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# Fitness Evaluation of *Phytophthora infestans* Isolates Collected from Punjab, Pakistan During 2017-18

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## ABSTRACT

Potato late blight (*Phytophthora infestans*) is an important disease causing severe damage in potato crop worldwide. Seventy seven isolates of *P. infestans* selected on the basis of aggressiveness were characterized for pathogen fitness test based on their lesion size, latent incubation period and sporulation capacity after their inoculation onto detached leaves of potato. The results showed that large variations in pathogen fitness were present among isolates for each regional *P. infestans* population studied. Those isolates selected during 2017-18 exhibited 85% and 74% successful pathogen fitness behavior respectively. Those isolates which had higher composite fitness index values were observed higher lesion size and spore count and same trend of pathogen fitness parameters was observed with respect to their districts during both the years.This is the first inclusive study to determine pathogen fitness of isolates in Pakistan. The experimental findings indicated that population of *P. infestans* in the Punjab province comprises diverse isolates with low to high fitness potential. A future challenge planned to combine our accumulated knowledge with that from other scientific fields to develop a disease management approach for late blight.


**Key words:** Fitness, Isolates, Late blight, Potato, Potential, Selected.

## INTRODUCTION

Potato (*Solanum tuberosum* L.) is cultivated for direct human consumption and grown on area of almost 70% across the world (Devaux *et al*., 2014). Potato crop is highly vulnerable to different biotic and abiotic factors which have significant impact on both tuber quality and yield (Cooke *et al*., 2006). Among biotic factors, potato late blight caused by *Phytophthora infestans* (Mont.) de Bary is an important disease (Haverkort *et al*., 2008). The epidemic of disease is so quick that it can cause almost 100 % disease severity in just few days to few months after primary infection on susceptible potato plants. Several studies have been made, yet knowledge is very scare about full biology of pathogen to control of disease in sustainable way. The most interesting thing about the *P. infestans* is its population diversity and frequently changes of the pathogen biology, making it really complicated for plant breeders to breed for crop durable resistance worldwide (Agrios, 2005; Flier *et al*., 2003; Agrios, 2005) while it can reproduce and survive both asexually as well as sexually when the two mating types of the pathogen coexist (Fry, 2008).Pakistan has cool climatic conditions and therefore it might be possible that zoospores are the main source of infection in the asexual cycle.

The population of *P. infestans* is totally diverse as compared to the population worldwide and both mating types are present, though frequencies vary in regions (Lehtinen and Hannukkala, 2004). The change in diversity might be result in change towards more destructive/aggressive populations having better fitness which has significant impact on the population biology on a global scale as well as a regional (McDonald and Linde, 2002; Lehtinen and Hannukkala, 2004; Fry, 2008). The production of spores gives the pathogen a better aptitude for long-time survival in potato growing areas worldwide (Lehtinen and Hannukkala, 2004; Widmark *et al*., 2007). Increased adaptability and fitness of the pathogen can have severe consequences in terms of disease control (Medzhitov *et al*. 2012). The rapid changes of the population structure can occur alternatively from one year to other followed by high assembly rate of spores.

Therefore, change in population can thereby happen by selection of better fit isolates across areas and meanwhile successful disease development does notes sentially involve the most aggressive isolates (Zwankhuizen *et al.,* 2000; Cooke *et al.,* 2012a).The virulence and fitness of a pathogen population is strongly linked, but really complex in nature (Cooke *et al.,* 2006).The fitness model of plant pathogen basically includes the capability to reproduce and survive in harsh conditions. Alternatively, highly aggressive isolates which are dominating in one season might be lost its virulence before the next season because survival of asexual structure is totally dependent on spread through seed tubers (Cooke *et al.,* 2006).Therefore, by evaluating the fitness of selected more aggressive isolates we can check the damage of disease so present study was planned to focused on the level of pathogen fitness of those isolates which are highly aggressive in potato growing areas of Punjab, Pakistan.

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## MATERIAL AND METHODS

The research work was conducted in the laboratory of Plant Pathology, College of Agriculture, University of Sargodha, Sargodha, Punjab, Pakistan during 2017-18 to evaluate the pathogen fitness level among more aggressive pathogen (*P. infestans)* in potato growing areas of Punjab, Pakistan.

### Sample collection

Diseased samples during occurrence of late blight of potato were collected during field visits to different areas of Punjab, Pakistan including Districts of Khushab, Sargodha, Chiniot, Jhang, Sahiwal, Okara (Fig 1).

The leaves with distinctive symptoms were selected from the field and put diseased pieces of leaves under potato tubers for sporulation. Samples were collected in plastic zipper bags and brought to laboratory for culturing *P. infestans* isolates to test aggressiveness components. After selecting the more aggressive isolates, having composite aggressiveness index more than CAI >500 range. Pathogen fitness level was assessed.

