Appraisal Of Plant Activators And Chemicals Against Brown Leaf Spot Of Citrus In Relation To Epidemiological Factors

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Abstract
Citrus is one of most important fruit crop of the world that belongs to family *Rutaceae*. It is a rich source of vitamins specially, vitamin C. It is consumed as a fresh fruit and also in processed form. Its production is affected due to various biotic (fungi, bacteria, virus and nematodes) and abiotic factors like extreme temperature, high humidity and rainfall. Among fungal diseases, Brown leaf spot caused by *Alternaria citri* is one of the most destructive diseases of citrus. *Alternaria* is a fungus that is present everywhere and associated with plants, soil and animals and many of them are plant pathogens, causing several diseases including fruit rots and immature fruit drops. This disease mainly attacks the twigs of plants, leaves and citrus fruit. Disease samples of typical symptoms of necrotic spots followed by yellow halo were collected from different citrus orchard. After collecting the samples these were brought in the Plant Disease Diagnostic Laboratory for further isolation, purification and identification of different pathogens associated with *Alternaria* brown spot. In vitro evaluation of different chemicals and plant activators were done by using different concentrations (200, 600 and 900ppm) against isolated pathogen by using poisoned food technique. Mancozeb and salicylic acid was highly effective against *Alternaria alternata*.

Introduction

Citrus is known as one of the most valuable fruit crop of the world which belongs to *Rutaceae* family and was originated in Southeast Asia (China and Indonesia) (Talat *et al.*, 2020). Several countries of the Mediterranean basin such as Greece, Italy, Spain, Tunisia, and Turkey are important producers of citrus fruit, as well as additional regions with Mediterranean climate such as Australia, California, Florida, and South Africa. (FAO, 2020). Pakistan ranks 12th among the citrus-growing countries in the world (Memon, 2017). Its production worldwide was 194.4 million tons from an area of 13.9 million hectares and in Pakistan citrus production was estimated as 2.29 million tones thousand tons from an area of 206.6 thousand hectares (FAOSTAT, 2020). Citrus has a great medicinal value; It is used to prevent cold and malaria and to promote blood coagulation (Anonymous, 2017). Several medicinally important compounds such as flavonoids, coumarins, vitamins, ascorbic acid, citric acid, minerals, and phenols are isolated from citrus fruit. Therefore, it has become an essential ingredient of soft drinks, alcoholic beverages, hot drinks (tea), pickles and spice (Liaquat *et al.*, 2021). Due to the presence of essential volatile compounds, it releases various bioactive compounds after addition with fermented food. Commercially, lemon fruit has a great market value. Due to the presence of citric acid, it is used as a food additive (Inglese *et al.*, 2019).

Citrus is a good sources of vitamin C with 5-9% sugar content. It also contains minerals such as phosphorus, iron and calcium. The major bioactive compounds present are Iso-limonene, citral, limonene, phenolics, flavonones, pectin, linalool, decanal, and nonanal, accounting for several health benefits. Pectin and heteropolysachharides also play a major role as dietary fibers. (Chhikara *et al.*, 2018). A number of diseases which are influencing citrus family are Melanose (Diaporthe citri), Canker (*Xanthomonas* citri pv. *citri*), Greening (*Candidatus Liberibacter asiaticus*), Wither tip (*Colletotrichum gloeosporioide* spenz), Scab (*Elsinoe fawceite*), Black pit (*Pseudomonas syringae*), *Alternaria* Brown Spot (*Alternaria alternate*), Gummosis (*Phytophthora nicotianae*) etc. (Thakur *et al.*, 2020). Among these Brown leaf spot caused by *Alternaria alternata* is the most drastic and damaging one and reduces 40% of citrus yield worldwide. (Aiello *et al.*,2020). *Alternaria alternata* has two distinct pathotypes that are linked to citrus and have been

classified on the basis of host specificity and toxin production. Environmental factors like amount of rainfall, duration of leaf wetness, Relative humidity and average daily temperatures has a great effect on disease severity. The infection rate is highest on days with optimum temperatures (20 to 28°C), and it is slightly lower on days with lower or higher temperatures. When the relative humidity is 74 percent, conidia production on the leaf lesion is reduced, but as the relative humidity rises from 85-100% it gets severe (Farooq *et al.*, 2018).

