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RESEARCH PAPER

Detection and monitoring of barley net blotch, *Pyrenophora teres*, in Erbil Province

Saya Nahro Shafeeq[1], Qasim Abdullah Marzani[1]
[1] Department of Plant Protection, College of Agricultural Engineering Sciences, Salahaddin University-Erbil, Kurdistan, Iraq.

A B S T R A C T:

The net blotch of barley *Hordeum vulgare,* which is caused by the ascomycete fungus *Pyrenophora teres,* is a significant disease in the province of Erbil and is one of the most severe limitations on barley production worldwide, producing between 10 and 40 percent yield loss. The objective of this work was the surveillance that determines the severity and incidence of net blotch of barley in four locations in the region. The survey was conducted, and the pathogen was isolated and identified then the disease was monitored and measured by the Area Under Disease Progress Curve (AUDPC). The identification results showed that the pathogen, causing net blotch in the province, was *Pyrenophora teres* and the identification was confirmed through specific primers using polymerase chain reaction (PCR). The results showed that the incidence and severity of the disease were variables according to the location. The highest disease severity (43.12%) was in JezhnikanNajim and the incidence (42%) was in SE Quchan while the lowest severity of 9.54 % and the lowest incidence of 12% occurred in Small Bnaslawa at the last of the season. The results of measuring the AUDPC were 283.09 and 946.13 in Bnaslawa and JezhnikanNajim villages, respectively, after 44 days from the planting date, while in Yedi Kizler village and SE Quchan village, the AUDPC reached the highest points after 59 days of the planting date with AUDPC values of 460.56 and 712, respectively.

KEY WORDS: *Barely Net Bloch, Monitoring, Disease severity and incidence, AUDPC*
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1.INTRODUCTION :

Barely *Hordeum vulgare* L. is the second most cultivated cereal crop after wheat. It is one of the oldest cultivated crops, likely domesticated around ten thousand years ago in the Fertile Crescent (Kurdistan) and then spread to the rest of the world (Kumar et al., 2018). Barley is generally cultivated as animal feed and as a source of malt for alcoholic beverages, but it is also extensively used in food and health items, such as bread, soups, and stews. Yet, barley production has numerous biotic and abiotic challenges (McDonald and Linde, 2002, Kuldeep et al., 2008, Liu et al., 2011a). Many diseases caused by fungi, bacteria, and viruses affect the yield and market value of crops can reduce the yearly average output of barley crops by up to 20%(Liu et al., 2011b) Barley leaf blotches are caused by the necrotrophic fungi *Pyrenophora teres f. teres* (Ptt) and *P. teres f. maculata* (Ptm), yield losses(McDonald and Linde, 2002) ranging from 10% to 40% (Liu et al., 2011a).

The ascomycete fungus *Pyrenophora teres* causes the net blotch of barley (NB) (Anamorph *Drechslera teres*) is a prominent barley disease that is the most severe in many parts of the world. The disease causes significant production and quality decreases in mature grain (McLean et al., 2009).

In the 1970s and early 1980s, it was widely spread throughout western Europe, where it caused considerable yield reductions(Skou and Haahr, 1987).In the 1980s, it became an increasingly significant pathogen in the United Kingdom, notably in the southwest(Jordan, 1981). In 2009, net blotch was observed in 86% of examined barley fields in Finland(Jalli et al., 2011).

During the growing period, the fungus *Pyrenophora teres* is capable of both asexual and sexual reproduction and is able to thrive in the stubble. Furthermore, it is a seed-borne pathogen (Duczek et al., 1999). This fungus survives the summer on infected straw debris, which allows the pathogen to persist between seasons and is the