### Isolation of *P. infestans*

Blighted leaves taken from the field was incubated overnight in humid boxes at 15-20°C to encourage sporulation. When sporulation developed, isolates were put onto antibiotic rye agar medium. This was done by cutting small pieces of infected tissue and place on the agar (or even under the agar), or second method was followed by cutting small cubes of antibiotic rye agar using a sterile scalpel and use these to pick up sporangia by lowering them gently onto the sporulation trying to avoid directly touching the infected tissue as far as possible. These rye agar cubes then was placed onto fresh plates of antibiotic rye agar containing 200mg sodium amplicillin L-1 and 50gm rifampicin L-1 and the plates were incubated at 15°C (light isn't needed) and checked regularly for growth of *P. infestans* hyphae from the cubes (using a lower powered microscope).

### Preparation of inoculation

Newly formed sporangia were collected by needle and add 10ml of distilled water to each peri dish and spore suspension were filtered through cheese cloth by making concentration of 60,000 sporangia/ml through haemocytometer. The collected fungal spores refrigerated at 4°C for 2 hours to release zoospores for use of inoculation (Pliakhnevich and Ivaniuk, 2008).

### Pathogenic fitness test

Isolates fitness was examined on individual healthy leaves of host plant by using sporangia which collected from the different diseased field samples. Plants were grown for 6 weeks in green house/field conditions at 18-20°C and grown in plastic pots containing peat/sand mixture. After 6 weeks, leaf disc was cut from fourth leaf of potato and placed, upper surface down in petri dishes having thick moistured filter paper. Each leaf disc was inoculating with 10µl droplet of original sporangial suspension of the isolates and incubated plates for four days at 20°C (12h light/day) as recommended (Fig 2).

Pathogen fitness test was calculated by:

$$\text{Pathogen fitness} = \frac{\text{lesion size} \times \text{sporulation capacity}}{\text{Latent period}}$$

While, (i) lesion size on leaves were measured 4 days after inoculation and discs were vortex for 1-2 minute to suspend sporangia and after this haemocytometer was used to count spores. (ii) Sporulation capacity was expressed as sporangia/square mm of leaf disc area of leaf (Three infected leaflets placed in a beaker with 10 ml of sterile distilled water and shaken for 2 min to dislodge sporangia. Sporangia were counted by using a haemacytometer (iii) Latent period is the time period between inoculation and emergence of symptom that assessed every 12h, apart from hypersensitive response (HR) (Flier and Turkensteen, 1999; Knapova and Gisi, 2002).

### Data analysis

The experiment was layout as CRD and ANNOVA was analyzed by using SPSS software v. 19 for measuring the variations among tested isolates against pathogen fitness while significant differences among the isolates means were evaluated by LSD at $p \leq 0.05$.

## RESULTS AND DISCUSSION

Pathogen fitness tests showed adaptation of *Phytophthora infestans* isolates and their differences were also indicated by detached leaf assays results which were consistent with those reported by Knapova and Gisi, (2002).

The detached-leaf technique can be used to supplement large scale field testing for resistance to late blight. It is rapid, requiring only 6 days from inoculation to completion. It is not plant destructive; therefore, the same

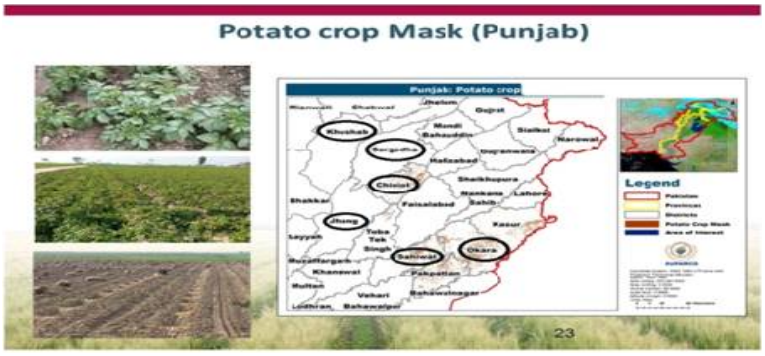



**Fig 1:** Potato crop mask of Punjab, Pakistan.

plants can serve as source material for screening different isolates of the pathogen or other pathogens (Goth and Keane, 1997).

All of the isolates tested in this study were derived from infected potato fields and exhibited host specificity, being highly pathogenic. Host adaptation of *Phytophthora infestans* has been extensively reported by Andrivon *et al*., (2004) who collected the isolates from the potato field and found more aggressive isolates while on alternate host like tomato, only less aggressive isolates were observed and vice-versa.

The pathogen, *Phytophthora infestans* causal agent of potato late blight was identified based on phenotypic characteristics of these isolates which were fluffy cottony mycelium with slightly striated pattern on Rye Agar media. The inoculated detached leaves showing sporulation which was clearly seen on inoculated leaves (Fig 2). Lesion size on leaves were measured after 4 days of inoculation and recorded with the help of ruler while latent infection period and spore count was measures as per described in material and methods section (Fig 3).

