

Modeling and mapping diversity of pathogenic fungi of wheat fields using geographic information systems (GIS)



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ABSTRACT

In this study, geographic information systems and remote sensing tools were integrated and used to investigate the diversity of pathogenic fungi of wheat fields in Qarasoo basin, Golestan province, Iran. Two distinct indices, Shannon–Wiener and Simpson, were employed in order to express species diversity. The relationships between these indices and a number of independent variables such as topographic, soil and climatic variables were investigated to understand the relationships between fungal pathogen diversity and the agroecosystem while 67 georeferenced wheat field data using 0.25 m² quadrats were used to calculate the indices. Mallow's Cp statistical method was employed to select the most suitable factors while multiple regressions served to select model development, with two diversity maps being produced using the developed models. Residual maps were used to test the validity of the models. Rainfall and soil factors (zinc and nitrogen) formed the most important components that are effective determinants of pathogenic fungal diversity. High Simpson (0.60–0.69) and high Shannon–Wiener (1.40–1.67) values were detected in areas with higher rainfall at higher altitudes which were considered to be the most endangered areas. Considering the different responses of the models, Shannon–Wiener was found to be suitable for rare species while the Simpson model might be appropriate for a single dominant pathogenic fungus in the study area.

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1. Introduction

Fungi are important plant pathogens that cause more significant yield losses than bacteria or viruses (Vadlapudi and Naidu, 2011). About 80,000 species of fungi have been described (Kirk et al., 2001) and Hawksworth (1991) gave a conservative estimate of about 1,500,000 species of fungi existing in the world. Among them, about 10,000 are known to be able to attack plants and an average of over 14% of world losses is attributed to plant diseases (Agrios, 2005), more than 50% of which are caused by fungi. In crop plants, fungi cause more economic damage than any other group of microorganisms, with annual losses estimated at more than 200 billion US\$ (Birren et al., 2002). The economic importance of fungi is related to their destructive effects on the ecological systems of

the most important agricultural product (i.e. human food). Due to their potential to reduce crop production, the importance of their diversity in agroecosystems is far greater than in natural ecosystems (Wood and Lenne, 1999).

Wheat, one of the most important staple food crops grown on more than 200 million ha of land worldwide, is a source of food and livelihoods for over one billion people in developing countries (Singh et al., 2008). Wheat diseases are one of the most important factors reducing wheat yield. Fungi are the largest, oldest, and most investigated group of wheat pathogens (Wiese, 1987). Globally important fungal diseases of wheat, caused by biotrophs (obligate parasites), include three rusts, powdery mildew, and the bunts and smuts, whereas those caused by hemibiotrophs (facultative parasites) include *Septoria tritici* blotch, *Stagonospora nodorum* blotch, spot blotch, tan spot, and *Fusarium* head blight (scab) (Singh et al., 2008). The extent of damage from wheat diseases in a given season depends on a number of factors, including the susceptibility of the wheat variety to specific diseases, the level of the pathogen inoculum

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present and the environmental conditions during that season (Conley et al., 2003). The role of the environment in this interaction is important because diseases need specific conditions to develop. Temperature and moisture are two of the most important environmental conditions that influence plant diseases (Hines, 2002). For example, if winter and spring are cool and/or dry, leaf diseases will usually be of little or no significance regardless of a variety's resistance while a warm, wet winter and spring would be favorable for infection by disease-causing fungi on wheat (Smith, 2011). Also, balanced and adequate soil fertility for any crop reduces plant stress, improves physiological resistance, and decreases the risk of disease. For example, wheat fields with low soil N often have higher levels of tan spot disease than adequately fertilized fields (Fernandez et al., 1998; Krupinsky et al., 1997).

To develop effective disease management strategies, there is a need to understand the relationship between species and the environment in which they exist so as to be able to decide which areas are the most important to protect. Consequently, mapping areas with high fungal pathogen diversity is a priority for crop managers. The effective management of diseases can thus only be achieved when this valuable spatial information exists. The most popular indices that have been used to quantify landscape composition are the Shannon–Wiener index and Simpson's index, emphasizing the richness component of diversity and the evenness component, respectively (Magurran, 1988; Nagendra, 2002). Both these indices of diversity combine evaluations of richness and evenness and are termed heterogeneity indices (Peet, 1974). Basically, the Shannon–Wiener index is sensitive to changes in the importance of the rarest species (Heuserr, 1998)