*** Corresponding Author:**
Qasim Abdullah Marzani
E-mail: qasim.marzani@su.edu.krd
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primary source of inoculum(Liu et al., 2011a, Ronen et al., 2019). Successful disease management requires accurate identification and quantification of pathogens, as well as the prompt implementation of control measures. Identifying diseases to the level (i.e., genus, species, or race) that may influence management decisions is beneficial. Monitoring plant diseases is a necessary component of any control scheme. Monitoring and recordkeeping, as well as an understanding of pathogen populations prior to planting, can aid in risk assessment and support crop selection and management decisions(Bockus et al., 2010). Traditional approaches for diagnosing fungal plant diseases focus on the evaluation of visible symptoms and/or isolation, cultivation, and laboratory diagnosis. The precision and dependability of these procedures are heavily dependent on the expertise and experience of the individual providing the diagnosis. Diagnostics requiring cultivation is inconvenient and time-consuming when immediate results are required (McCartney et al., 2003) .Therefore, it is desirable to create a reliable, quick, and sensitive diagnostic approach for early detection of infection with this pathogen'. PCR method has been utilized to rapidly detect, characterize, and identify a vast array of species. The creation of appropriate PCR primers that aid in species-level identification of the pathogen is one of the most vital procedures in PCR diagnostics research.

The severity of a disease is typically stated as a percentage or proportion of plant area or fruit volume damaged by a pathogen. Typically, disease evaluation scales ranging from 0 to 10 or 1 to 4 are employed to describe the relative proportions of afflicted tissue at a given period. At a specific growth stage, disease yield loss is assessed based on progressive disease assessments at various phases of a crop's development, or by computing the area under a disease progress curve (AUDPC), i.e. the area between the disease progress curve and the X axis of the graph. AUDPC is determined using a formula that takes into account the number of times the disease severity was measured, the severity disease at each evaluation period, and the duration of the epidemic(Agrios, 2005). Long have plant-disease epidemiologists been fascinated by the temporal progression of disease, as represented by a disease-progress curve. Straightforward descriptive growth models have frequently been used to characterize the temporal patterns of disease spread. The objective of our study was to identify the barley net blotch pathogen and to track the disease using (AUDPC).

2. Materials and Methods

2.1 Field Survey

Surveys were conducted in four fields located in Erbil province during mid-March to the end of 2022 season. The four locations were Yedi Kizler village (southwest of Erbil, latitude 35.98999, longitude 43.85592), SE Quchan village (South of Erbil, latitude 35.83315 Longitude 44.12675), JezhnikanNajim village (North of Erbil, latitude 36.36192 Longitude 43.9978), and Small bnaslawa village (East of Erbil, latitude 36.15727 longitude 44.14613). In each location, samples were taken randomly following the corners and center method. During March, April, and May, 2022, At regular intervals, disease incidence and severity were monitored by means of measurements and samplings. Using this formula, the incidence of disease was computed (Galano et al., 2008)

$$\text{Disease incidence} = \frac{\text{DP}}{\text{TP}} * 100$$

Where:

DP = Number of diseased plants

TP = Total number of plant samples

The disease was visually evaluated using the proposed numerical reaction scale (1- 10) (Tekauz, 1985).The severity of the disease was then determined and computed using the following formula(Azmy and Korra, 2010).

$$\text{Disease severity} = \frac{\sum(n*k)}{N*K} * 100$$

Where: **n**= number of infected leave, **k**= disease rate, **N**= number of examined leave, and **K**= maximum disease rat

2.2. Disease diagnostics

2.2.1. Isolation and identification of the pathogen

Collected samples of infected leaves of barley were isolated, pure-cultured, and then identified microscopically. The identification was done under light microscope depending on the descriptions in text books and characteristics of the fungus(Brown, 1924) .To validate the identity of the fungal infection, polymerase chain reaction (PCR) was utilized for molecular identification. To accomplish this, DNA was isolated from the fungus cultures using the method described by (Cenis, 1992). PCR amplification for ITS partial gene was done in 50 µl of reaction mixture containing; 2x Taq DNA Polymerase Master Mix (AMPLIQON A/S Stenhuggervej 22), 10 Picomol (pmol) of forward primer LSU

(ACCCGCTGAACTTAAGC), 10 pmol reverse primer LSU (CGCCAGTTCTGCTTACC), DNase free water and template DNA by Bioresearch PTC-200 Gradient thermocycler. Temperature profile included an initial denaturation at 95 C for 5 min, followed by 35 cycles of a denaturation at 95C for 45 second, a primer annealing at 58C for 45 sec., an extension at 72C for 1 min and final step is an extra extension at 72C for 10 min. The PCR products were visualized on gel electrophoresis, then the PCR products were sequenced and the blast was run on NCBI (National Clast was made on enter for Biotechnology (https://blast.ncbi.nlm.nih.gov/Blast.cgi) website for comparing and alignment laboratory or query sequence with other biological sequence to find out more similarity with other targets.