### Evaluation of pathogen fitness

For each of the isolates tested, disease development varied on inoculated leaves of potato kept under laboratory or left attached to the host plant. Disease development was significantly faster during 2017 collection with more pathogen fitness index value of top three isolates are Oka-13 (6391.33) followed by Jhg-10 (5968.6), Cht-6 (5352.83) while least

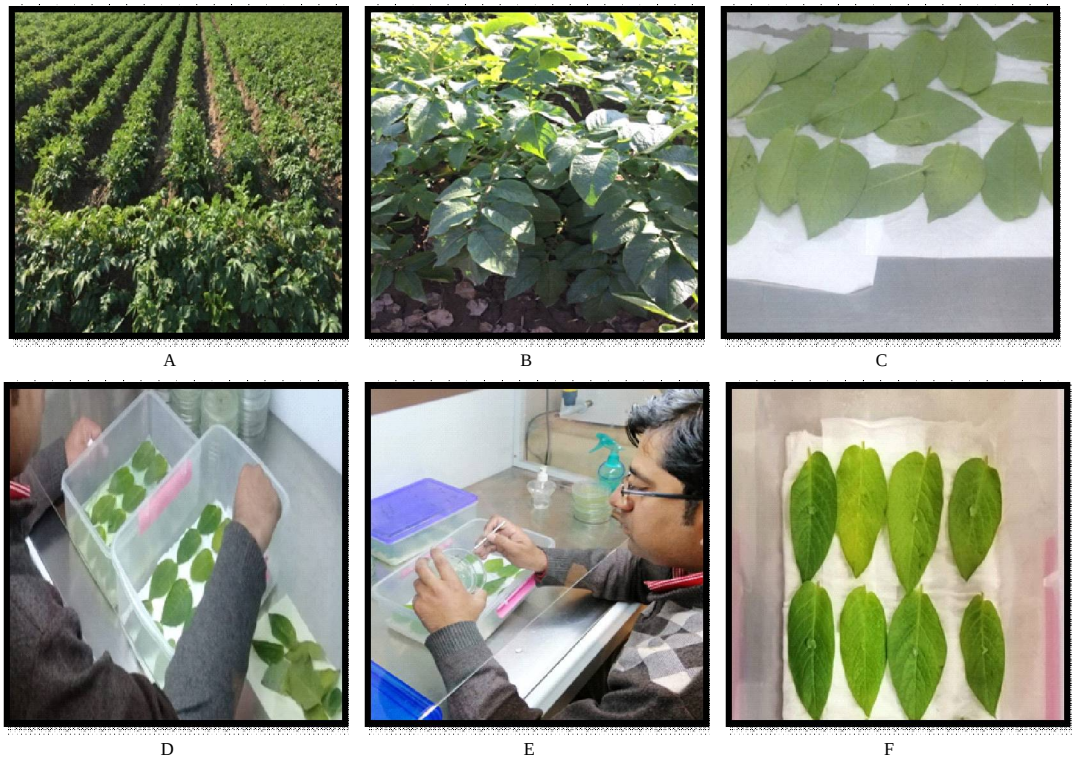



**Fig 2:** (A) Potato crop grown in greenhouse (B) Selected healthy potato plant (C) Healthy leaflets brought in laboratory and remove excess moisture (D) Placed detached leaflets in moist box (E) Inoculation of pathogen plugs from pure culture of *P. infestans* (F) Representative inoculated detached leaflets.

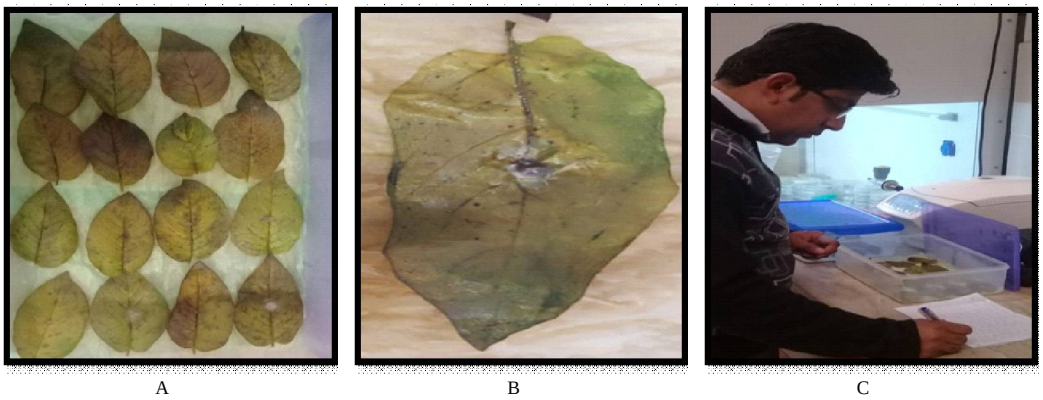



**Fig 3:** (A) Leaves showing lesions on representative inoculated detached leaflets (B) Representative inoculated leaf showing sporulation (C) Data recoded.