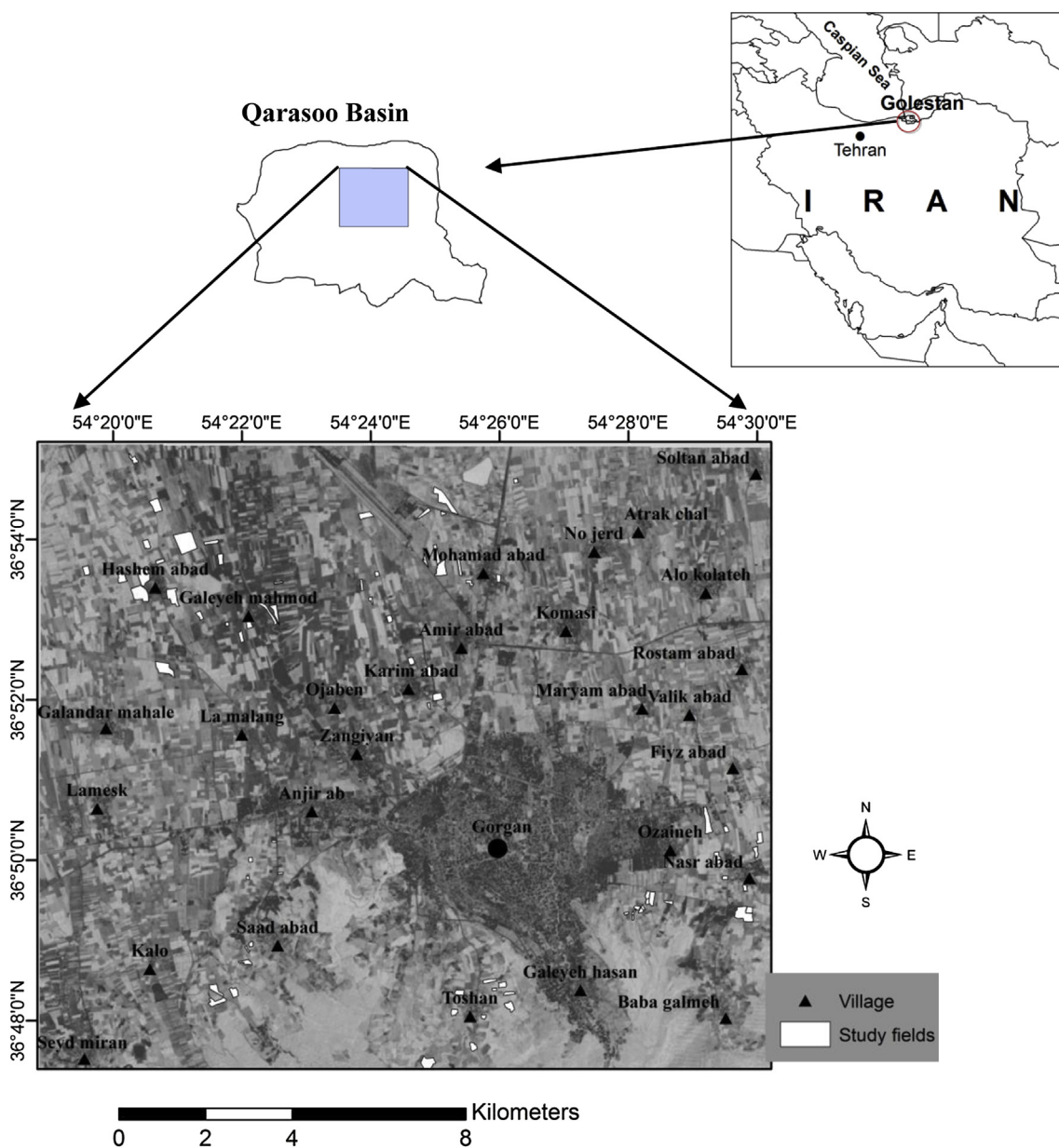


Fig. 1. Location of the 67 study fields of wheat at a part of Qarasoo basin (Golestan province, Iran).

while Simpson's index is considered to be a measure of species dominance (Magurran, 1988). By choosing the appropriate methods and tools, such indices could have the potential to map diversity. Global Positioning Systems (GPS) and Geographic Information Systems (GIS) technologies can be utilized to geospatially reference information from disease forecasting models, disease surveys and then be used to accurately define prescription management zones (Nutter et al., 2011). Within this framework, the aim of this study was to create a new approach to conventional diversity indices (Shannon–Wiener and Simpson) using GIS and remote sensing (RS) tools. For this purpose, the diversity of pathogenic fungi of Gorgan wheat fields in the 2010–11 growing seasons was modeled and mapped using this new approach.

2. Materials and methods

2.1. Study area

A survey was conducted from November through May 2010 to determine the diversity of fungal pathogens of 67 wheat fields located in a part of the Qarasoo basin, Golestan province, Iran (Fig. 1). The geographical location is in the range of 36° 46' 27"–36° 55' 29" North latitude and 54° 18' 59"–54° 30' 46" East longitude. The study area covers an area of 242.52 km². There are 27 settlement areas in the study area, the majority of them being small villages. Wheat accounted for the largest area under cultivation in Golestan province from 1982 to 2009 (JAGO, 2009). Wheat varieties planted in the study area included N-81-18, N-80-19, Koohdasht

