

Thermal Imaging as a New Approach for Determining Bio-Thermogenesis of *Cercospora beticola* in Vitro

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Abstract: *Cercospora* leaf spot disease is one of the most diseases causing great losses in sugar beet yield which causes by *Cercospora beticola* fungus. The anti-fungal effective of three plant extracts in growth of *Cercospora beticola* have been determined by the thermal imaging of colony temperature and linear growth curves of the fungus. In vitro study three plant extracts *Haloxylon* spp, *Chicory Cichorium* spp and *Capparis* spp were used with different concentration to inhibit the linear growth of *Cercospora beticola*. All plant extracts with high concentration inhibited fungal growth of *Cercospora beticola* and increasing the colony temperature of *Cercospora beticola* whereas with the low concentration of three plant extracts the colony temperature was lower but relatively higher than the colony temperature of control which not treated with plant extracts. Results revealed that the use of the thermal imaging offers an alternative method to identify the interaction between plant extracts and linear growth of *Cercospora beticola*. Moreover; it could apply this technique as a new innovative approach in plant pathology field.

Keywords: Thermal camera; *Cercospora beticola*, plant extracts, colony temperature

INTRODUCTION

Cercospora leaf spot (CLS) caused by *Cercospora beticola* fungus is one of the most widespread and destructive foliar diseases that were causing a reduction in root weight and sucrose content (Smith and Martin, 1978). Moreover, loss of 30% percent in recoverable sucrose were common under moderate disease conditions (Lamey, 1996). From practical point of view, applying information technology, especially remotesensing techniques could help to detect Bio-Thermogenetic of fungi. Thermography is the science of infrared imaging and highly specialized in capturing of thermal images to determine infrared energy or heat radiated from objectives (Awad *et al.*, 2015). It is a promising technology to conduct a proper inspection more efficiently of raising temperature of buildings, machinery, equipment, or even live bodies such as plant, or fungi for early prediction of problems and maintenance (Awad *et al.*, 2015). Thermal imaging is widely applied in several fields such as veterinary and agriculture as. Usage of thermal camera is easy and can be accurately measured emitting temperature from object without requiring an illumination source when compared to other imaging system. Recently, thermal imagery is a promising alternative technique to conventional methods for measuring respiration rate (Ruminski and Kwasniewska, 2017) and Bio-Thermogenetic which Means heat production, the term is usually applied to microorganisms that produce heat in their environment (Yu-Hua *et al.*, 2010). In this study, we investigated the temperature of *Cercospora beticola* fungus colonies and attempted to Determine Bio-Thermogenetic of *Cercospora beticola* and effect three plant extracts in their colony temperatures. For this study, we investigated the colony temperatures of *Cercospora beticola* isolated from infected leaves of sugar beet.

MATERIALS AND METHODS

Collection of plant material

The fresh healthy leaves and flowers of Three wild plants Bunge (*Haloxylon* spp.), Chicory (*Cichorium* spp.) flowers, and Caper (*Capparis* spp.) leaves were collected between May and August from different location 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 department of botany at the faculty of science at Suez Canal University.

Preparation of plant extracts

Leaves and flowers of collected plants were dried at room temperature for 7 to 10 days without direct sun light. Where 50 gm of the grounded plant was extracted in a Soxhlet using 300ml of methanol solvent for 72 hours at a temperature does not exceed the boiling point of the solvent than extract 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.

Measuring the effect of three plant extracts on the linear growth of *Cercospora beticola* by thermal imagery

Preparing plant extracts and *Cercospora beticola*

Three plant extracts flowers of *Haloxylon* spp. and *Cichorium* spp. and leaves of *Capparis* spp. leaves were used in the present study. Six concentrations for each plant extract were prepared by adding (0, 2, 3, 3.5 and 4 ml) of plant extract solution to 90 ml of PDA (Potato Dextrose Agar) media to prepare 0%, 20%, 30%, 35%, 40%, previously. Zero concentration represents control treatment. After addition of all plant extracts, mycelial discs (5 mm) were taken from the edge of an actively growing PDA culture of *Cercospora beticola* fungi was placed at the centre of the prepared Petri dishes then incubated at 25±2°C.

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Three replicates were used for each treatment of plant extract a mentioned before. All treatments consisted of three replicates, and experiments were repeated two times.

