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ORIGINAL PAPER

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SPATIAL DISTRIBUTION OF EARLY BLIGHT DISEASE ON TOMATO, CLIMATIC FACTORS AND BIOEFFICACY OF PLANT EXTRACTS AGAINST *Alternaria solani*

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ABSTRACT

Information on the spatial variability in plant disease is essential for location-based disease management. In the current study, the spatial distribution of tomato early blight disease was ascertained in District Gilgit, Gilgit-Baltistan, Pakistan. The comprehensive field survey was carried in two growing seasons (2014–2015), whereas in each growing season, 62 tomato fields were surveyed. Based on the distribution of disease, the respective thematic maps (incidence and severity) were prepared using Arc Map 10.1 with spatial analyst function of Arc GIS software by means of the inverse distance weight (IDW) interpolation method. Results indicate that early blight of tomato is spatially distributed in both growing seasons. However, in the first growing season, the disease incidence ranged from 10.22% to 44.16% and during later season 14.03–49.16%, whereas 5.37–16.40% and 6.52–26.94% severity was recorded. Furthermore, this information indicates that higher disease infestation occurred in 2015 in relation to 2014. This information linked to metrological data (temperature, precipitation and relative humidity), seemingly favored the early blight development during the growing period. Seven botanical extracts were tested against pathogen *Alternaria solani* at different concentrations (4, 6 and 8%). Results revealed that all tested plant extracts showed antifungal activities. However, at 8% concentration of plant extract, *Datura starmonium*, *Berberis orthobotry*, *Podophyllum emodi* and *Uretica dioica* exhibited >60%, while *Peganum harmala*, *Artemisia maritima* and *Capparis spinosa* <60% antifungal properties. The information generated due to this study could help the tomato growers regarding disease management and selection of resistant cultivars, improving profitability and food security in the Gilgit region.

Key words: tomato disease, *Alternaria solani*, GIS, spatial analysis, plant extract, District Gilgit, Pakistan

Abbreviations: GIS-Geographic l Information System, IDW-Inverse distance weight, GB-Gilgit-Baltistan, KIU-Karakorum International University

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is source of income and food security for small-landholder throughout the world. In Pakistan tomato is the most essential vegetable after potato considered as highly nutritive value. In Pakistan annual production is approximately 574.052 thousand tons in 58.196 thousand hectare lands

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[AGRISTAT 2013]. Whereas in Gilgit-Baltistan tomato is produced 6,455 metric tons on 805 hectare of which 3194 MT were consumed, 2293 MT were marketed, and 968 M.T were wasted [Agric Stat 2009]. Despite it is one of the most common and popular vegetable, used in Pakistan as well as in Gilgit-Baltistan. There has been a progressive increase in its production and area under cultivation but its production is far lower as compared to the other countries. There are many constraints for its low production including lack of technical knowledge, infestation of weed, low yielding varieties and disease and insects etc. [Year book… 2006]. Tomato is also prone to biotic and abiotic stress similar to other vegetables [Abdel-Sayed 2006]. Among the biotic constraints early blight of tomato caused by Alternaria solani one of major common threats, which cause >70% yield reduction [Tewari and Vishunavat 2012]. Brown to dark necrotic spots on leaf stem and fruits is most common symptoms after pathogen infestation [Singh 1987]. GIS maps of disease distribution provides key information to manage and policy formulation for sustainable crop production in near future [Nelson et al. 1994, Orum et al. 1997]. The climatic factors like warm temperature, extended period of leaf wetness and crowded tomato plantation are supportive for development of early blight disease [Cerkauskas 2005, Momel and Pemezny 2006]. An understanding of the role of environmental conditions and its consequence on infection and survival of the pathogen is needed to develop disease management practices. Earlier investigations described that environmental factors such as temperature, precipitation, relative humidity (RH) and leaf wetness significantly contribute to disease development [Vieira 2004]. Management of early blight tomato is done by good sanitary practices which leads to reducing amount of inoculum in the next growing season as well as removing the dead plants would help minimize infection. The most common technique for controlling of early blight tomato disease is the use of fungicides. Though, they are not only costly but also capable of creating problems on the environment, human and animal health. Application of plants extract to control many plant disease are most effective and environmental friendly [Thobhunluepop 2009, Duru and Onyedineke 2010].