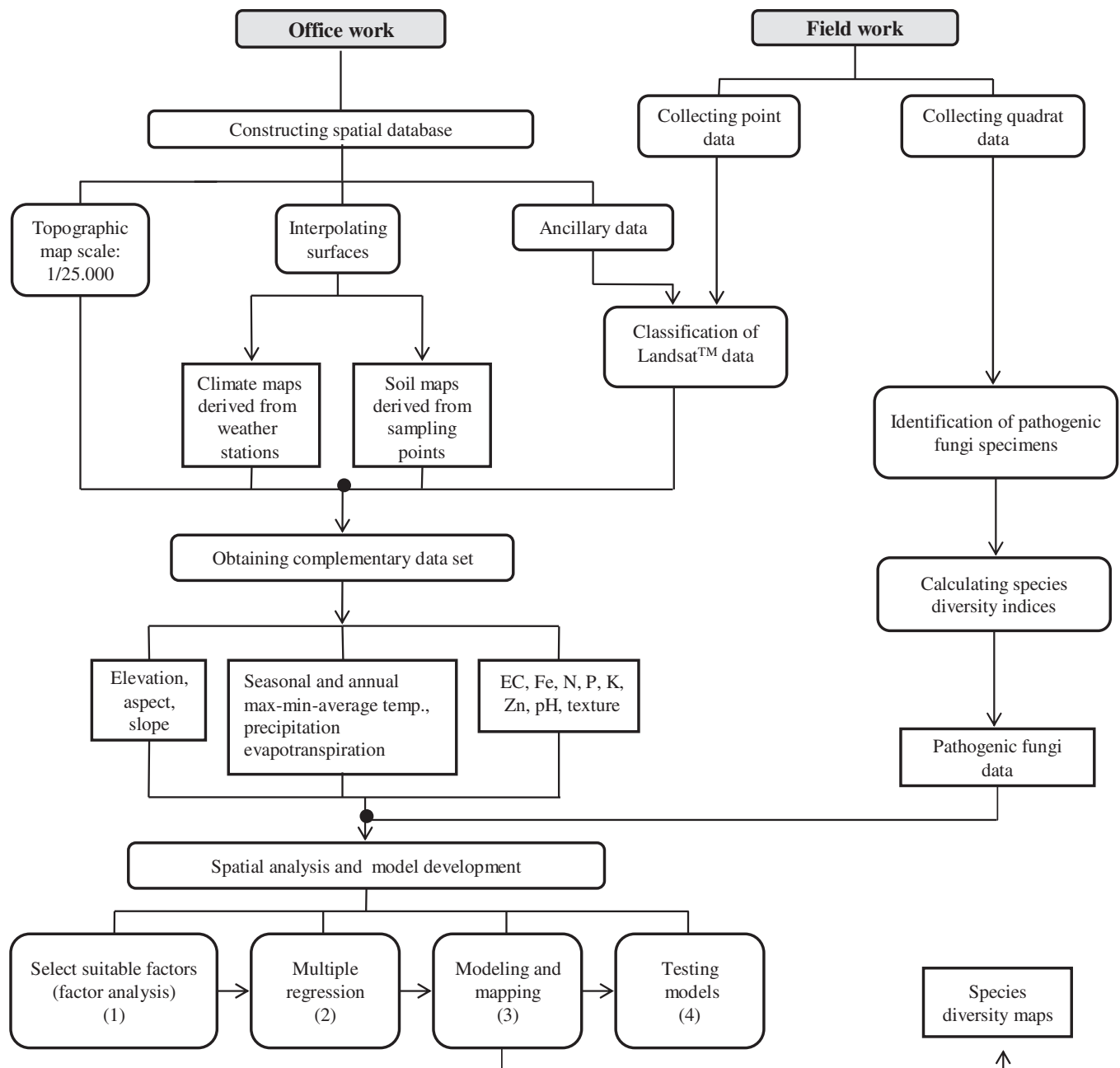


Fig. 2. Flowchart of the methodology. Rounded rectangles indicate the analyses and processes while rectangles show output products.

and Zagros, and their resistance to fungal diseases was ranked as: N-81-18 > N-80-19 > Koohdasht > Zagros (JAGO, 2009). Golestan has a moderate and humid climate known as “the moderate Caspian climate.” The factors affecting such a climate are the Alborz mountain range, the direction of the mountains, the neighboring Caspian Sea, vegetation surface, local winds, altitude and weather fronts. As a result of these factors, three different climates exist in the region: plain moderate, mountainous, and semi-arid. Average temperatures during the survey period ranged from 9 to 27 °C, total rainfall measured during the survey period was 170–250 mm, and relative humidity was on average 64%. Also, the altitude of the study area ranges between –25 and 422 m.

2.2. Sampling scheme

A flowchart of the methodology is provided in Fig. 2. In this study, 80 wheat fields in the study area were randomly selected from eight villages (Karim abad, Ghaley mahmod, Hashem abad, Mohamad abad, Toshan, Saad abad, Nasr abad and Ozaineh) that were important with respect to wheat production in the basin. Villages were selected so that the geographical position of selected fields was located in all directions and covered the entire study area (North, South, East and West regions) and then sampled. We select those fields which farmers claimed they were doing their best to eliminate the application of fungicides. Moreover, information was gathered about all practices conducted during the wheat-growing season in all sampled fields. Sampling was conducted in two stages of wheat growth due to the appearance and spread of pathogenic fungi in two stages: 1) from flag leaf visibility to very early dough (GS 37-81, Zadoks et al., 1974), and 2) from soft dough to hard kernels (GS 81-90, Zadoks et al., 1974). Hau et al. (1982) and Lin et al. (1979) reported a comparison between different sampling methods such as diagonal path, Z-shaped path, W-shaped path, three-diagonal path, diamond-shaped path and random samples for surveying diseases. They indicated that the precision of sampling method was of the same magnitude for all methods under random disease conditions. In addition, the random distribution of diseases can be caused by seed-borne infection or by air-borne inocula being introduced from a source which is a considerable distance away (Brown and Ogle, 1997). Since pathogenic fungi studied were from air-borne fungi, and since to the W-shaped sampling path covered the whole field (Hau et al., 1982; Lin et al., 1979), we thus selected the W-shaped sampling path instead of other sampling methods. In each field, 10 sampling points were chosen in joints and along the arms of a W pattern, covering the entire field except for 15–20 m away from field margins. Sampling was performed using 0.25 × 0.25 m quadrats based on Basu et al. (1973). System coordinates and altitude were recorded for each quadrat by the GPS GARMIN MAP 72CSX model. The second stage of sampling was conducted at the same points sampled from the first sampling stage, and GPS was used to find points sampled from the first stage. In each quadrat, wheat plants with symptoms of fungal diseases on the leaves and spikes were collected. Samples of each field were placed separately in plastic sacks and labeled, then brought to the laboratory for isolation and identification. In addition, the abundance of suspected symptoms of each disease was recorded in each quadrat (i.e., number of infected plants for each disease). From those 80 selected wheat fields, 13 fields were excluded due to a loss of sampling time.