Thermal image data acquisition

Digital thermal images were acquisition using Thermal Imager Ti32 (Fluke Ti32, Thermography, Germany), equipped with a 320×240 pixels microbolometer sensor. Sensitive in the electromagnetic spectrum ranged from $7.5\text{-}13\ \mu\text{m}$ wave lengths with calibration of -10.0°C to 600.0°C and 9 Hz image speed data. The camera has ability capture and creates an image of an object by using infrared radiation emitted from the object in a process that is called thermal imaging. The created image represents the temperature of the object. Digital thermal images were acquisition at the same time as the linear growth of the fungi is measured. This camera was using to measure temperature emitting from the colony of fungi in the target Petri dish which treatment by three plant extracts. For more accuracy, the span of the auto-adjusted thermal image is manually set, in addition to the level of the displayed as an important camera feature to detect maximum and minimum temperature of the entire display. A thermal camera was kept at a constant distance 50cm from lens of camera to the target Petri dish. Four scans for each Petri dish replicates were acquired to obtain the average temperature. During thermal imaging capture humidity and air temperatures in the laboratory were periodically measured using Humidity and Temperature Meter Jenway, model 5075, serial No.: 43424, the USA. Smart View®Software was using to convert emitting temperatures to images based on colors of electromagnetic spectrum.

RESULTS AND DISCUSSION

The use of thermal imaging offers an alternative method and an Innovative approach to measure and

determine effects of three-plant extracts at different concentration on the colony temperatures and linear growth. Where Agerskan (1975), Gonzalez and Woods (2004) and Chelladurai *et al.*, (2010) mentioned that thermal imaging is a non-destructive and non-contact infrared sensing technique and allowed to see variations in temperature as the amount of radiation emitted by an object usually increases with temperature. And reported that thermal infrared region is very useful in imaging application that use temperature or heat measurements. Lilleskov (2017) reported that fungal respiration contributes substantially to ecosystem and respiration measurement provides a window into the ecophysiology of fungal.

The results in Fig (1) illustrate the variations of colony temperature of *Cercospora beticola* when treated with plant extracts and without plant extracts (control C) for each tested plant extract. Data in table (1) shows raising of colony temperature of fungus by increasing the concentration of plant extracts. With plant extracts of *Capparis* spp. media temperature without fungus is 32.55°C at 20%, 32.63°C , at 30%, 32.73°C at 35% and 32.85°C at 40% of plant extract. Whereas plant extract plus fungus of *Cercospora beticola* is 34.64°C at 20%, 35.63°C at 30%, 35.63°C at 35% and 35.71°C at 40%. Also, with plant extract of *Cichorium* spp., media temperature without fungus is 33.30°C at 20%, 33.56°C at 30%, 33.75°C at 35% and 33.90°C at 40%. Whereas the temperature with fungus is 35.48 at 20%, 35.45 at 30% and 35% and 35.66 at 40%. With plant extract of *Haloxylon* spp., media temperature without fungus is 33.30 at 20%, 33.46 at 30%, 33.85 at 35% and 33.98% at 40%. Whereas the temperature with fungus is 33.30 at 20%, 35.75 at 30% and 35% and 35.79 at 40% of plant extract.

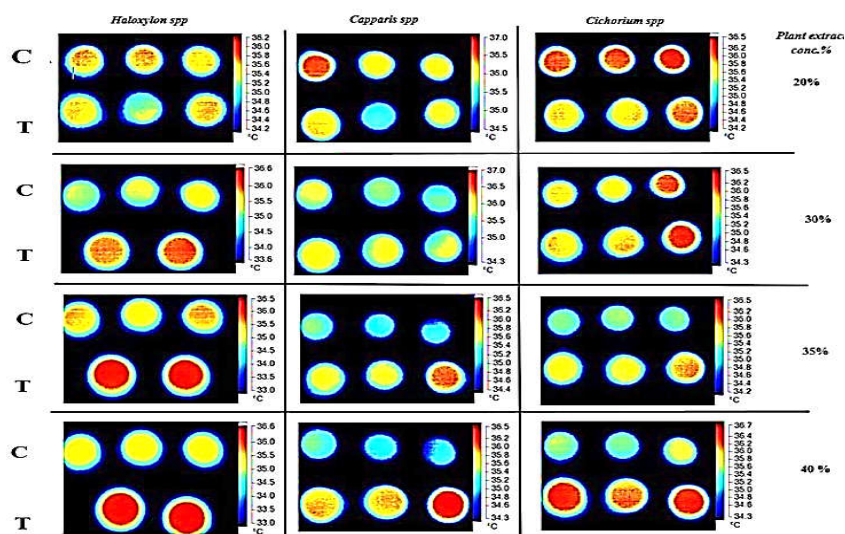


Fig. (1): Thermography of colony of *Cercospora beticola* was control treatment (without plant extracts) T: treatment plant extracts