The present investigation was carried out to assess tomato early blight disease distribution in district Gilgit. The obtained information was link with meteorological data. Furthermore the study also focused the bio-efficacy of plant extracts against *A. solani* to develop appropriate management program of the disease.

MATERIALS AND METHODS

Description of study area. Gilgit district is administrative division and is located in Gilgit-Baltistan, Pakistan. The estimate terrain elevation above sea level is 2731 m and lat. 36°15'0", lon. 74°15'0". The district connected with boundaries of Afghanistan, China and India (Fig. 1). According to the 1998 census Gilgit District had a population of 243,324.

Spatial distribution of early blight disease of tomato. An extensive survey of spatial distribution (incidence & severity) of tomato early blight disease were carried in different tomato field of district Gilgit, Gilgit-Baltistan Pakistan in two growing seasons (2014–2015). A total 124 fields were selected and in each year 62 fields were taken into consideration. Within each field ten tomato plants were randomly selected and all the leaves were examined for recording incidence and severity of disease. Disease incidence (DI) was calculated by using following formula:

$$DI = \frac{NIL}{TNL} \times 100$$

where: – disease incidence; NIL – number of infected leaves plants; TNL – total number of leaves. Disease severity was determined according to scale of Christ [1992]. The disease severity index percentage (DSI) was calculated by using following formula:

$$DSI = \frac{\sum AR}{TNR\ (MD)} \times 100$$

where: (DSI = disease severity index; ∑AR = sum of all disease rating; TNR = total number of rating; MD = maximum disease grade).

Climate data were obtained from the Department of Meteorology, Gilgit-Baltistan, Pakistan.

Geographic information system. A data base comprises of X and Y coordinate in the study site was cre-