2.3. Disease Monitoring

Area Under the Disease Progression Curve (AUDPC) was computed utilizing the following equation: AUDPC was determined by monitoring net blotch at four places every 15 to 20 days and scoring the disease severity using the method described in section 2.1. (Shaner and Finney, 1976).

$$A.U.D.P.C. = \sum_{i}^{n-1} \{\frac{Yi + Y(i+1)}{2} * (t(i+1) - ti)\}$$

In which *Yi* = disease severity on the *i*th date, ti = *i*th day, *n* = number of dates on which net blotch was recorded.

2.4. Data analysis

Statgraphics centurion XV.I software was used for statistical analysis of variance (ANOVA), and the means were compared using Fischer's least significant difference (LSD) test at a significance level of P (0.05).

3.Results

3.1. Disease diagnostics

The symptoms and microscopic and cultural characteristics were showed that the fungal pathogen was *P. teres* (Figure 1). The mycelia are septate and brown and the conidia have 2-5septa. Furthermore, the PCR using universal primers and NCBI blast have confirmed the identity of the fungus (Figure 2).

Figure 1 Fungi isolates from barely disease plants *(P.teres*),where: the disease symptoms (left) and the fungus mycelia and conidia (right).

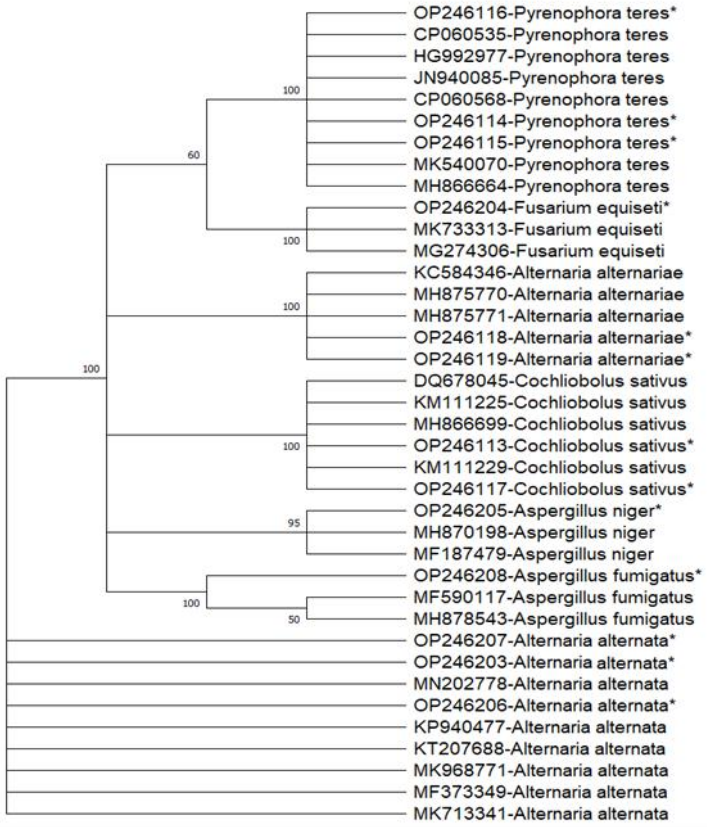

Figure 2: Fungi sp. samples from Iraq: Kurdistan area (Erbil) phylogenetic tree. The phylogenic tree was generated using the Maximum Likelihood technique with the Tamura-Nei model in MEGA11 software and bootstrap analysis with 100 replicates. Partial DNA sequence.