Successful infection of *Alternaria alternate* requires temperature of 25-27 °C with leaf wetness duration of 10hrs (Farooq *et al.*, 2018). As the temperature drops, longer wetting durations are required for infection. Infection is rare at 32 °C. Small levels of infection can arise after 4–8 hours of leaf wetness, but significant infection generally requires 10–12 hours of wetness.

Different management strategies against Brown leaf spot is use, including chemical control, use of antibiotics, nutritionals, biological control, phyto extracts and plant activators but the most effective strategy is the use of resistant varieties. Citron, Mandarins. Calamondin and kumquat are few resistant varieties of citrus. While the important mandarin cultivars, such as 'Emperor', 'Fortune', 'Nova' and 'Murcott', are susceptible to ABS (Arlotta *et al.*, 2020). In order to assess source of resistance screening was done on ten different varieties of citrus. Brown Leaf Spot has become a serious threat to citrus industry in recent years. Chemical application is important in such situation. The use of chemicals has been reported to have positive effect and are believed to be the most effective way to control the disease (khadka *et al.*, 2020).

Another useful strategy for disease control is the use of plant activators. The plant activator acibenzolar (Bion) is a non-fungicidal compound that can be used to combat plant pathogens by inducing the host plant's natural defence mechanisms. Miles *et al.* (2005) used plant activator (acibenzolar), copper hydroxide, captan, iprodione and chlorothalonil/pyrimthanil in the field as a control of *Alternaria* brown leaf spot. Plant activators help the plants to produce defensive responses that can prevent or delay pathogen infection, and some of them have been shown to increase yield. These activators improve the defence system of plant by involving in defense mechanism. Plant activators represent to be environment friendly compounds capable of inducing resistance against many plant pathogens. Earlier studies showed that foliar spray of plant defense inducers could slow down *Alternaria* brown leaf spot disease progress (Rahman *et al.*,2019) Hu *et al.* (2018) used four different plant activators including salicylic acid, oxalic acid, acibenzolar-S-methyl and potassium phosphate provided significant control on disease progress. These treatments not only increased yield but also better fruit quality.

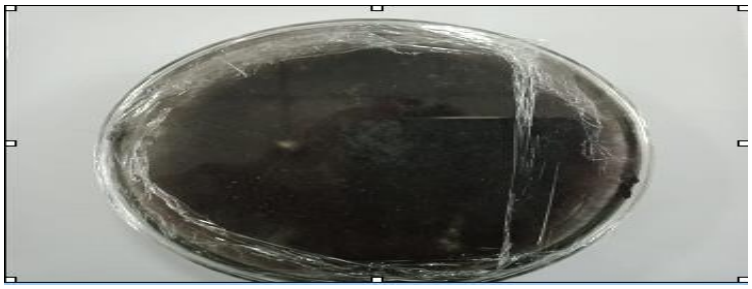
Keeping in view above mentioned facts, present research was made with the following objectives.
- Assessment of citrus germplasm against brown leaf spot to find out source of resistance.
- Evaluation of chemicals and plant activators against brown leaf spot of citrus.
- Determination of environmental factor conducive for the development of brown leaf spot through co-relation and regression analysis.

Methodology

Collection of samples

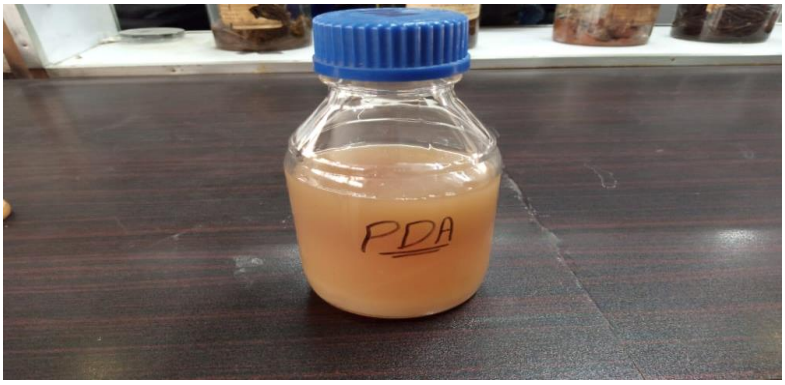
Infected samples of brown spot disease after visiting different citrus orchards. Samples of citrus leaves and fruits having brown spot were collected from different locations. The samples were collected in brown paper bags and marked their location and then these samples were brought to plant pathology lab.