2.3. Isolation and identification of pathogenic fungi from wheat plants

The isolation and identification of pathogenic fungi followed the method given by Solarzka and Grudzińska (2005). Diseased

foliar and spike samples were cut into small pieces, which were submerged in 10% sodium hypochlorite for 3–5 min. After this treatment, they were extensively washed with sterile distilled water and placed on plastic Petri dishes (9 cm diameter) containing potato dextrose agar (PDA, Liofilchem, Italy), corn (maize) meal agar (CMA, Central Drug House, India), malt extract agar (MEA, Liofilchem), yeast extract agar (YEA, Liofilchem) and Czapek dox agar (CZA, HiMedia Laboratories, India) amended with 30 mg/L streptomycin sulfate (I.E. Ulagay, Turkey) to eliminate bacterial contamination, with 3–5 pieces of tissue per Petri dish, then incubated at 25 °C for 72 h according to Ismail and Aly (1997), Montealegre et al. (2003) and Abou-Zeid et al. (2004). The isolated fungal strains were purified and identified according to Alexopoulos and Mims (1979), Barnett and Hunter (1998), Booth (1977), Burgess et al. (1988), and Ellis (1976). Rusts are obligate pathogens and therefore cannot normally be grown in pure culture, so rust-infected samples were identified by optical microscopy.

2.4. Supervised classification

To prepare land use maps and to separate wheat fields from other crops (Fig. 3), the supervised classification method (maximum likelihood parametric rule) was employed. For this purpose, a Landsat 5™ image acquired in June 2010 was used. The image was rectified to Universal Transverse Mercator (UTM) zone 40N and to World Geodetic System WGS-1984 data. The 7 bands (1–7) were stacked, and two spectral enhancement procedures including normalized difference vegetative index (NDVI) and principal component analysis (PCA) layers were also combined. Before implementing image classification, selection of good-quality training samples was critical. Training samples were taken from 361 points recorded by GPS and Google Earth software. A classification was established and five classes could be distinguished: 1 – urban, 2 – agricultural land, 4 – forest land, 5 – water, 7 – barren land. The classification was performed based on the United States Geological Survey (U.S.G.S) (<http://landcover.usgs.gov/classes.php>) method. To simplify the process for demonstration, the number of classification groups was reduced to two from the original work (wheat fields and non-wheat fields) (Gumz and Weller, 2011). The

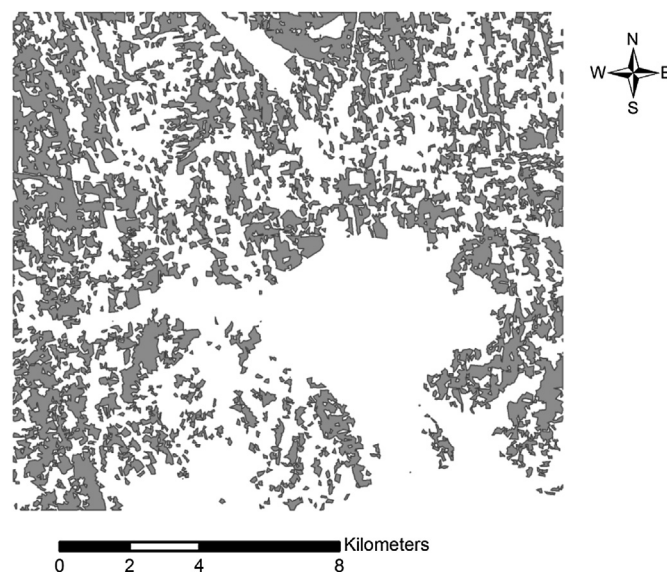


Fig. 3. Wheat fields map of the study area acquired from supervised detection method from the analysis of Landsat 5™ data (228 ground control points were used).

classification was conducted in Erdas Imagine 9.2 software (Leica Geosystems, 2008). Classification accuracy assessment was performed using Arcview GIS 3.3 software (ESRI, 2002). Supervised classification obtained 88% overall accuracy with a Kappa coefficient of 0.74, and produced a reliable result. Then other maps were extracted based on the wheat map.

2.5. Climate database

Historical meteorological data were obtained from seven weather stations within and close to the study area. The number of recorded years was different for each station and ranged between 4 and 56 years. These data were interpolated to calculate meteorological data for all the map squares. For this purpose, a multiple regression method was applied. In this case, each variable (including mean temperature, minimum temperature, maximum temperature and precipitation) were related by corresponding coordinates and elevation data. Then different combinations were tested to obtain the least squared difference between observed data minus the simulated one. Then a 10×10 net was constructed on a digital elevation model (DEM) to extract centroid points. For this purpose, “Add XY coordinates” and “extract values by points” functions were used. Then all meteorological variables were calculated by the “raster calculator” procedure and were interpolated by the spline function. All climatic layers were combined by a cell statistics tool that calculates a per-cell statistic from multiple rasters, as needed. These data were combined to calculate mean temperature values (average, minimum and maximum values) and the sum of precipitation during the wheat-growing period (Fig. 4), which ranged in the study area from November to May. The rainfall raster layer in the study area was divided into four classes and most rainfall was observed in many parts of South, Southeast and East sides of the study area. In addition, rainfall and altitude patterns were the same, and, together with increasing altitude from the north to the south side, rainfall also increased.

2.6. Soil database

Soil information was obtained from sampling points at the regional level by the Jihad-e-Keshavarzi (JAGO, 2009). Classical and

geostatistical interpolate functions were used to interpolate soil maps and raster layers were prepared. For this purpose, the Kriging function was used to prepare layers of magnesium (Mg), potassium (K), phosphorus (P), nitrogen (N), and pH, while the Cokriging function was used to prepare the iron (Fe) layer, and the inverse distance weighted (IDW) function for the layers of zinc (Zn) (Fig. 5) and EC.

2.7. Relief database

The slope (expressed as a percentage) and the aspect information were obtained from the DEM (Fig. 6) using spatial analyst functions in GIS media. The altitude layer in the study area was divided into three classes of -25 to 46 , $47-142$ and $143-422$ m. More than half of the wheat fields were located in the range of -25 to 46 m. The maximum altitude ($143-422$ m) was located in the southern part of the region, which was also the lowest extent of the study area.