3.2. Disease incidence and severity

The results of disease incidence were revealed that there were variations among the locations (table1). On the other hand, the maximum disease incidences were recorded in Yedi Kizler, SE Quchan and Small bnaslawa village on 29-March-2022 with incidences of 42, 37, and 20%, respectively, then disease was decreased in its progress in April and May in the same locations. However, in Small bnaslawa it is started to decrease in April. The results also showed that the lowest disease incidence were in Small bnaslawa village (1.2%) with significant differences between the locations (table 2).

The disease severities were also varied among locations. The highest disease severity was in JezhnikanNajim (43.12%) then followed by SE Quchan with no significant differences between them while the lowest disease severity was in Small bnaslawa village (9.54 %) with a significant difference with other location (Figure.3).

Table 1 Disease incidence in 4 locations in Erbil Province

Location		Disease incidence (%)			
		17-Mar-22	29-Mar-22	11-Apr-22	5-May-22
1	Yedi Kizler	13%	37%	10%	4%
2	SE Quchan	37%	42%	13.3%	2.2%
3	Jezhnikan Najim	16%	15.2%	31.2%	30 %
4	Small bnaslawa	*……..	20%	5.4%	1.2%

Table (2): ANOVA Table for Dis SEV by location

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	10760	3	3586.67	5.28	0.0021
Within groups	65207.8	96	679.248		
Total (Corr.)	75967.8	99			

*The survey in Bnaslawa started in 29-Mar-2022

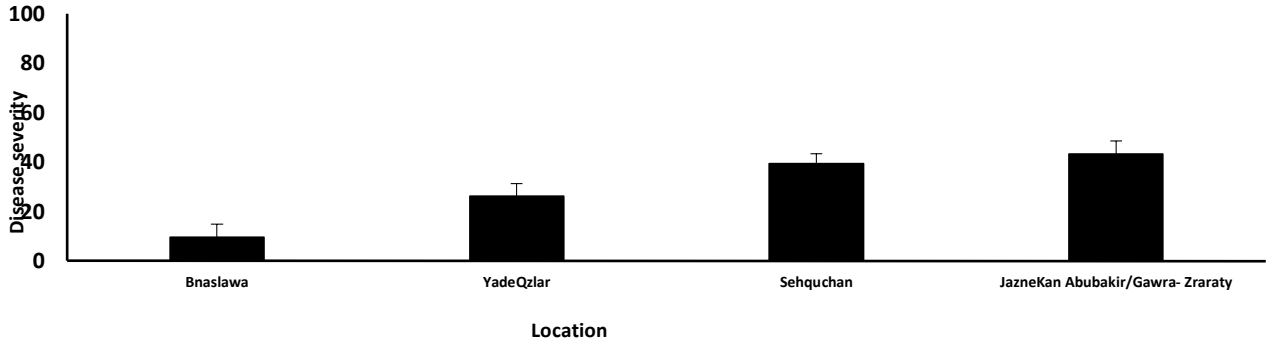

Figure 3 Disease severity of locations in Erbil providence during First of March until 15-May 2022

3.3. Disease Monitoring

Recorded were the findings of monitoring NB from the beginning of the season until the end of the season and calculated through AUDPC. The highest AUDPC were in Small bnaslawa and Jezhnekan Najim at 44 days after of the planting date with AUDPC values of 283.09 and 946.13, respectively (figure 4 c and d) while in Yedi Kizler and SE Quchan the AUDPC were reached to the highest points after 59 days of the planting

date with AUDPC values of 460.56 and 712,respectively (figure 4 a and b).The results also showed after these dates the disease progress curves were declined in all locations until the disease was checked.