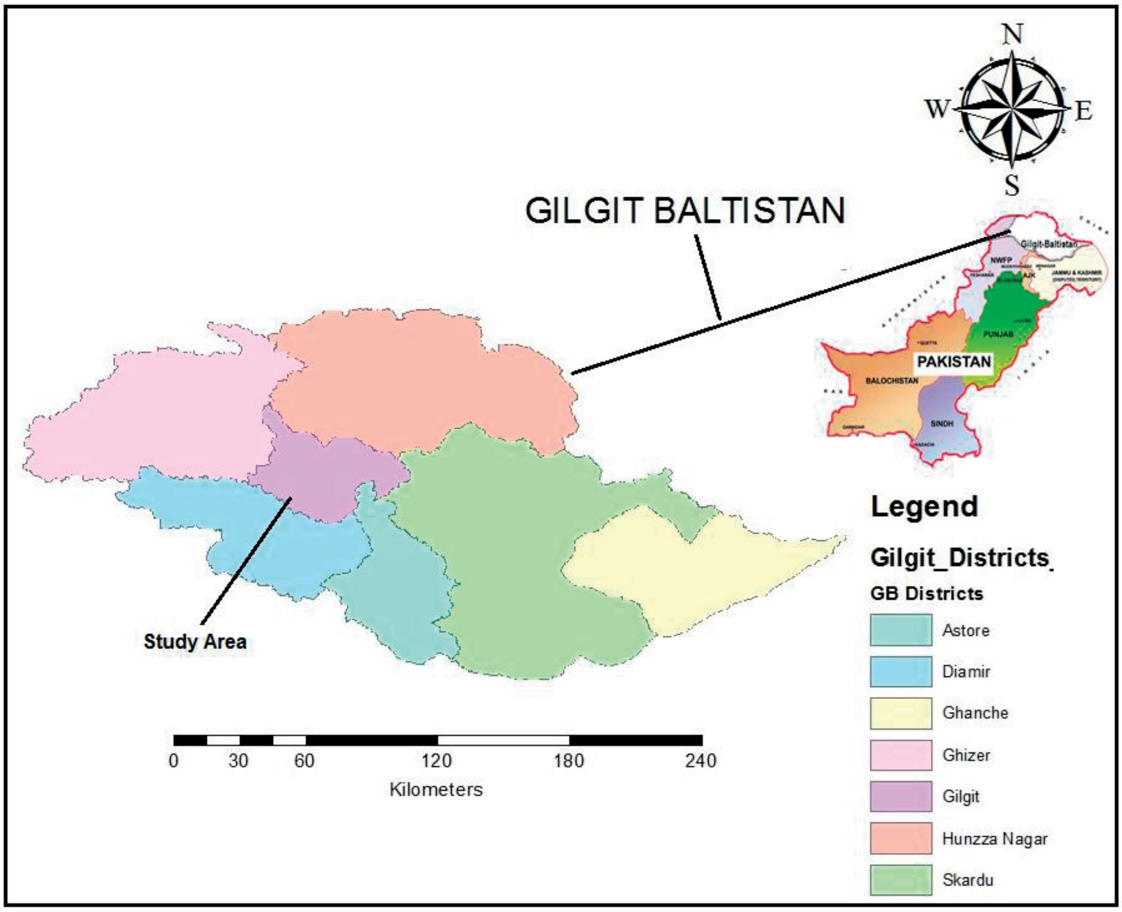

Fig. 1. Map of study area

ated. After that shape file of District was open in a GIS software (Arch 10.1). In the projects three fields X, Y and Z was opened. In X-field, X-coordinate, Y-field, Y-coordinate was selected whereas in Z field disease data was placed. Arc view spatial analyst "Interpolate grid option" was selected. On the output "grid specification dialogue", output grid extend chosen was same as District Gilgit boundary and the interpolation method employed was inverse distance weight (IDW).

Isolation of *Alternaria solani*. Pathogen *Alternaria solani* was isolated from infected tomato leaves collected during survey which showing typical symptoms of disease. The infested leaves were methodically washed under running tap and distil water and made small pieces (about 2 mm) of infected leaf. Then these small pieces were surface sterilized as described by Arunakumara, [2006]. The small infected pieces were then plated on potato dextrose agar medium at 27°C for 7 days and sub cultured until pure isolate of *A. solani* was obtained and identified as *A. solani* according to Simmsons [2007].

Plant materials used in the experiments. Seven plant leaf samples were collected during field and brought to laboratory. These samples were washed with water and shade dried. After drying grounded to make fined power. Fifty gram of powder mix with ethanol and distill water at ratio 20 : 80 v/v for twenty minutes then left in dark glass bottles for three days. Filter the extract through thin cheesecloth sheets. The final extracts were exposed to 60°C in water bath for 30 min for ethanol evaporation. The extracts were then stored in a refrigerator at 5°C until use.

In vitro bioassay. Food poison technique was applied to evaluated bioefficacy of plant extract as describe by Manmohan and Govindaiah [2012]. Three concentration (4, 6 and 8%) with three replications of each of different botanicals extracts were used. Potato dextrose agar (PDA) as nutrient medium. The vigorously growing culture of *A. solani* was prudently cut using a gel cutter and moved aseptically to the center of each Petri dish comprising the poisoned solid medium. Control was sustained by growing the cultures on PDA without the botanical extract. All the petri dishes were incubated at 27 ±2°C for seven days. After incubation period, bio efficacy was calculated terms of percentage growth inhibition according to the following formula [Taskeen et al. 2011, Sallam and Kamal 2012]:

$$GIP = \frac{MGC - MGT}{MGC} \times 100$$

where: GIP – growth inhibition percentage, MGC – mycelial growth in control, MGT – mycelial growth in treatment.

