The Inhibitory Effect of *Haloxylon, Cichorium spp* and *Capparis spp* on Cercospora leaf spot disease on sugar beet *in vitro* and *vivo*

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**Research Article**

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Abstract

Sugar beet (Beta vulgaris L.) is one of the most important sugar crops in the world, which considered the second important sugar crop in Egypt after sugar cane. In Egypt, sugar beet is attack by Cercospora leaf spot disease caused by pathogen Cercospora beticola fungal, which caused losses can reach to 40% of yield. In the present study, three plant extracts of Flower of Haloxylon spp, Cichorium spp, and the leaf of Capparis spp were used. Different concentration of the three plant extracts (10%, 20%, 30%, 35% and 40%) previously were used in experiment in vitro to determine Antifungal activity on the linear growth of Cercospora beticola. While in vivo, the concentration which recorded highest effect against Cercospora beticola in vivo were tested. In vivo experiment conducted in two treatments, the first one sprayed sugar beet plants by plant extracts before the inoculation and the second treatment sprayed plant extracts after the inoculation. Experimental result show under laboratory, all tested plant extracts at concentrations (30, 35 and 40%) showed highly effective on mycelial growth of Cercospora beticola and inhibited fungal growth compared to the control treatment and (10 and 20%) concentration. While in vivo result show that the treated sugar beet plants with plant extracts before inoculated then by Cercospora beticola give the best result for reduction the disease severity of CLS disease comparing with the control. In addition to result revealed that the treated sugar beet plants with tested extracts after inoculated by Cercospora beticola gave the best result for value total of chlorophyll a and chlorophyll b which recorded the highest value comparing with the control treatment.

Introduction

Sugar beet (Beta vulgaris L.) is belonging to family Chenopodiaceae. It is a biennial plant and one of the most important sugar crops in the world (Eweis et al., 2006), which considered the second important sugar crop in Egypt and all over the world after sugar cane. Sugar beet covers approximately 35% of the global needs of sugar, where global production of sugar beet in 2019 amounted to 278.5 million ton (FAOSTAT, 2020). In Egypt, sugar beet is one of the most important crops for sugar production and it was ranked as the second crop after the sugar cane for sugar production (Memon et al., 2004 and Eweis et al., 2006). The total production of sugar beet in Egypt from 2010 to 2019 was about 7.84 to 10.53 million tons (Wu et al., 2013 and FAOSTAT, 2020). In Egypt sugar beet is sowing from September to November and harvest from March to April. The area from 1984 to 2016 has increased by about 90% from 152 hectares in season 2011 to 211.81 hectares in 2014 (FAO, 2019). However, area was increasing from 56,984 hectares in 2000 to 207,527 ha in 2019 by an average annual rate of 7.56% (FAOSTAT, 2020). Under seasonal climate conditions in Egypt, sugar beet crop is attack with several pathogenic fungi causing serious diseases i.e., CLS disease caused by pathogen Cercospora beticola, where it is considered the most important and most destructive foliar disease of sugar beet worldwide. The fungus belonging to the kingdom Fungi, order Capnodiales, Family Mycosphaerellaceae (Chupp, 1954 and Skaracis et al., 2010) and considered the primary leaf pathogen of sugar beets, (Bleiholder and Weltzien, 1972 and Holtschulte, 2000). It can cause serious yield losses in the absence of treatments, ranging from 25 to 50% (Byford, 1996). In Egypt, sugar beet under field conditions, is infecting by Cercospora beticola Sacc; Crop losses of sugar beet can reach 40% and result in complete yield loss in the absence of fungicide treatments (El-Kholi, 2000 and Rossi et al., 2000).