isolate which was marked and not selected for population analysis experiment is Shl-7 having value only 302.8. Progress of disease and pathogen fitness index was observed highest during 2018 collection of Oka-24 (8542) followed by Shl-25 (7250.83) and Jhg-22 (6140.17) while only 94.13 fitness index value was observed of tested Cht-20. The most common observation which was observed during both years collection, that Okara district isolates showed more pathogen fitness in 2018 collection than last year may be due to more aggressive strains prevailed in this district or the isolate become more aggressive with time period depend on variations in the aggressiveness of the isolates.

The isolate from district Oka-13 formed more spores and lesion area followed by Jhg-10 and this trend was observed as same while isolates collected during the year of 2018. The pathogen composite fitness index (CFI) values are directly proportional to the larger lesion produced by tested isolates.

Based on individual performances of districts during 2017, district Khushab, the Khb-9 showed more (CFI) value (3984.83) followed by Khb-1 (2925.5) with more lesion area and sporulation count respectively. Among Sargodha isolates, the isolate Srg-1 was showed best CFI value (4124) with highest lesion size (42mm) and spores count (196.33) while least was observed from isolate Srg-11 (366.06) and lesion size of (21.33mm). In case of district Sahiwal, when the isolates tested having more CFI value, the isolate which observed as top was Shl-10 (4943.5) while least performance was observed from Shl-7 having just 302 index value. The Okara district isolates overall expressed more CFI values than other districts and therefore, isolates from this district was more aggressive in nature due to favorable environmental conditions. The isolate Oka-13 which was also showed best among all districts during 2017 having more CFI value to be considered for further experiments related to study. The district Jhang and Chiniot isolates showed significant performance having CFI values, 5968.5 & 5352.83 respectively. Fig 5 showed the variations in pathogenic fitness behavior and composite fitness index of the isolates collected during 2018 from 6 potato growing areas of Punjab. The district Khushab isolate Khb- 21 showed higher fitness having (CFI 5170.67) with lesion size (44mm). The lesion size was observed directly proportional to CFI and spores count as last year and same trend has been observed during this year. The other districts from Punjab, Sargodha isolate Srg-14 observed highest composite fitness index (5333.83) followed by Sahiwal isolate, Shl- 25 (7250.83), Okara district, Oka-24 (8542), Jhang district, Jhg-22 (6140.17) and district Chiniot, Cht-18 (5526.5) (Fig 4 and 5).

The study revealed that those isolates selected from last experiment (to determine aggressive isolates) were also observed having more pathogen fitness values known as composite fitness index. All the selected isolates showed substantial variation of pathogen fitness components. The significant differences were observed for lesion area, spores count and latent infection period and composite fitness index among isolates. The isolates which had higher CFI values were observed significantly almost same level of pathogen fitness with respect to their districts during both the years 2017-18 of isolates tested.

Based on adaptation phenomenon, it is also more apparent that during 1980's renewed global migration of both A1 (asexual) and A2 (sexual) mating types rapidly displaced the old clonal lineages (Garelik, 2002, Ryu *et al*., 2003). The driving force behind this adaptation might be due to the high level of aggressiveness and pathogen fitness in new populations (Flier and Turkensteen, 1999).

Similar to our experiment protocol, Brooks, (2008) has privileged the detached-leaf assays for quantitative assessments of pathogen fitness parameters specifically *P. infestans*. According to the findings of Naerstad, (2000) who observed lesions induced on detached potato leaves by aggressive isolates expanded faster collected from potato and theses finding is consistent with previous reports and supported by Naerstad, (2000). The variations among different isolates and survival abilities might be possible due to differences in response to different climatic factors (Pariaud *et al*., 2009). This information is essential to propose integrated management strategies that include the populations actually concerned in inciting the disease on host plant.

Identification of suitable methods for testing the pathogen fitness on the basis of aggressiveness potential of different isolates of *P. infestans*, disease reactions are necessary for cost management (Brooks, 2008).

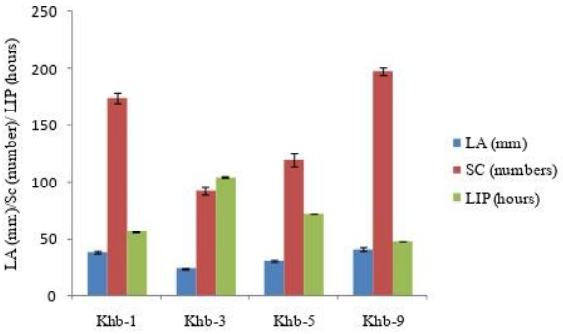
Our results agreed with previous studies (Huang *et al*., 2005; Foolad *et al*., 2015) those described detached leaflet methods as accurate, reliable, repeatable and consistent to field screening for the aggressiveness, partial resistance studies and virulence testing of *P. infestans*.