RESULTS AND DISCUSSION

Tomato is one of the most common vegetables grown all over the world, including Pakistan. It is vulnerable to many pathological problems especially early blight disease which is most serious and significant in dropping yield and triggering economic losses. For assessment of early blight incidence and severity a comprehensive survey was carried in two growing years (2014–2015) in District Gilgit, Gilgit-Baltistan Pakistan. Geographic Information System (GIS) was applied for preparation of disease distribution map from data obtained during field survey. Distribution map of early blight occurrence and sternness in the two study years are portrayed in Figures 2–3. Results showed that in the growing season 2014 and 2015 disease incidence ranged from 10.22–44.16; 14.03–49.94% while disease severity was 5.37–16.40; 6.52–26.95%. Mean early blight incidence and severity 24.87 and 9.36% in 1st year survey while 29.67 and 12.62% in 2nd year. Comparison of year-wise data revealed that high disease incidence and severity of disease was recorded in 2015 as compared to 2014. Meteorological data including temperature ranged approximately 25–30°C, precipitation 10–20 mm and relative humidity >72% in growing periods of study area (Figs 4–5). This indicates that climatic data favored the development of early blight of tomato. The increasing disease trend is distressing for tomato growers of study area. This study found spatial variation in disease incidence and severity of early blight of tomato in district Gilgit. Spatial variation in disease may indicate the existence of isolates differing in virulence. Another reason for variation in disease may be the environmental conditions (temperature, precipitation and humidity) and production practices (lack of resistant varieties, time of sowing and inappropriate management strategy) that influence the final disease outcome. This statement was agreement with various researcher [Rouselle et al. 1999, Cerkauskas 2005, Afaq et al. 2014]. Temperature is probably one of most important environmental variable with significant effect on the development of plant disease especially early blight of tomato. Several researchers reported that highest growth of pathogen *A. solani* occurred at 28°C [Krishna et al. 2009]. Naik and Sinha [1997] also stated that optimal temperature for growth of *A. solani* is 28–30°C. Stevenson and Pennypacker [1988] reported optimum temperature of 25°C for germination of *A. solani*. In current studies, He et al. [2012] described 30°C as the optimum temperature for the germination of *A. solani.* In epidemiology relative humidity plays key role of early blight disease of tomato and potato. >85% relative humidity highest disease infestation was recorded however at 75% RH germination of pathogen was also noted. Krishna et al. [2009] reported that sprouting of *A. solani* also observed at 75% of RH. Ganie et al. [2015] reported that mean temperature, relative 26.47 and 27.32°C, >90% relative humidity and precipitation 8.68 and 6.42 mm, favored the maximum development of early blight of tomato which was the agreement of current study. Besides climatic data deficiency of soil nitrogen makes tomato plants weaker, slower growing and faster aging. In such a condition plant becomes more susceptible to pathogen *A. solani* [Agrios 2005]. This statement is in agreement with previous findings that Gilgit-Baltistan soil is deficient in soil nitrogen [Baber et al. 2000, Azhar et al. 2016]. The applications of Geographical Information System (GIS) and geostatistics are the increasing demand for spatial pattern and hotspot analyses over large regions and can be used to analyze and manage plant disease information. For distribution and mapping of