In the last few decades, it's one of the most important applications and perceived to increase crop productivity through the provision of accurate, targeted, and up-to-date crop information, as well as reduce costs of disease control and environmental contamination associated with excessive pesticide applications (Laudien et al., 2004 and Barbedo, 2013). Unwise human activities in using fungicides to protect and treatment of Cercospora leaf
spot (CLS) in sugar beet are considered the second problem due to cause environmental pollution. Therefore, it must be applying a plan to control and treat CLS disease and reduce pesticides to the lowest level to preserve the environment, as well as biological diversity, which is affected very significantly as result of the use of pesticides. Therefore, using of compounds resulting from natural plants was one of the most important things that should be used due to good effect in preserving the environment and as one of the alternative means for pesticides. *Haloxylon* spp *Cichorium* spp and *Capparis* spp, it is considered a wild plant, which have adaptation under water limiting, nutrient poor and environmental conditions of arid regions. These plants were used as a feed, medicine and in restoration of degraded arid lands. And it has been many beneficial effects on fungi and bacteria growth (Eddouks et al., 2004 and Levizou et al., 2004). These plants have been reported as a potential source to restrain bacterial growth. extracts of these plant were reported to be effective to inhibit the growth of wide range of microorganisms (Perez et al., 2005; Ilic et al., 2004 and Mandalari et al., 2007). the main goal of this research is to evaluate the effect of the *Haloxylon* spp, *Cichorium* and *Capparis* spp extracts on the linear growth of *Cercospora beticola* and evaluate the efficacy extracts in control CLS disease.

**Materials And Methods**

**Collection of plant material**

The fresh healthy leaves and flowers of three wild plants *Haloxylon* spp, *Cichorium* spp flowers, and *Capparis* spp leaves were collected between May and August 2019 from different locations in Egypt, South Sinai governorates (Nuweiba, Sant-Katrina, and Dahab), each plant was labeled and notes with date and location of collections. Plants were identified at Botany of the Faculty of Science at Suez Canal University.

**Preparation of extracts**

Leaves and flowers of collected plants were dried at room temperature for 7 to 10 days under lab conditions. 50 g. of the grounded plant was extracts in a Soxhlet using 300mL of methanol solvent for 72 hours at a temperature that does not exceed the boiling point of the solvent, then extracts was filtered using filter paper. Concentrated and removed alcohol in a vacuum using a rotary flask evaporator. The extracts were kept in black, brown bottles at 4°C until use.

- **Antifungal activity of three extracts on the linear growth of Cercospora beticola**

*In vitro*, three extracts, flowers of *Haloxylon* spp, *Cichorium* spp and leaves of *Capparis* spp were evaluated their effect on the linear growth of *Cercospora beticola*. The original extracts were considered as 100% concentration, six concentration of each test extracts were prepared by adding 1,2,3,3.5 and 4 mL to 90 mL of PDA media to obtain 10%,20%,30%,35% and 40% previously. The extracts were added to conical flasks containing sterilized PDA before solidification to obtain the proposed concentrations of 10%,20%,30%,35% and 40%. Twenty mL of amended media were poured into 9cm diameter petri dishes for each treatment, 3 replicates were used. All plates were inoculated individually with 0.5 cm diameter disc of *Cercospora beticola* then incubated at 25 ± 2°C the control plates were kept without any extracts. The linear growth of fungi was measured each two days, after inoculation. All plates were arranged in a complete randomized design. Mycelial growth reduction is taken as growth of fungi as percentage of growth on PDA according to the equation of Abo-Elyousr et al.,(2014) as the following equation
Where \( R = \frac{C-B}{C} \times 100 \) Where \( R \) = % of growth inhibition, \( C \) = growth in the control, and \( B \) = growth in the treatment.

Extracts were rated for their inhibitory effects using the scale described by Sangoyoni,(2004) as followed:

1. \( \leq 0\% \) inhibition not effective
2. >0-20 inhibition slightly effective
3. >20-50 inhibition effective
4. >50<100 effective and 100% inhibition highly effective

**Efficacy of three extracts on the control of CLS in vivo.**

The experiment was carried out at the experiment of farm of the faculty of Agriculture at Suez Canal University in Ismailia, in pots. The experiment was conducted to evaluate the efficacy of the tested three extracts flower of *Haloxylon spp*, *Cichorium spp* and leaves of *Capparis spp* and one fungicide (Score [difenoconazole]) in controlling CLS disease caused by *Cercospora beticola* using sugar beet variety Misbal (poly). All compounds were applied at the recommended dose with four replicates for each treatment. Forty-eight pots (50cm³ in diameter) contain a mixture of clay sandy soil by rate (1:1) were cultivated with five seeds from variety Misbal (poly). All plants were watered as necessary and fertilized weekly with NPK, plants after reaching growth stage GS16 (60 days after sowing) were divided into four group as follow:

The first group, consisting of twelve pots plants was inoculated with *Cercospora beticola* by rate (3\( \times \)10 spore/mL) as mentioned before. Ten days from inoculation plants were treated with extracts 100 ml/L (each extract was sprayed individually over sugar beet plants with 4 replicates).

The second group, consisting of twelve pots, sugar beet plants were sprayed with extracts 100 ml/L (each extract was sprayed individually over sugar beet plants with 4 replicates). Then after ten days sugar beet plants were inoculated with *Cercospora beticola* by rate (3\( \times \)10 spore/mL) as mentioned before.

The third group, consisting of twelve pots, sugar beet plants were inoculated with *Cercospora beticola* by rate (3\( \times \)10 spore/mL) then after ten days, plant were treated with fungicide Score.

The fourth group, consisting of twelve pots, sugar beet plants were used as control treatment. these pots were watered at regular intervals and the other agriculture practices were routinely performed.

The experiment was laid out in Randomized Complete Block Design (RCBD) with four replications. DS percentages were recorded each four days. The Efficacy of each treatment in reducing CLS severity % was calculated using the following formula Abd El-Nasser et al.,(2017):

\[
\text{Efficacy\%} = \left( \frac{DSC-DST}{DSC} \right) \times 100
\]

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

**Determination of photosynthetic pigment**
The pigments chlorophyll a and b were determined in green leaves from all treatments. Half gram of fresh leaves ground in 10 mL of 85% aceton with calcium then centrifuged at 3000 rpm for 5 min. The optical density of sample was then measured against blank at wavelength of 662,644 and 440.5 nm. The concentration of chlorophyll a and b were calculated as follows:

chlorophyll a = \((9.784 \times E_{662}) - 0.99 \times E_{644}\) = mg 100\(^{-1}\) FW

chlorophyll b = \((21.426 \times E_{644}) - 4.65 \times E_{662}\) = mg 100\(^{-1}\) FW

E = optical density at wavelength indicated (Amon, 1967) total chlorophyll were calculated as sum of chlorophyll a and chlorophyll b.

**Result**

**Efficacy of three extracts on linear growth of** Cercospora beticola **in vitro.**

Antifungal activity of three extracts of *Haloxylon spp*, *Cichorium spp*, and *Capparis spp* were evaluated under laboratory conditions on mycelial growth of *Cercospora beticola* at different concentrations of 0, 1, 2, 3, 3.5 and 4 (v/v). Data in Table (1) show that all tested extracts differed significantly in reducing the mycelial growth of *Cercospora beticola* as compared to the control treatment. *Haloxylon spp* and *Cichorium spp* were the most effective in reducing mycelial growth being 61.97% and 61.87% at concentration respectively followed by *Capparis spp* being 14.65%. On the other hand, from concentration 3 mL / (v/v) and above from all extracts were effective to reduce the mycelial growth of *Cercospora beticola* by a 100% reduction. According to the rate of extracts for their inhibitor effects using the scale, to determine the efficacy %, all extracts inhibited fungal growth of fungi *Cercospora beticola* in concentrations 3, 3.5 and 4 mL and were highly effective against pathogenic.

