowers composed chemically of anthraquinones (rhein and nodososide) [51]; flavonoids (quercetin rhamnoside & kaempferol glucoside) [52, 53]; isoflavones [52] and fatty acids [54].

Leaves contains flavonoids (quercetin arabinoside and kaempferol rhamnoside) [55]**.** Most of the previously mentioned pure constituents were reported to possess antifungal activity [56-60].

6. Conclusion

The different experiments of *in vitro* and *in vivo* types were revealed that we could consider the use of stem bark *n*-butanol extract of *Cassia nodosa* as antifungal agent for cultivated plants as it was the most effective extract. It was also observed that methanolic extract of flowers and ethyl acetate extract of stem bark either alone or in combination with salicylic acid were the most effective treatments. Salicylic acid combined with flowers methanolic or leaves ethyl acetate or stem bark ethyl acetate and *n*-butanol extracts demonstrated a synergistic effect. A highly significant negative correlation was found between CLS severity % and all of root weight, sucrose % and biochemical components of sugar beet leaves.

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