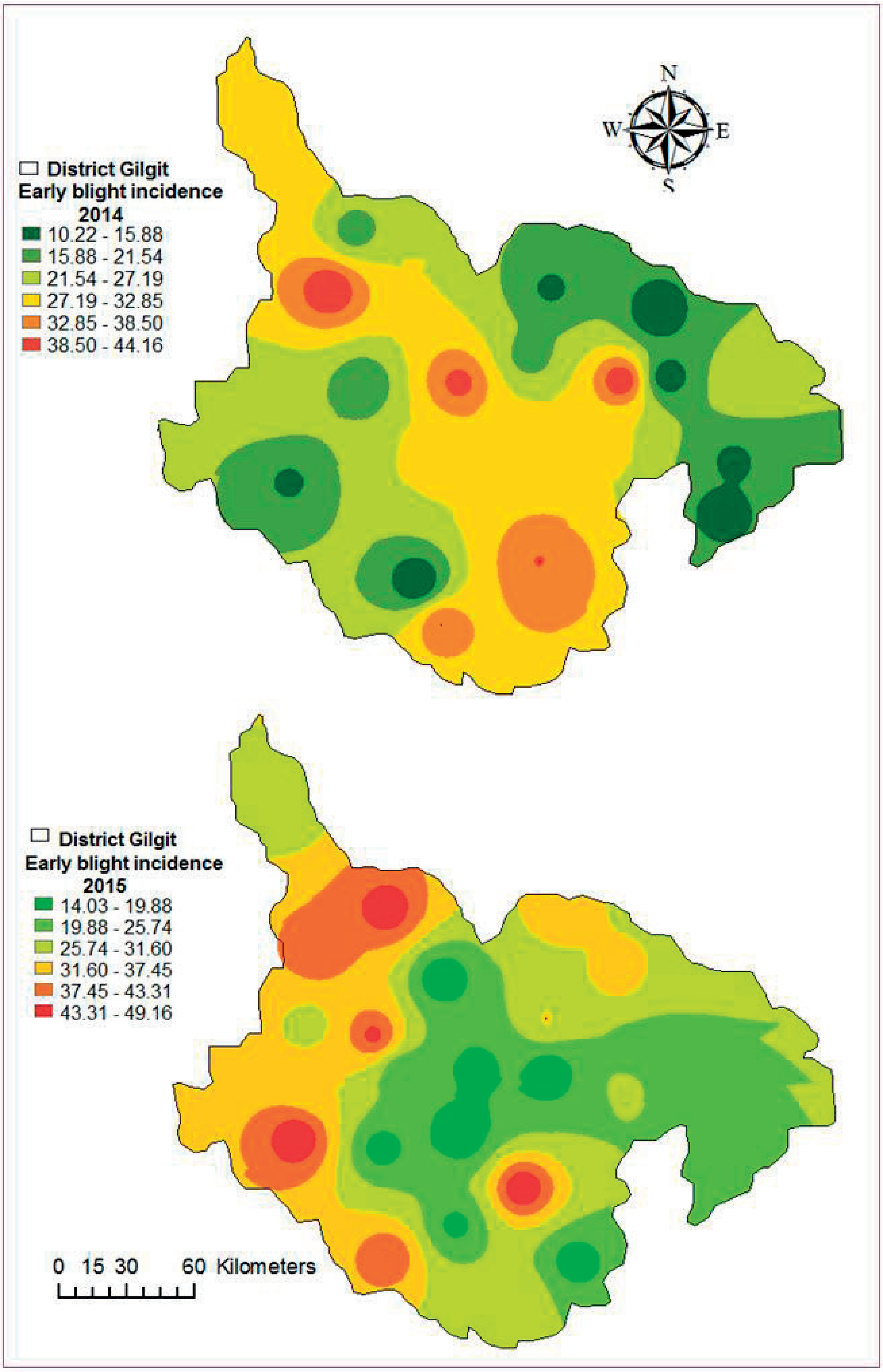

Fig. 2. Spatial distribution of early blight incidence in year 2014–2015

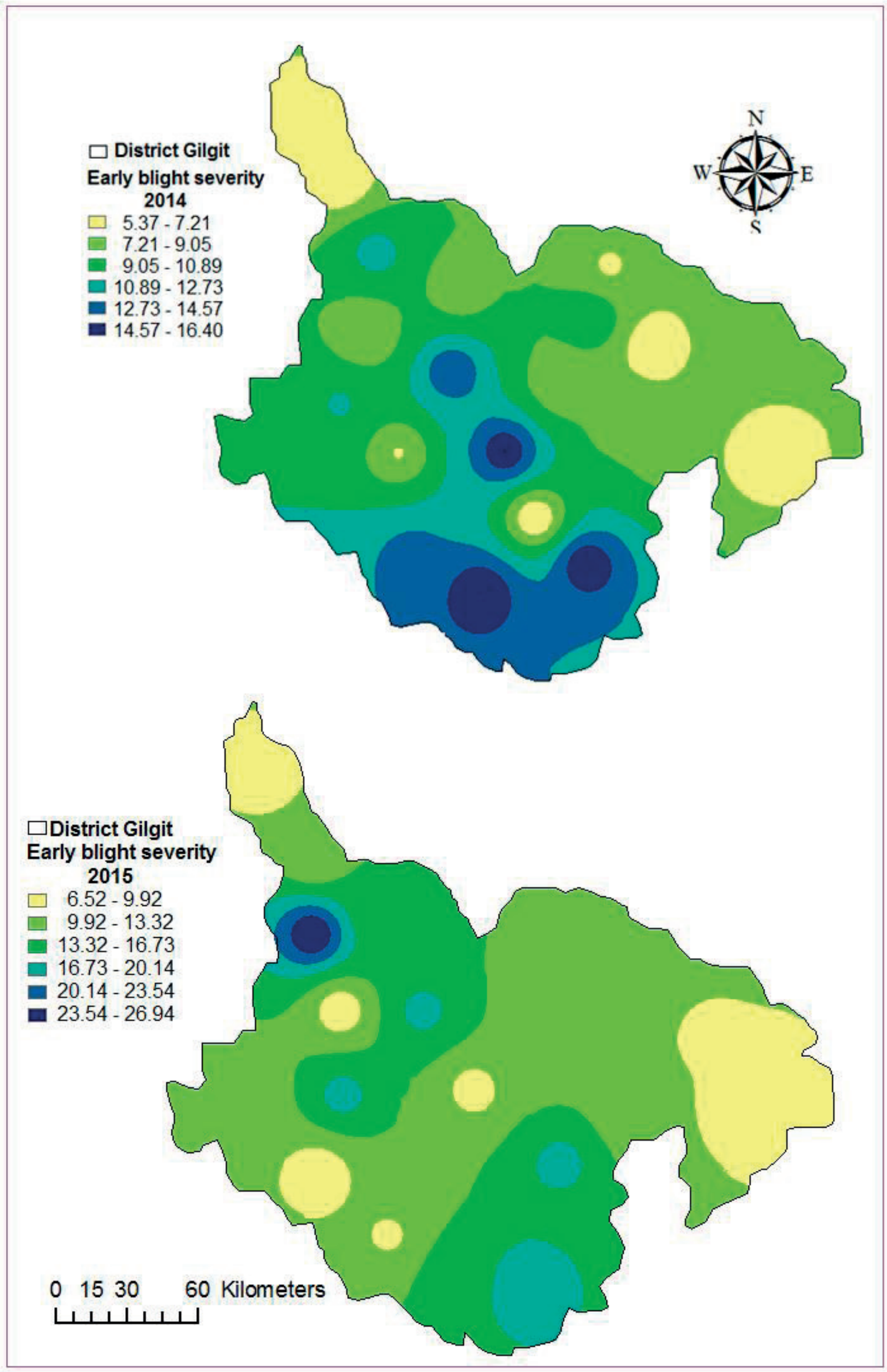