**Table 1**

<table>
<thead>
<tr>
<th>The concentration of extracts</th>
<th>% Reduction mycelial linear growth of C. beticola</th>
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<tbody>
<tr>
<td></td>
<td>Haloxylon spp</td>
</tr>
<tr>
<td>0%</td>
<td>0.00</td>
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<tr>
<td>10%</td>
<td>10.48</td>
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<tr>
<td>20%</td>
<td>61.97</td>
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<tr>
<td>30%</td>
<td>100</td>
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<td>35%</td>
<td>100</td>
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<td>40%</td>
<td>100</td>
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</tbody>
</table>

**Efficacy of extracts on the control of Cercospora leaf spot** in vivo.

The experiment was conducted to evaluate the efficacy of the tested three extracts flower of *Haloxylon spp*, *Cichorium spp* and leaves of *Capparis spp* and fungicide (Score [difenoconazole]) in controlling CLS disease caused by *Cercospora beticola* and control using sugar beet variety Misbal (poly) planted after reaching growth stage GS16 (60 days after sowing) were divided into four groups in Table (2) show that all the tested extracts are
significantly reduced the disease severity percentage of CLS in both treatments (before and after inoculation of *Cercospora beticola*), but the treated sugar beet plants with tested extracts before inoculated then by *Cercospora beticola* give the best result for reduction the disease severity of CLS disease comparing with the control the most effective extracts is *Haloxylon* spp give the highest reduction of CLS disease in both treatments before and after by rate (95.93%, 70.13%) respectively. Whereas *Capparis spp* and *Cichorium spp* are also effective to reduce the CLS disease when treated the sugar beet plants before inoculating with *Cercospora beticola* by rate (94.18%, 92.96%) respectively. While, when treated after inoculation with *Cercospora beticola* the reduction of CLS disease is (60.20%, 50.90%) respectively. Comparing with control data in the same table show that fungicide (Score® 50cm³ /100L) is very effective to reduce the CLS disease in both treatments (before and after) it gave 95.24% reduction of the CLS disease compared with all tested extracts and control.

<table>
<thead>
<tr>
<th>Table 2</th>
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<tbody>
<tr>
<td><strong>Effect of extracts and Score fungicide on CLS disease of sugar beet plants</strong></td>
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<tr>
<td><strong>Extracts</strong></td>
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<td></td>
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<tr>
<td><em>Haloxylon</em> spp</td>
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<tr>
<td><em>Cichorium</em> spp</td>
</tr>
<tr>
<td><em>Capparis</em> spp</td>
</tr>
<tr>
<td>Score®</td>
</tr>
<tr>
<td>Control</td>
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</tbody>
</table>

**Effect of extracts and fungicide treatment on leaf pigment content chlorophyll a and b**

Data in the Table (3) show that all the tested extracts are significantly affected in chlorophyll a and chlorophyll b in both treatments (before and after inoculation of *Cercospora beticola*), but the treated sugar beet plants with tested extracts after inoculated by *Cercospora beticola* gave the best result for value total of chlorophyll a and chlorophyll b which recorded the highest value comparing with the control treatment. the highest value of total of chlorophyll a and chlorophyll b was in *Haloxylon* spp give the highest value in both treatments before and after by rate (0.391, 0.475 mg/g) respectively. Whereas *Capparis* spp and *Cichorium* spp are also affected the total of chlorophyll a and chlorophyll b when treated the sugar beet plants before inoculating with *Cercospora beticola* by rate (0.376, 0.414 mg/g) respectively. While, when treated after inoculation with *Cercospora beticola* the total of chlorophyll a and chlorophyll b (0.432, 0.434 mg/g) respectively. Comparing with control data in the same table showed that fungicide (Score® 50cm³ /100L) is very effective in the total of chlorophyll a and chlorophyll b in both treatment (before and after) it gives 0.429 mg/g compared with all tested extracts and control.
Discussion