2.8. Species diversity

Species diversity indices were calculated for each field sampled using the Ecological Methodology ver. 6 software (Krebs, 1999). Two indices were used to calculate diversity, Shannon–Wiener index, H' , as:

$$H' = - \sum_{i=1}^S P_i \ln P_i$$

where P_i is the proportion of total sample belonging to i th species, S is the number of species; and Simpson index, $1 - \hat{D}$ as:

$$1 - \hat{D} = 1 - \sum_{i=1}^S [n_i(n_i - 1)/N(N - 1)]$$

where n_i is the number of individuals of species i in the sample, N is the total number of individuals in the sample. To determine the superior model including soil, weather and topographic factors, several model selection methods (forward selection, stepwise selection, backward elimination and Mallows' Cp

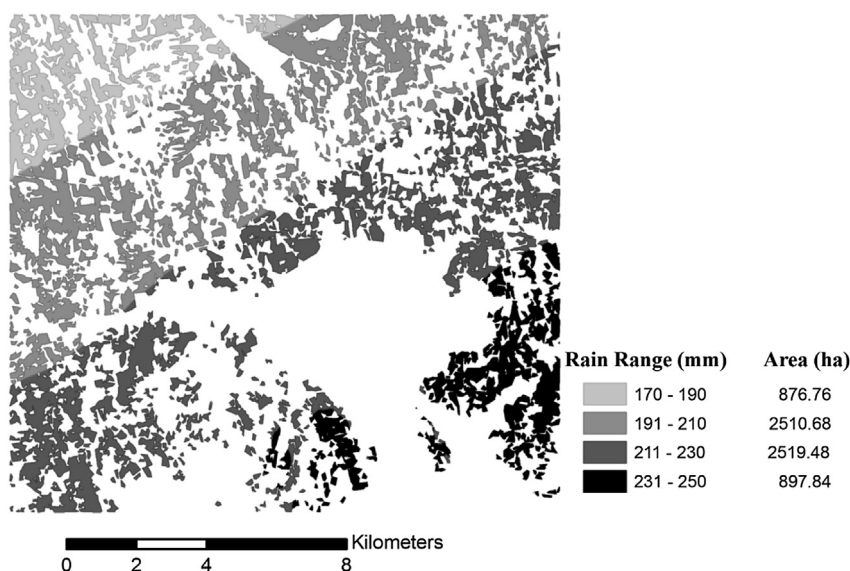


Fig. 4. Rain map of the study area during the wheat growing period (November to May), interpolated by spline and extracted based on the wheat fields map.

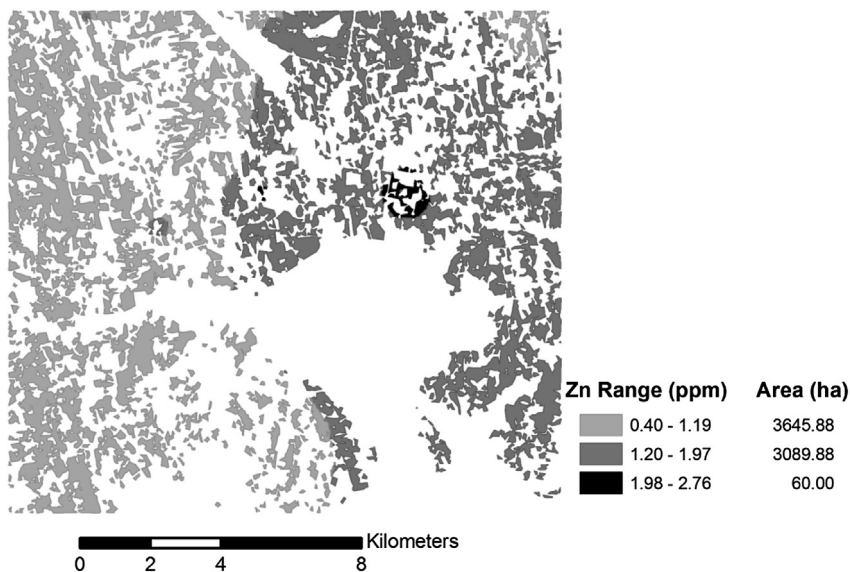


Fig. 5. Soil Zn map of the study area, interpolated by inverse distance weighted and extracted based on the wheat fields map.

selection) were used. Among them, Mallow’s Cp method had a better result based on statistical indices, thus it was used as the best model in SAS software, ver. 9 (SAS Institute, 2002) (Mesdagh, 2004). Multiple regression, regressing a dependent variable on a series of independent variables (Sokal and Rohlf, 1995), was chosen to formulate the relationship between the Shannon–Wiener and Simpson indices (each as a dependent variable) and soil, topographic and climatic factors (as independent variables). Applying the models, species richness maps were produced in ArcMap™ ver. 9.3 (ESRI® Institute). The reliability of the maps was tested by residual maps and ecological interpretations. Residuals were calculated by using the observed and computed values of indices in each quadrat. To create residual maps, spline and inverse distance weighted (IDW) interpolation methods were evaluated. The IDW method estimates

the values of an attribute at unsampled points using a linear combination of values at sampled points weighted by an inverse function of the distance from the point of interest to the sampled points. The assumption is that sampled points closer to the unsampled point are more similar to it than those further away in their values (Li and Heap, 2008). Splines consist of polynomials, which describe pieces of a line or surface, and they are fitted together so that they join smoothly (Webster and Oliver, 2001). We tested IDW and spline methods by storing many data as a test database. Then, generated values were plotted against observed values and root mean square and *a* and *b* coefficients, corresponding to a fitted line on observed versus generated data, were tested with respect to significances at 0 and 1, respectively. The IDW method was selected as the superior interpolation method. To evaluate different indices in the same