Diseased samples



Preparation of PDA media

Potato dextrose agar (PDA) medium was prepared to isolate the fungus. For the preparation of one-liter media such as PDA, 250g peeled potato were boiled in 1000mL of distilled water in the pan for 10-15 minutes in order to get starch. After cooling of water, the remaining ingredients i.e. 10g Dextrose and 10g Agar-agar were added into starch and mixed thoroughly. After this the media was autoclaved at 121 ºC maintaining the 15 Psi for 30 minutes.



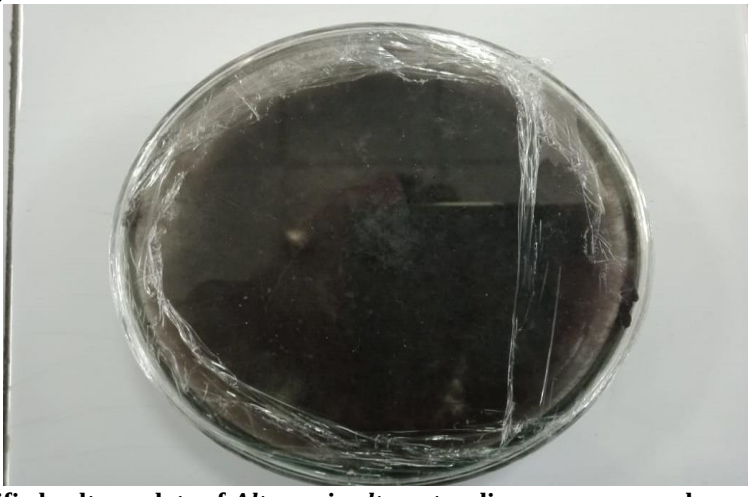
Preparation of Potato Dextrose Agar medium

Isolation and purification

The diseased samples along with some healthy portion were cut into small pieces of 2-3mm size. 1% of the NaOCl solution was prepared as disinfectant. The cut parts were dipped for 30 seconds in sodium hypochlorite (NaOCl) and then three washing of distilled water was given. After washing, samples were dried on sterilized tissue paper. All the work was done in laminar flow (RTVL-1312, Robus United Kingdom). Then with the help of sterilized forceps, the samples were placed on already prepared media. After wrapping the plates were placed in incubator at 28℃. The fungal growth was observed after 24 hours. Purification of fungus was done by using the single spore method. By using the sterilized needle, fungal spore was picked and transferred on a new media plate. The single spore was selected after 24 hours of incubation and was placed on another media plate for further purification.

Identification of pathogen

The purified fungal colonies were identified under microscope on the basis of colony color, growth pattern and spore shape. Dark brown conidia in chains were observed ranging in sizes from 5 to 35 µm. The conidia surface was smooth to verruculose, slightly constricted with 4–6 transverse septa, the lower part of each portion had one or two longitudinal septa (Basım *et al.*, 2018).



Purified culture plate of *Alternaria alternata*, olivaceous green colony color

Pathogenicity Test

Pathogenicity of isolated pathogen of brown spot was done to confirm the disease. One-year-old citrus plants were collected from the research area of institute of horticultural sciences and planted in 8-inch-diameter pots containing sterilized sandy loam soil, which were then brought and maintained in the green house for better growth. The surface of the leaves was sterilized with 70% ethanol before being washed with distilled water. Suspension of the test pathogen was prepared. A hemocytometer (QJ1102, Qiujing) was used to measure the pathogen suspension at a rate of 1x104spores/mL Early in the morning, a suspension of the test pathogen was sprayed onto the leaves. Four plants were inoculated, with one serving as a control, which was sprayed with sterilized water. On a daily basis, the inoculated plants were observed. The symptomatic plant parts were taken after 2-3 weeks of infection symptoms showed on inoculated plants. The pathogen was re-isolated, purified, and injected at 27°C on PDA media. After confirming that the isolated pathogen was identical to the previous one, the pathogen was multiplied and stored for future experiments.