Evaluating the efficiency of three extracts, the flower of *Haloxylon spp*, *Cichorium spp* and leaves of *Capparis spp* with six concentrations (0, 10, 20, 30, 35 and 40% under laboratory conditions on mycelial growth of *Cercospora beticola* was done. All extracts inhibited fungal growth of *Cercospora beticola* with the concentrations 30, 35 and 40% and were highly effective against *Cercospora beticola* and gave highly significant decreasing and have antifungal activities. This is in agreement with Ilic et al., (2004) and Mandalari et al., (2007). They found the antimicrobial potential of different parts of *Capparis spp* and exhibited their ability to inhibit the growth of microorganisms. Mali et al., (2004) ; Hong et al., (2006) and Ibrahim, (2012) studied antifungal activities of *Capparis spp* extracts and found *Capparis spp* extracts have different degrees of antifungal activity against the tested fungi. Tlili et al., 2011, Asl et al., 2012 reported that *Capparis spp* demonstrated significant resistance to different biotic and abiotic stresses. Al-Askar, (2012) found the antifungal activities of ethanolic extracts of three Saudi plants, camel thorn *Capparis spp* was investigated in vitro against *Alternaria alternata, Fusarium oxysporum, Phoma destructiva, Rhizoctonia solani, and Sclerotium rolfsii*. These results are agreement with Cavin et al., (2005); Mulabagal et al.,(2009) reported that the bioactive phytochemicals described in *Cichorium spp*. Ferioli et al., (2015) and Papetti et al.,(2017) were tested for their antimicrobial effect against a panel of microorganisms. Results offer insights into the antimicrobial potency of this Egyptian local plant and provide a basis for further phytochemical and pharmacological research. For *Haloxylon spp* Aldoweriej, (2016) mentioned that bio-active properties, *Haloxylon spp* was used in the treatment bacterial infection. Brantner et al., (1996) mentioned that the common herbs contain phenols, which are effective against bacteria and fungi where their extracts were toxic to microorganism. In vivo all the tested extracts are significantly to reduce the disease severity percentage of CLS in both treatments (before and after inoculation of *Cercospora beticola*),but the treated sugar beet plants with tested extracts before inoculation by *Cercospora beticola* gave the best result for decreasing the disease severity of CLS disease compared with the control. The useful of performance of extracts as an effective control measure against fungi and their plant diseases was mainly interpreted on the presence of active substance and or change in biochemical compound in plant tissues that agreement with Matsuyama et al., (2009) and Tlili et al., (2010) who found that the chemical composition of different extracts from *Capparis spp* have reported the presence of numerous beneficial compounds such as polyphenols, flavonoids and vitamins, that known for their antioxidant properties. However ,Gull et al.,(2015) reported that *Capparis spp* possesses different

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Chlorophyll(a) Before Inoculation</th>
<th>Chlorophyll(b) Before Inoculation</th>
<th>Total Chlorophyll (a + b) Before Inoculation</th>
<th>Chlorophyll(a) After Inoculation</th>
<th>Chlorophyll(b) After Inoculation</th>
<th>Total Chlorophyll (a + b) After Inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cichorium</em></td>
<td>0.241</td>
<td>0.172</td>
<td>0.414</td>
<td>0.269</td>
<td>0.165</td>
<td>0.434</td>
</tr>
<tr>
<td><em>Haloxylon</em></td>
<td>0.244</td>
<td>0.147</td>
<td>0.391</td>
<td>0.286</td>
<td>0.189</td>
<td>0.475</td>
</tr>
<tr>
<td><em>Capparis</em></td>
<td>0.229</td>
<td>0.146</td>
<td>0.376</td>
<td>0.252</td>
<td>0.179</td>
<td>0.432</td>
</tr>
<tr>
<td>Score®</td>
<td>0.265</td>
<td>0.163</td>
<td>0.429</td>
<td>0.265</td>
<td>0.163</td>
<td>0.429</td>
</tr>
<tr>
<td>Control</td>
<td>0.207</td>
<td>0.109</td>
<td>0.316</td>
<td>0.207</td>
<td>0.109</td>
<td>0.316</td>
</tr>
</tbody>
</table>