Fig. 3. Spatial distribution of early blight severity in year 2014–2015

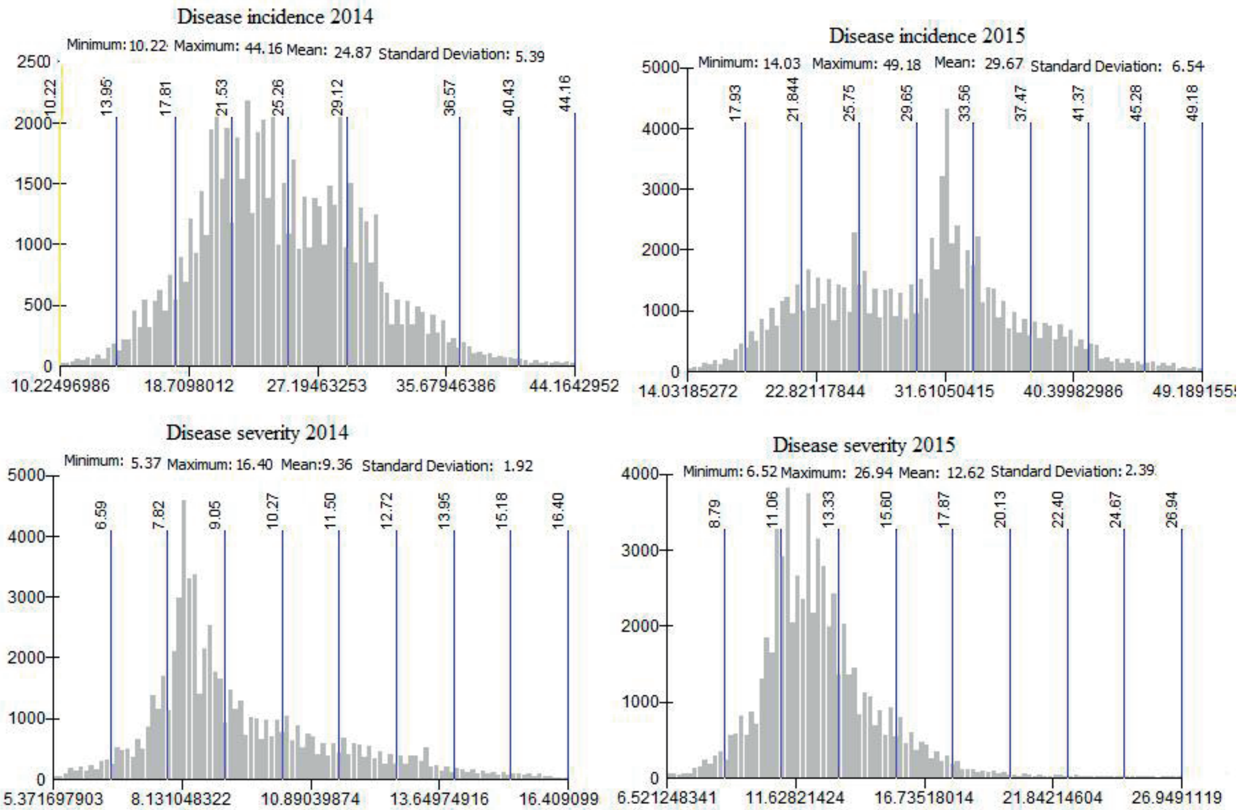

Fig. 4. Maximum, minimum and mean disease incidence and severity during year 2014–2015