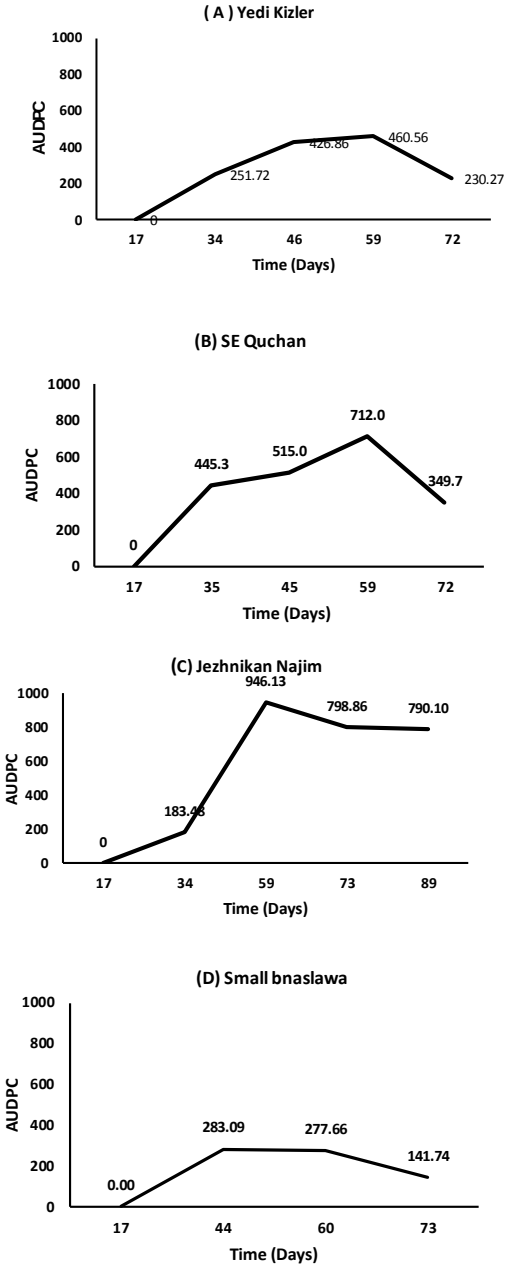

Figure 4: the AUDPC of NB of Barely During growing season 2021-2022 in A: Yedi Kizler, B: SE Quchan ,C:Jezhnekan Najim, and D: Small bnaslawa village

4. Discussion

Symptomatic leaf specimens were gathered for disease isolation. The direct identification of the pathogen which isolated from infected barely leaves was performed with the aid of cultural and microscopical characters. This is compatible with that of Wu et al. (2003), (Liu et al., 2011a) who described similar characteristics of the pathogen of In the current investigation, the fungus was also identified by PCR to validate its pathogen status. PCR was also used to validate a similar diagnosis of barley net blotch (Lightfoot and Able, 2010, Liu et al., 2012).

In the current work the highest disease incidence was reached to 42% after 45 days from planting in SE Quchan while the disease severity 43.12% after 59 days from planting in Jezhnekan Najim. In agreement to our results Jordan and Allen (1984) also found that the incidence and severity depending on ploughed or unplowed fields, barley plants were infected within 27 to 42 days. These differences in both disease incidence and severities in the location may refer to the differences in weather conditions of temperature, humidity, and rainfall. For instance, Launay et al. (2014) it was said that in the spring, net blotch of barley, the climate risk for infection is anticipated to occur earlier, but would result in a reduced infection pressure in May. Another factor may be that in the locations in south of the province Yedi Kizler (latitude 35.98999, longitude 43.85592) and SE Quchan (latitude 35.83315 Longitude 44.12675) may be the early cultivation and the suitability of weather conditions made the disease appearance and progress early in March but in the locations North of Erbil province the cultivation is late and weather conditions is mild compare to South part of the province.

The use of AUDPC is an efficient tool to measure the disease development in plants. The method has been used by many researchers. AUDPC is a useful tool for assessing the epidemic development of the wheat foliar disease *S. tritici* Paraschivu et al. (2013) and on stripe rust (Jeger et al., 2001). The results of the current study showing that the disease progress was increased with time in all locations. This may refer to the availability of we table environmental conditions in these locations during March and April but the curve was started downward after that in May. In a similar work on spot blotch disease on barley, Prasad et al. (2013) found the range in mean AUDPC percent days and days to maturity of the best-performing 25 entries was 250–463 and 88–111.

5. Conclusions

It can be concluded that the AUDPC can be dedicated for measuring foliar plant diseases such as barley net blotch. Where a progress curve can be made showing the development of the disease clearly throughout the season.

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