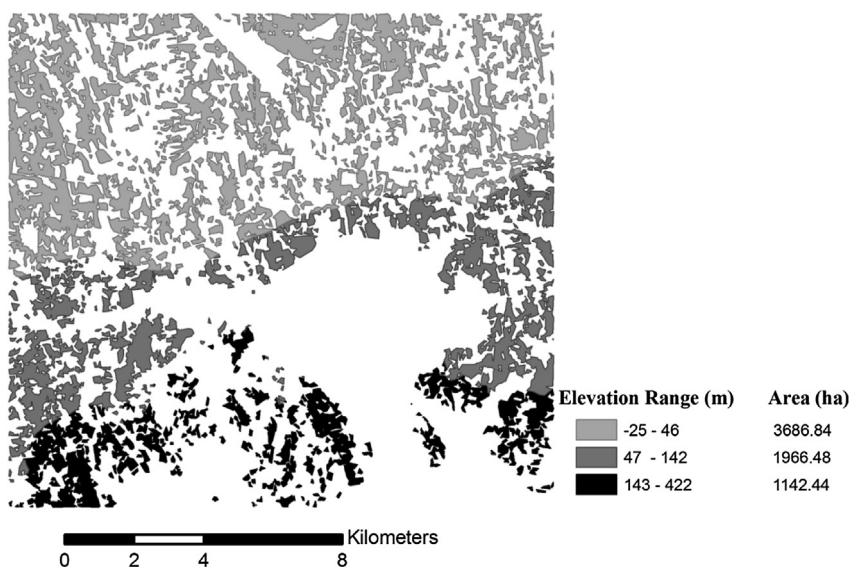


Fig. 6. Digital elevation model map of the study area, extracted based on the wheat fields map.

base, surfaces of the residuals were interpolated by using standard deviation values of each index (Dogan and Dogan, 2006).

3. Results

3.1. Fungal species

A total of 5 fungal species were determined in the study area: stripe rust (*Puccinia striiformis* f. sp. *tritici*), leaf rust (*Puccinia triticina*), glume blotch (*Stagonospora nodorum*), head blight (*Fusarium graminearum*) and spot blotch (*Bipolaris sorokiniana*). In the villages surveyed, stripe rust fungus at the lowest presence and frequency (%) was found relative to other pathogenic fungi, while spot blotch and glume blotch fungi had the most presence and frequency, respectively (Table 1).

3.2. Modeling and mapping

Among the aforementioned independent variables, nitrogen and zinc content in soil and rainfall as independent variables had the greatest impact on the dependent variables (Shannon–Wiener and Simpson indices). The results of linear (multiple) regression are summarized in Table 2 in two important domains, namely Analysis of Variance (ANOVA) and Model Summary for Simpson and Shannon–Wiener indices. The ANOVA table tests the acceptability of the model from a statistical perspective. In the ANOVA table, the regression row displays information about the variation accounted for by the model, while the residual row displays information about the variation that is not accounted for by the model. The model summary table reports the strength of the relationship between the model and the dependent variable. Moreover, the significance values of the *F* statistic were less than 0.01 in all models, indicating that the variation explained by the models was not due to chance (Table 2). The unstandardized coefficients, which were defined as the coefficients of the estimated regression model, were used in the developed models (Table 3). Based on the model developed in each diversity index, nitrogen and rainfall variables had the highest and lowest coefficients, respectively, so they had the most and the least impact on the diversity indices. Consequently, species diversity maps of focused indices were developed (Fig. 7).

3.3. Validity of interpolation method

The reliability of species diversity maps was questioned by residual maps. Residual maps predict the locations where the models work perfectly. The residual from regression is simply the difference between the observed and the computed value (Berry and Marble, 1968; McGrew and Monroe, 1993), and is a good indicator to show where the models work perfectly or imperfectly. In

Table 2

The statistics of linear (multiple) regression models.

ANOVA					
Simpson (1/D)^a					
Model 1	Sum of squares	df	Mean square	F	Significance
Regression	0.328	3	0.109	7.567	0.0002
Residual	0.911	63	0.014		
Total	0.911	66			
Model of summary					
Model 1	R	R²	Adjusted R²	Standard error	
	0.515	0.265	0.230	0.120	
Shannon–Wiener^b					
Model 1	Sum of squares	df	Mean square	F	Significance
Regression	1.948	3	0.649	7.738	0.0002
Residual	5.287	63	0.084		
Total	7.236	66			
Model of summary					
Model 1	R	R²	Adjusted R²	Standard error	
	0.519	0.269	0.234	0.290	

Variables were selected base on Mallow's Cp method.

^a Dependent variable: Simpson. Independent variables, RAIN, Zn, N.

^b Dependent variable: Shannon–Wiener. Independent variables, RAIN, Zn, N.

general, low residual values indicate robust models. The residual maps of the two models that resulted from interpolation by spline and inverse distance weighted methods are shown in Fig. 8. The percentage area covered by each distinct residual class indicates the credibility of the two models. The less predictive areas for both models were only accounted for by small percentages (Simpson: 1.73% and Shannon–Wiener: 0.88%) while strongly predictive areas were accounted for by significant parts (Simpson: 89.37% and Shannon–Wiener: 86.06%). Moderately predictive areas were also present for each index: 8.89% for Simpson and 13.04% for Shannon–Wiener. When strongly and moderately predictive areas were collectively considered, it appears as if each model runs very well on its own.

4. Discussion

In this study we tried to quantify diversity indices of pathogenic fungi of wheat fields. For this purpose, both GIS and RS as important tools for data acquisition and processing were applied. Then provided maps were used after validation of interpolation method by residual maps. The overall results of this study indicate that the Simpson model worked as effectively as the Shannon–Wiener model (Fig. 7). High Simpson (0.60–0.69) and high Shannon–Wiener (1.40–1.67) values were detected in areas with higher rainfall which also corresponded to higher altitudes. The relationships between these indices and rainfall can be recognized when the rainfall (Fig. 4) and diversity maps (Fig. 7) were examined together. A direct relationship between rainfall and

Table 1

Frequency (%) of pathogenic fungi species in wheat fields sampled from 8 villages at a part of Qarasoo basin (Golestan province, Iran).