Regardless of the large amount of research work that has been and currently being done on the understanding population dynamics of *Phytophthora infestans* worldwide but we are still a long way from a compact assessment of the precise factors that contribute to the evolution of the populations in the different geographical as well as ecological situations where the pathogen prospers.

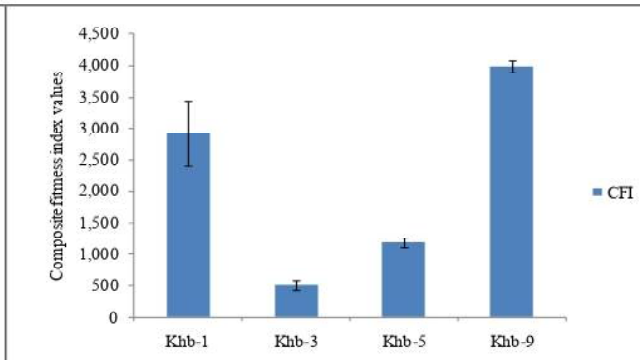
The possible restraint during detach leaf assay according to study of Pettitt *et al*., (2011) is the possibility of pathogenicity reactions and the probable descent of detached leave parts before the assay may be successfully concluded. Mainly, the plant resistance tends to be compact after excision and in bioassay that presented here might be given the impression that non-pathogenic isolates were pathogenic in nature. Contrary to this impression we reject this hypothesis due to the speed of infection caused by the *Phytophthora* isolates after inoculation with pathogen suspension negated the need for intricate procedures to avoid their decline.

Actually it was unknown which of the fitness component has the great influence on over all pathogen fitness. A lot of researchers stated the difficulty of defining the relationship among single fitness components and overall fitness. Therefore, the overall pathogen fitness of one area population relative to other probably tricky to predict exclusively on the basis of knowledge related to pathogen fitness components. Consequently, we attempted to combine the fitness component parameters in a manner that could