Table 3

Effect of extracts and fungicide on leaves pigment concentration on CLS disease of sugar beet plants.
pharmacological effects including antioxidant effects. Chemical studies have reported that alkaloids, lipids, polyphenols, flavonoids, indole, and glucosinolates were isolated from *Capparis* spp extracts (Tlili et al., 2010). The richness of the *Capparis* spp plant with the total phenolic compounds, rutin, tocopherols, carotenoids, and vitamin C could be the main factor in its antimicrobial effects (Mahbouib et al., 2013). However, Sathiyamoorthy et al., (1999) mentioned that the phytochemical analysis of the aerial parts of *Haloxylon* showed the presence of alkaloids, sterols, cardiac glycosides tannins, volatile bases, and volatile oils. This is agreement with .Brantner and Grein,(1994) who reported *Haloxylon* contains phenolic compounds, which was isolated and identified as an antibacterial substance. Phenyl propane-derives compounds that are in the highest oxidation state, two types of alkaloids compounds of *Haloxylon* have been isolated. Abbas et al.,(2008) showed the exhibited importance medicinal of the extract of *Haloxylon* plant through antimicrobial activity. Microbs showed a variable susceptibility for different concentrations of *Haloxylon* extracts. Some of the simplest bioactive phytochemicals consist of a single substituted *Haloxylon* ring which is in the highest oxidation state. Brantner et al.,(1996) reported that the common *Haloxylon* contain phenols, which are an effective against bacteria and fungi *Haloxylon* extracts shown toxic to microorganism. Ferioli et al., (2015) and Papetti et al.,(2017). reported bioactivities have been described in *Cichorium* spp hydroxycinnamic acids, flavonoids, anthocyanins and coumarins and showed that the discovery of novel and more efficient antimicrobial agents from natural sources like plants is one of the most important ways through which the growing threat of antibiotic-resistant pathogens can be overcome. Amer, (2018) reports the potential antimicrobial activity of *Cichorium* spp. Abbas et al.,(2008) illustration the mechanisms antimicrobial activity of the phenolic extract to be responsible for phenolic toxicity to microorganisms including enzyme inhibition by the oxidized compound, possible reaction with sulphydryal groups or through more nonspecific interaction with the proteins. It can be concluded that these extracts have antifungal activities that might be due to the damage structure and function of fungi enzymatic bioactivity and the fungal growth damages caused by extracts might be due to their capabilities to penetrate fungi cells and the phenolic compounds have toxic effects. That agreement with Voragen ,(2005). Several studies have reported that phenolic compounds are mainly responsible for the antimicrobial properties ,and promising phenolic compounds to control fungi by affecting the growth rate and characteristic of the antifungal activity (Dixon and Paiva, 1995; Solecka, 1997 and Helle et al., 1998). Increasing the phenol components surely gave an increase in the capability of the plant to defend against the disease infection process and its development. Where effect of phenolic components in plants acting through effecting in the structure and enhance host resistance by stimulating host defense mechanisms. The results of the present study revealed that chlorophyll a + b was decreased after infection while in all the tested extracts are significantly affected in chlorophyll a and chlorophyll b in both treatments (before and after inoculation of *Cercospora beticola* ) ,but the treated sugar beet plants with tested extracts after inoculating by *Cercospora beticola* gave the best result for value total of chlorophyll a and chlorophyll b which recorded the highest value compared with the control treatment that might to inhibitor effect of extracts on CLS compared to free infection by CLS which led to damage of cellular pigment and degradation pigment in leaves .This is in agreement with Carter and Knapp,(2001); Coops et al., (2003) they reported that the content of leaf pigments change when plants are subjected to CLS that produce chlorotic and necrotic symptoms. Pigment content of sugar beet leaves inoculated with *Cercospora beticola* was decreasing during disease development. Nevertheless, the progress of disease symptoms influenced leaf total chlorophyll content. This effect was decreased in pigment content at high infection rates with symptoms. Due to high variation between sampled drops infected with *Cercospora beticola*, no significant differences in pigment content were reported in their study. However, according to Malthus and Madeira (1993), a slight increase in pigment content has been observed at early infection stages.
Conclusions

The three plants *Haloxylon, Cichorium and Capparis* had a very significant impact on the linear growth of *Cercospora beticola vitro* at high concentration of extracts were led to inhibit the growth of *Cercospora beticola* completely as well as decreasing disease severity of Cercospora Leaf Spot on *vivo* spray on plants either after inoculation or before inoculation.

Declarations

The author who name is listed immediately below certify that they there is no funding and or conflicts of interests /competing interests in the subject matter or material discussed in this manuscript

Author name : Ahmed Ameen Abdullah

References