Pathogen fungi	Villages							
	Karim abad	Ghaleye mahmod	Hashem abad	Mohamad abad	Toshan	Saad abad	Nasr abad	Ozaineh
<i>Bipolaris sorokiniana</i>	100	100	100	100	100	100	100	100
<i>Stagonospora nodorum</i>	100	100	100	100	90	100	100	100
<i>Fusarium graminearum</i>	100	100	33.33	87.50	0	70	88.89	0
<i>Puccinia triticina</i>	83.33	70	88.89	37.50	80	100	100	100
<i>Puccinia striiformis</i> f. sp. <i>tritici</i>	0	0	0	0	20	0	0	33.33

Table 3

Developed models of each index (Shannon–Wiener and Simpson). This table states the models (regression equations) according to the results of multiple regression. In the equations, the understander coefficients are the coefficients of the estimated regression model. Each model also has a constant value such as; -1.719 for Shannon–Wiener, -0.306 for Simpson.

Models
Simpson index = $-0.306 + (0.003 \cdot \text{RAIN}) - (0.214 \cdot \text{Zn}) + (0.431 \cdot \text{N})^a$
Shannon–Wiener index = $-1.719 + (0.008 \cdot \text{RAIN}) - (0.531 \cdot \text{Zn}) + (2.256 \cdot \text{N})^b$

Standard error of the significant predictive independent variables for models of each index.

^a Standard errors were 0.0009, 0.051, 0.64 for RAIN, Zn and N, respectively.

^b Standard errors were 0.002, 0.12, 0.56 for RAIN, Zn and N, respectively.

indices was detected. In general, frequent showers and high humidity produce conditions that favor disease development (Turkington et al., 2006). Rain-splash is an important mechanism for the dispersal of spores of plant pathogens (Fitt et al., 1989). When a raindrop strikes an infected plant, splash droplets can entrain and disperse spores and thus can introduce inoculum into areas where the pathogen is not present (Fitt and McCartney, 1986). Considering the range (170–250 mm) of rainfall in the

study area, both the Simpson and Shannon–Wiener indices showed a monotonous increase as rainfall increased. Also, an inverse relationship between soil Zn (Fig. 5) and both indices was detected. High Simpson (0.60–0.69) and high Shannon–Wiener (1.40–1.67) values were detected in areas with lower levels of soil Zn (0.40–1.19 ppm). Zinc deficiency renders the plants more susceptible to various diseases, many of them of fungal origin (Graham, 1983). As a result, an increase in the diversity and spread of pathogenic fungi causes an increase in the values of diversity indices.

Depending on these results, an important question arises: which model has the capacity to delineate the real situation in the field? The difference between the models comes from their inherent characteristics. Basically, the Shannon–Wiener index gives more importance to the richness component and to rare species while the Simpson index weighs the evenness component and dominant species (Molles, 1999). Therefore, the appropriateness of the models depends on the aims sought by decision-makers. The Shannon–Wiener model might be useful to detect areas where rare species are the focus. On the other hand, the Simpson model could be best fit to determine areas where dominant species are concentrated.