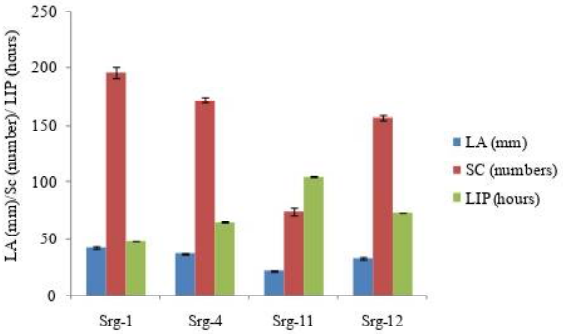
## Pathogen Fitness test Results-2017



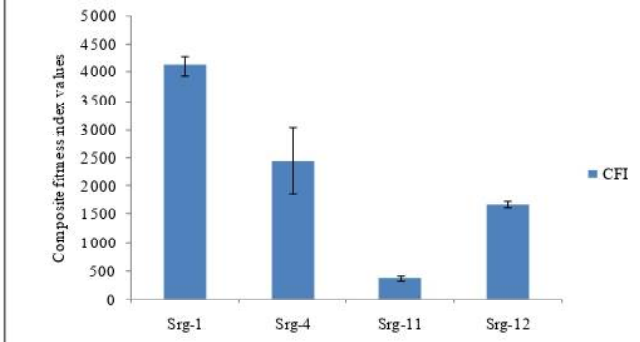

A (Isolates collected from district Khushab-2017



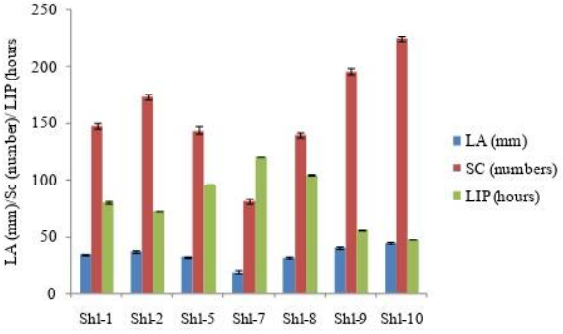

B (Composite fitness index of district Khushab-2017



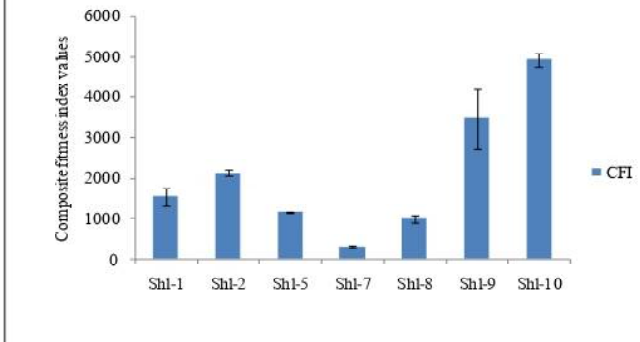

C (Isolates collected from district Sargodha-2017



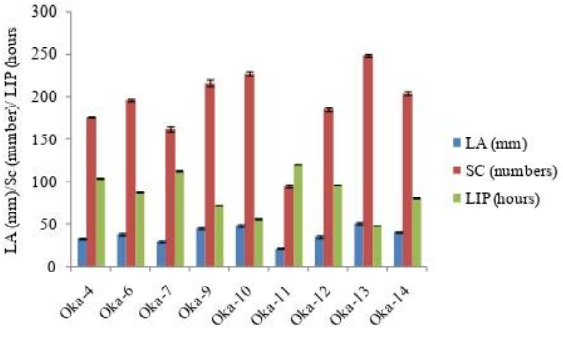

D (Composite fitness index of district Sargodha-2017