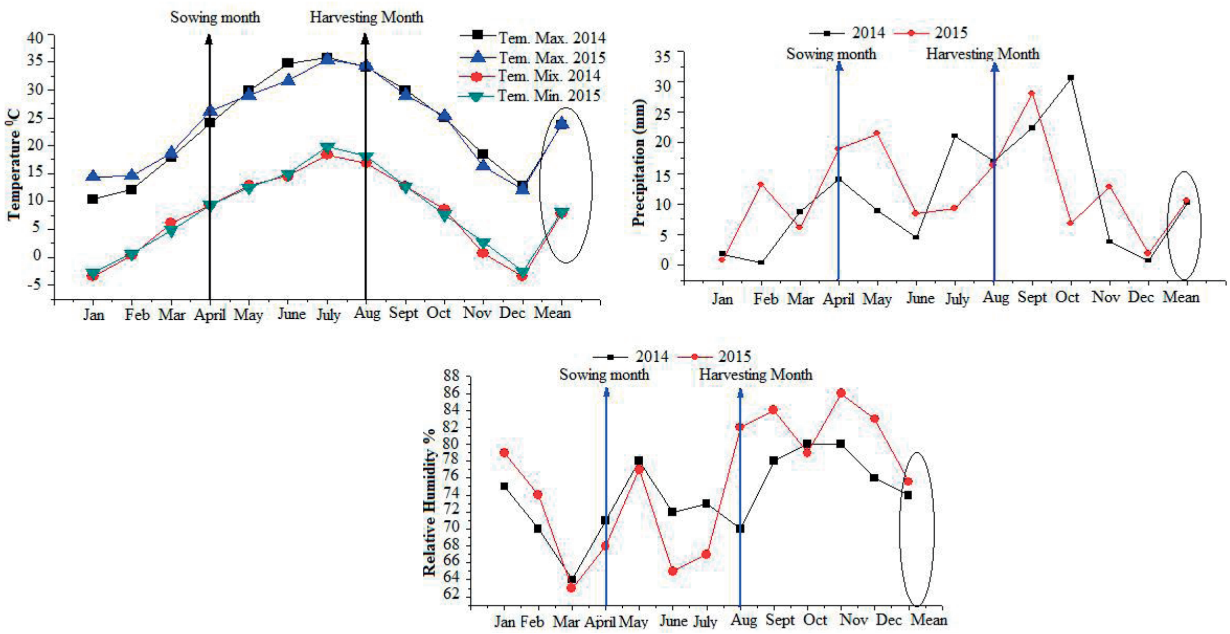

Fig. 5. Mean monthly temperature (°C), precipitation (mm) and relative humidity (%) 2014–2015

Table. 1. *In vitro* screening of botanical extracts against *Alternaria solani* by poison food technique

Plant extract	Concentrations of plant extracts		
	4%	6%	8%
Podophyllum emodi	28.13^{C}	42.40^{B}	61.96^{B}
Peganum harmala	19.28^{D}	35.05^{C}	55.50^{C}
Datura starmonium	33.48^{B}	49.66^{A}	66.61^{A}
Uretica dioica	33.30^{B}	48.61^{A}	61.86^{B}
Artemisia maritima	29.12^{C}	41.83^{b}	57.69^{C}
Berberis orthobotrys	40.40^{A}	53.47^{A}	63.51^{AB}
Capparis spinosa	27.60^{C}	37.22^{BC}	55.79^{C}
LSD	3.78	5.37	3.1