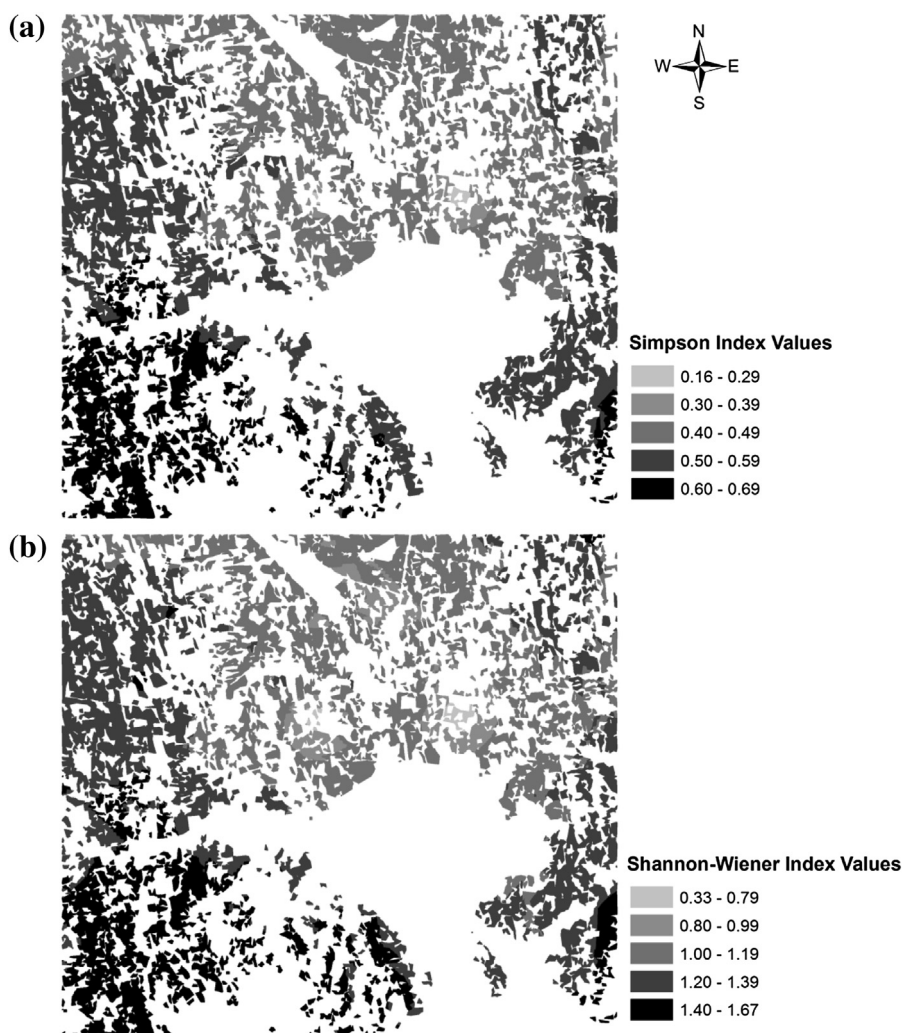


Fig. 7. Pathogen fungal species diversity maps according to Simpson (a) and Shannon–Wiener (b) indices, extracted based on the wheat fields map.

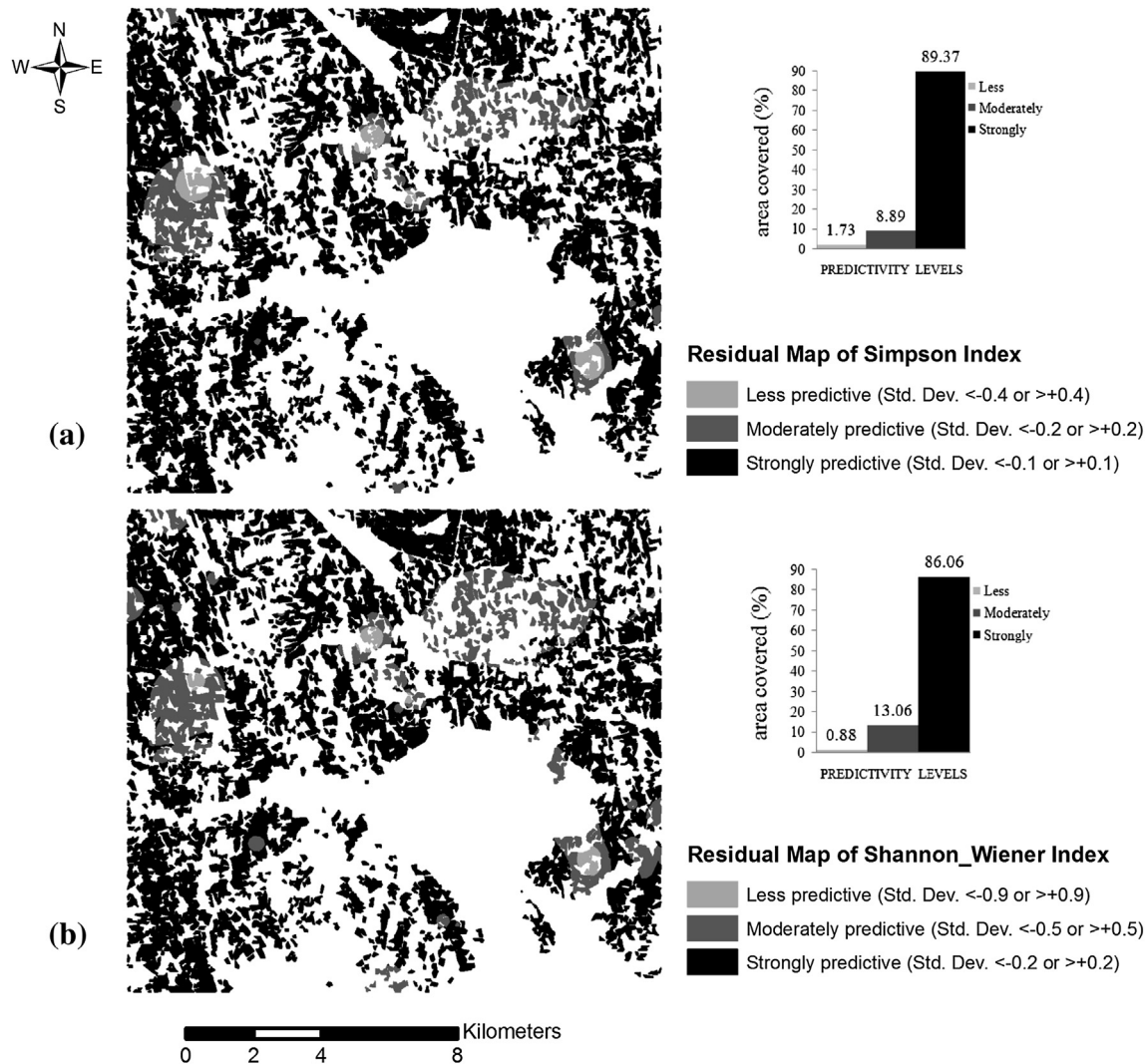


Fig. 8. Residual maps of the two models interpolated by inverse distance weighted: a) Simpson index model, and b) Shannon–Wiener index model.

5. Conclusion

In this study, two diversity indices were modeled and mapped and the relationship between pathogenic fungi biodiversity with the factors (climate, topography and soil) that affected them were investigated. Comprehensive data on topography, soil and climate were used to provide maps as the main inputs of spatial analysis and modeling stages. According to the results, Shannon–Wiener was suitable for rare species, while the Simpson model is more appropriate for a single dominant pathogenic fungus. These maps could help policy makers to detect more vulnerable areas to fungal infection and to determine risk zones. Using wheat varieties more tolerant to fungi, sowing date management and soil fertilization could be advisable options to alleviate fungus-induced injuries. Undoubtedly, this type of study, which is based on on-farm and spatial data, can provide more reliable outputs. When such a study is matched with geospatial analysis tools such as GIS, it can provide visual maps which are important and informative in disease management.

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