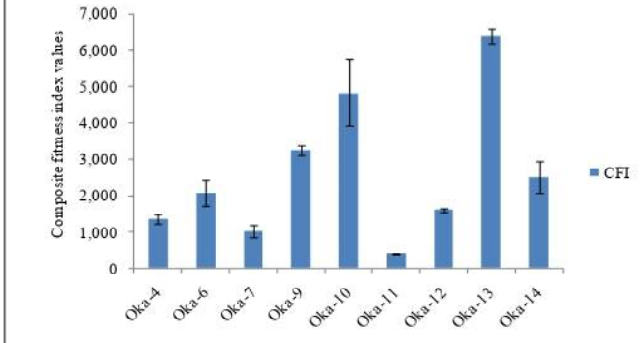

D (Isolates collected from district Sahiwal-2017




E (Composite fitness index of district Sahiwal-2017




E (Isolates collected from district Okara-2017




F (Composite fitness index of district Okara-2017

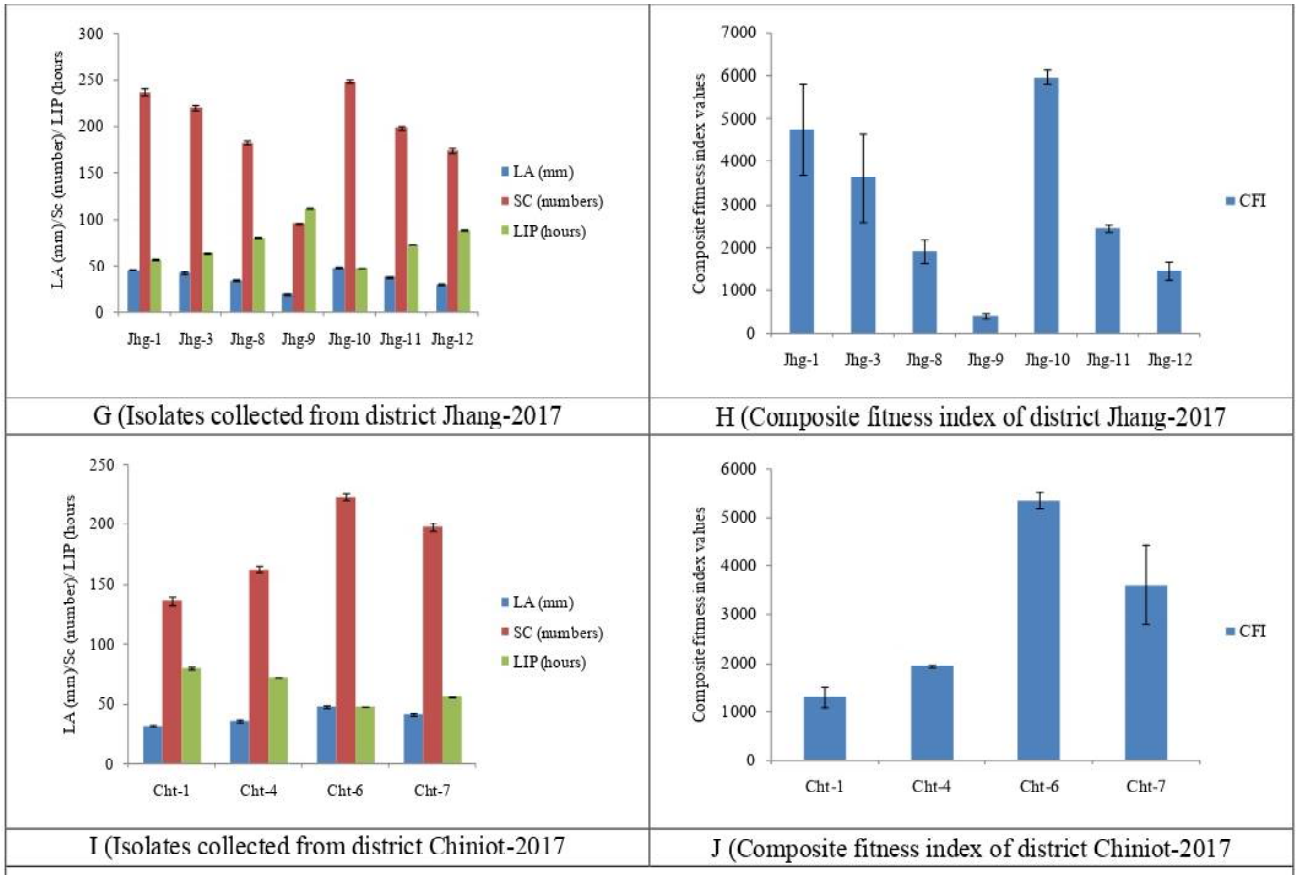
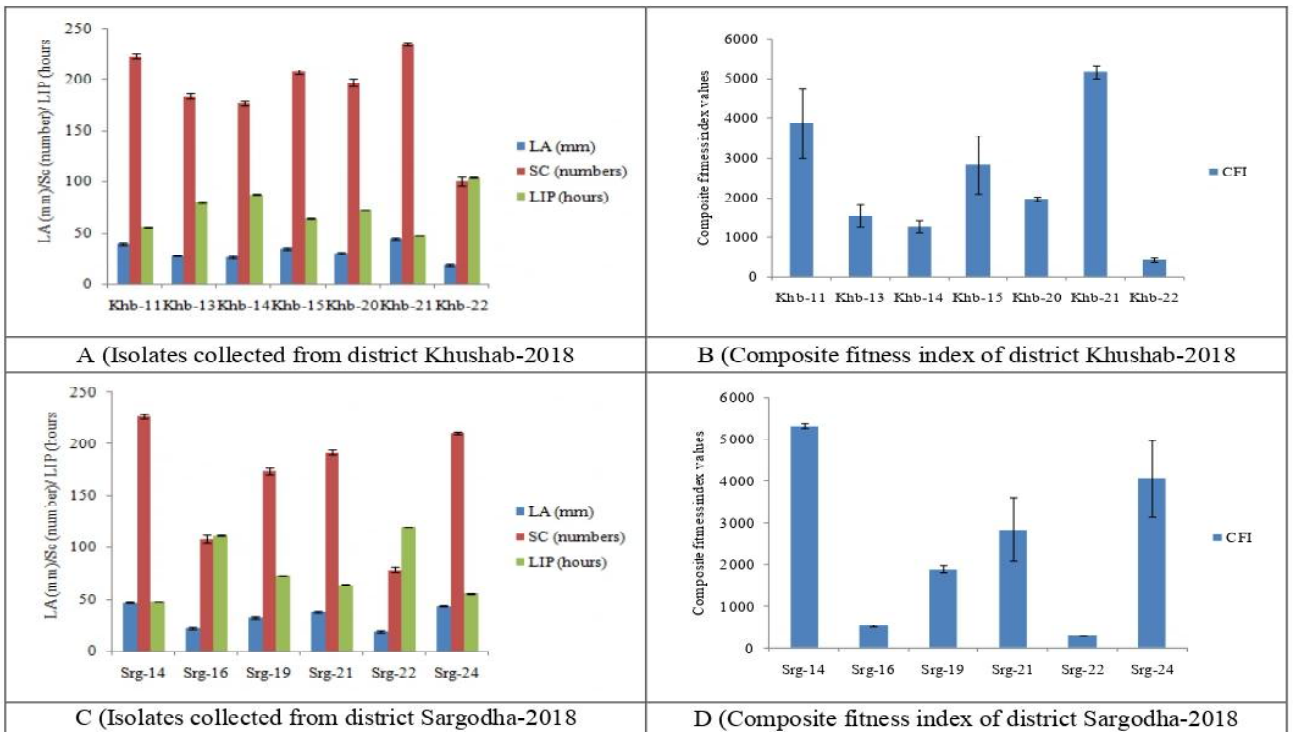



**Fig 4 (A-J):** Pathogen Fitness test and Composite Fitness Index of isolates collected from potato growing areas of Punjab during 2017.