This may be due to high levels of phenols compounds in media due to the use of high concentrations of plant extracts as this led to the death of fungi and pressure due to presence of these substances' phenolic compounds, which have an effect as antifungal, and affected the breathing of fungi represented in the temperature emission from PDA media. Where in the present study treatment PDA media by plant extracts without fungi colony increasing media temperature with increasing the concentration of plant extracts compared to PDA media only. Where the PDA media only without fungi temperature recorded 33.23°C while media with fungi recorded 33.68°C. That might be Fungi respiration process and metabolism of fungi in media that led to rising colony temperature compared to media without fungi. Using this technique measure and determine effect of plant extracts on fungal activity agreement with Li and Wadsö (2007) mentioned that thermal measurement of fungal activity provides continuous,

quantitative data that can be used for predictive modeling of microbial activity. Thermal power can in principle be measured from all types of samples irrespective of if they are opaque or transparent, liquid, or solid, with surface growth or internal growth. Thermal measurements can be a valuable addition to the measurement techniques for predictive microbiology. Chang-li and Xie (1990) mentioned that thermograms contain enough information about bio-thermogenetic metabolic processes to allow us to calculate the rate constant and activation energy parameters from this thermogenesis. This is very significant for microbiologists and bio-thermochemists.

Nunomura and Fujit (1981) reported that, the patterns of heat production varied with the physiological state of cells, which were influenced by several factors including culture conditions, methods of storage, starvation, temperature, and the suspending medium used for calorimetric measurements.

Table (1): The effect of different concentration of three plant extract on the linear growth of *Cercospora beticola*

Extract	Plant Extracts Concentration									
	0%		20%		30%		35%		40%	
	Media Only	Media with Fungi	Without Fungi	With Fungi	Without Fungi	With Fungi	Without Fungi	With Fungi	Without Fungi	With Fungi
Control	33.23°C	33.68°C	-	-	-	-	-	-	-	-
<i>Capparis</i> spp	-	-	32.55°C	34.46°C	32.63°C	35.63°C	32.73°C	35.63°C	32.85°C	35.71°C
<i>Cichorium</i> spp	-	-	33.3°C	35.48°C	33.56°C	35.54°C	33.75°C	35.54°C	33.9°C	35.66°C
<i>Haloxylon</i> spp	-	-	33.3°C	35.3°C	33.46°C	35.75°C	33.85°C	35.75°C	33.98°C	35.79°C

CONCLUSIONS

Despite using infrared thermal imaging in measuring effective and the interaction of plant extracts in rate linear growth of fungi in vitro is not famous, lack of researchers and research have obstructed being it a valuable tool, but it could help to implement appropriate to Update the views towards in laboratory experiments specialized in the study of innate growth and the impact of plant extracts on growth and breathing rates to keep pace with the rapid development in other areas. Data concluded that thermal imaging has the potential to identify the interaction between three plant extract and rate of linear growth of *Cercospora beticola* fungi in vitro by measurement the change in colony temperature.

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التصوير الحراري كنهج جديد لتحديد التولد الحراري الحيوي في فطر *Cercospora beticola* في المعمل

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مرض تيقع الأوراق السركسيوري هو واحد من أكثر الأمراض التي تسبب خسائر كبيرة في محصول بنجر السكر الذي تسببه فطر *Cercospora beticola*. تم تحديد فعالية مضادات الفطريات لثلاثة مستخلصات نباتية في نمو *Cercospora beticola* من خلال التصوير الحراري لدرجة حرارة المستعمرة ومنحنيات النمو الخطي للفطر. في دراسة المختبر تم استخدام ثلاث مستخلصات نباتية *Haloxylon* و *Cichorium* و *Capparis* بتركيز مختلف لمنع النمو الخطي ل *Cercospora beticola*. جميع المستخلصات النباتية ذات التركيز العالي منعت النمو الفطري لـ *Cercospora beticola* وزادت من درجة حرارة مستعمرة *Cercospora beticola* بينما مع انخفاض تركيز ثلاث مستخلصات نباتية كانت درجة حرارة المستعمرة أقل، ولكنها أعلى نسبياً من درجة حرارة مستعمرة المرجعية التي لم تتم معالجتها بالمستخلصات النباتية. وكشفت النتائج أن استخدام التصوير الحراري يوفر طريقة بديلة لتحديد التفاعل بين المستخلصات النباتية والنمو الخطي لـ *Cercospora beticola* علاوة على ذلك يمكن تطبيق هذه التقنية كنهج مبتكر جديد في مجال أمراض النبات.

الكلمات المفتاحية: التصوير الحراري - *Cercospora beticola* - المستخلصات النباتية - حرارة المستعمرة.