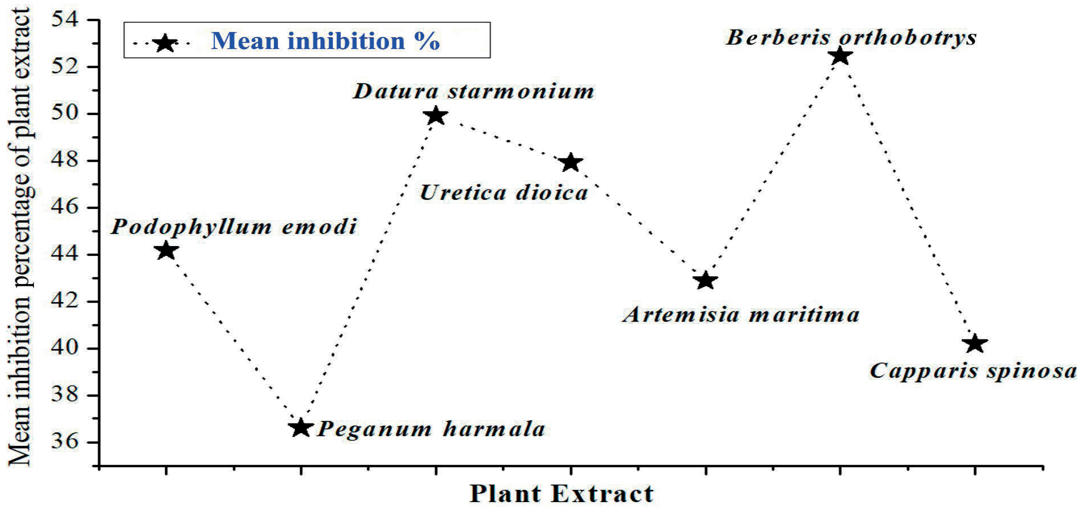

Fig. 6. Mean inhibition percentage of botanical extract against pathogen *A. solani*

disease or specific genotype of plant pathogen GIS has been used extensively [Nelson et al. 1994]. The current study showed that early blight was widespread throughout the study. The interpolation maps indicated that higher incidence and severity was in year 2015 compared to 2014.

In vitro bio efficacy of plant extracts against *Alternaria solani* was presented in Table 1. An insight into the data discloses that all the plant extract significantly inhibited mycelial growth of pathogen at all concentrations tested. *Datura stramonium* showed considerably superior to all other plant extracts exhibiting 66.61% of mycelial growth inhibition of the test fungus at final concentration. This was followed by *Berberis orthobotrys* (63.51%), *Podophyllum emodi* (61.96%) and *Uretica dioica* (61.86%). *Peganum harmala* and *Capparis spinosa* showed comparatively least inhibition over the control at all test concentrations. In general basis, the of mycelial growth inhibition by the test plant extracts enhanced with increase in their concentration. It was notice that irrespective of concentration the highest average percentage of mycelial growth inhibition was observed in *Berberis orthobotrys* (52.46%) followed by *D. stramonium* (49.92%) and *Uretica dioica* (47.92%). The least averge mycelial inhibition percentage was exhibited by *Peganum harmala* (36.61%) (Fig. 6). The utilization of plant or their natural products is gaining a lot of attention for plant disease management due to no adverse side effects on human health, environment friendly and cheaper compared to synthetic chemicals. Recently, efforts are being made to manage plant diseases through the use of different plant extracts or their products. A number of plants have been reported to possess antifungal activity [Neeraj and Verma 2010, Derbalah et al. 2011]. In the present investigation, efforts were made to explore the possibility of using extracts of locally and easily available plants for the management of early blight of tomato. The findings showed that all the plant at various concentration inhibit the mycelial growth of the pathogen *A. solani* significantly ($p < 0.05$). However, mycelial growth inhibition increased by increasing the concentration of plant extracts which was the agreement of several researchers [Latha et al. 2009, Goussous et al. 2010]. It was recorded that *Berberis orthobotrys* irrespective of concentrations, exhibited maximum average mycelial growth inhibition followed by *Datura stramonium* and *Uretica dioica.* Similar properties of numerous other plant products effective against *Alternaria spp.* were reported by several researchers [Latha et al. 2009, Goussous et al. 2010]. The least average mycelia growth inhibition percentage was found in *Peganum harmala.* The inhibitory effect of the plant extract might be attributed to the presence of secondary metabolites of medicinal plants.

CONCLUSIONS

This is the first ever research on spatial distribution of early blight of tomato and its management in mountain region Gilgit-Baltistan, Pakistan. This research documented that early blight disease is widespread in tomato fields as well as weather factors influence for the development of disease. The information generated through this study could help the tomato growers regarding disease management and selection of resistant cultivars, improving profitability and food security in the Gilgit region. The outcomes of current endeavor clearly indicated the prospects of plant extracts to control plant pathogens. These plant extract would be helpful for development of plant based biopesticides in future research.

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