## Pathogen Fitness test Results-2018



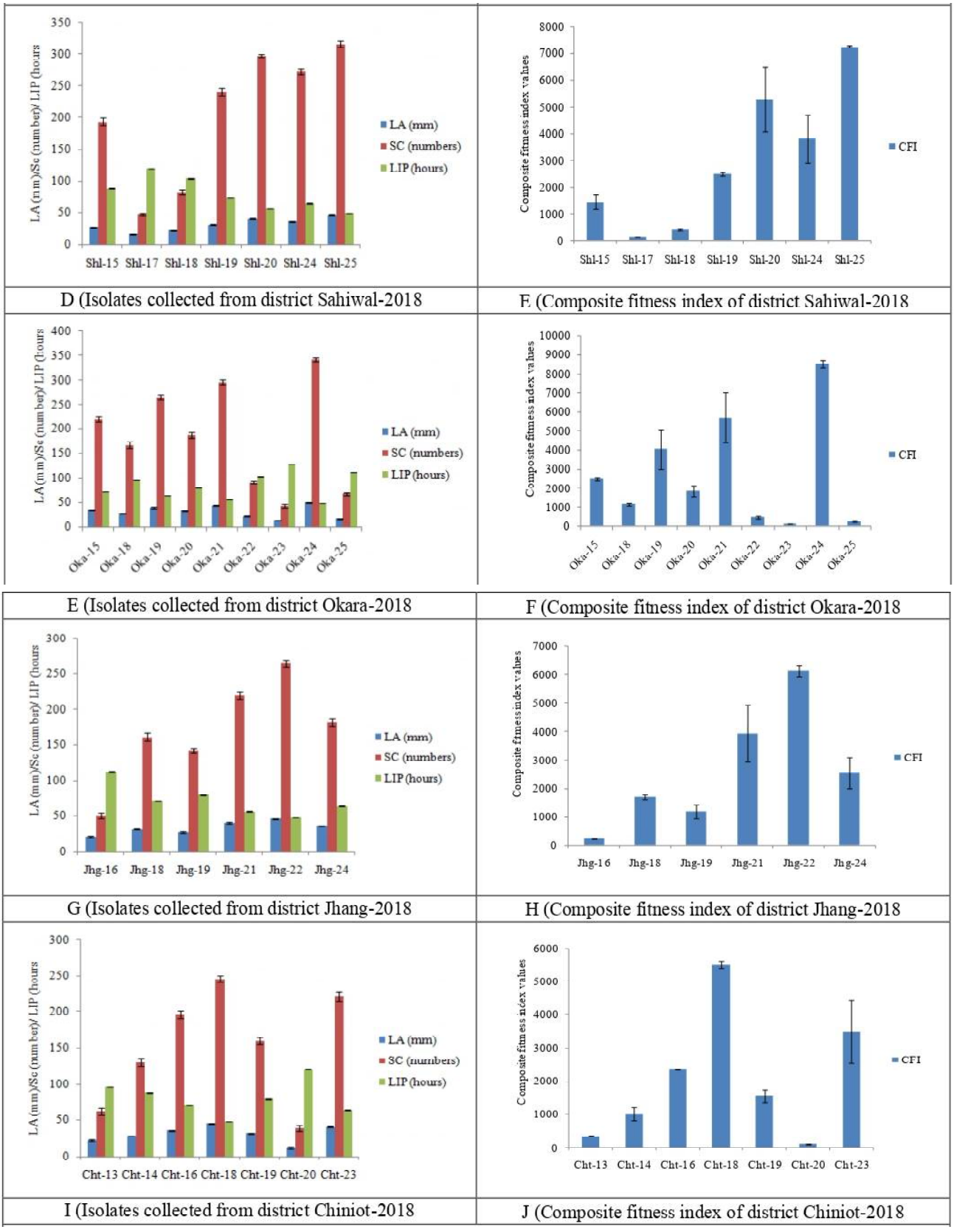



**Fig 5 (A-J):** Pathogen Fitness test and Composite Fitness Index of isolates collected from potato growing areas of Punjab during 2018.

logically estimated overall fitness by calculating the composite fitness index.

In present study, all selected aggressive isolates tested gave positive necrosis reactions on detached leaves and the indications are that isolates have low virulencemay also be indicated by low incidence of infection and also slowed down rates of necrosis and vice versa, although additional investigation would be essential to firmly set up this basic concept.

However, pathogen fitness test on detached leaf assays clearly offer a reliable preliminary test of virulence which is rapid almost 2 min to set up a single leaf assay while second to assessed it later definite and requires minute inoculum. According to our findings, detached leaf assays are useful tool for epidemiological studies, where large numbers of isolates from potato growing areas need to be assessed.

## CONCLUSION

The findings have implications in terms of justification of change in population structures of *P. infestans* across the world, but also in terms of epidemiology and disease management strategies.

Host adaptationhas significant epidemiological consequences in those areas where two or more potential hosts grow which is often the case in Punjab, where two hosts like potato and tomato are often grown in the same field. The prevalence of isolates, including some with a low aggressiveness could also result from differences in survival abilities. Considering this fact that the pathogen, *P. infestans* is a poor saprophyte, its populations undergo extreme demographic changes when the hosts are absent. In such circumstances, balanced pathogenicity and to asses' pathogen fitness on detached leaf assay is valuable in the long term, since it has increased the possibility for the pathogen to control hosts and hence reduce the effects of an unfavorable period in its life cycle of the pathogen. Differential survival abilities may also be related to aggressiveness which is some evidence that variation exists among *P. infestans* isolates.

## ACKNOWLEDGEMENT

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