Correlation of environmental factors and disease development:

Environmental factors (temperature, relative humidity, rainfall and wind speed) were observed under field conditions. Data were monitored hourly in field with automated meteorological stations, including sensors located within the row in the experimental area for air, temperature, relative humidity, rainfall and wind speed (Bassimba *et al.* 2017). Infection period were monitored weekly from September 2020 to December 2020. Data was collected on weekly basis from metrological station and evaluated by using disease rating scale. The environmental factors were correlated with brown leaf spot disease incidence through correlation and stepwise regression analysis (Zhang *et al.*, 2019). Finally, disease predictive model was developed based on environmental conditions having a significant influence on brown leaf spot disease development through stepwise regression.

Evaluation of plant activators in vivo condition:

Citrus plant of 1-year age of susceptible varieties (rough lemon, mandarin, sweet orange, grapefruit) were collected from research are of institute of horticulture sciences and planted in the field area under RCBD design. All the horticulture practices were done at proper time to keep plant in good condition. Three different concentration (0.25, 0.5and 0.75%) of plant activators (Salicylic acid, Potassium dihydrogen phosphate, Benzoic acid, Di-potassium hydrogen phosphate, Calcium Chloride and Citric Acid) were made by adding 5, 7.5 and 10g into each bottle of an activator, having 1 liter of distilled water and then applied on plants early in the morning with the help of nozzle sprayer. Distilled water as control was applied on plants for comparison. Weekly basis data were recorded to evaluate the efficacy of plant activators against brown leaf spot. The recorded data was analyzed through Statistical Analysis System.

Different plant activators and their active ingredients

Serial No	Name Of Chemical	Active Ingredient	Company Name
1.	Salicylic acid	Beta hydroxyl acid (BHA)	Seatle Pharma Pakistan (pvt) Ltd
2.	Potassium dihydrogen phosphate	Monopotassium phosphate	Emsure
3.	Benzoic acid	Sodium benzoate and potassium benzoate	Glaxo SmithKline Pharmaceuticals Ltd
4.	Di-potassium hydrogen phosphate	Potassium, Hydrogen phosphate, Phosphoric acid	Fisher Chemical
5.	Calcium Chloride	Hydrochloric Acid, sodium hydroxide	Novartis Ltd
6.	Citric Acid	Citric acid	Ashahi chemicals

Preparation of Stock solution

To prepare stock solution, 1g of pure active ingredient mix into 100 mL of sterilized water. This stock solution is used for making further concentration. For making 200,600 and 900 ppm concentration, 2,6 and 9 mL of stock solution is mixed into 100 ml of sterilized water. of water.

Evaluation of Chemicals In-vitro Condition against brown leaf spot:

Six chemicals (Topsin M, Cabriotop, Forum top, Fossil, Excel and Kocide) were evaluated at different concentration against brown leaf spot with poisoned food technique. To obtain better results, three replications of each concentration were prepared by adding 4mL, 6mL, and 9mL, stock solution each chemical in 100mL of distilled water. These concentrations were prepared and mixed with PDA. These amended media of different concentration were poured into petri plates of 90mm diameter in laminar flow chamber (RTVL-1312, Robus United Kingdom) and was solidified.9mm fungal discs were taken with the help of sterilized coke borer and inoculated in plates of different concentration while control only contain media. After 48 hours, 72 hours, and 96 hours of incubation, data was collected. The inhibition of mycelial growth showed the effect of different chemicals and concentrations. From the below-mentioned formula, the inhibition of mycelial growth was checked.

Inhibition of mycelial growth (%) = (C – T/C) x 100 'C' is the mean diameter of the fungal colony in control plates and 'T' is mean diameter of the fungal colony in poisoned plates (Gupta and Tripathi, 2011)

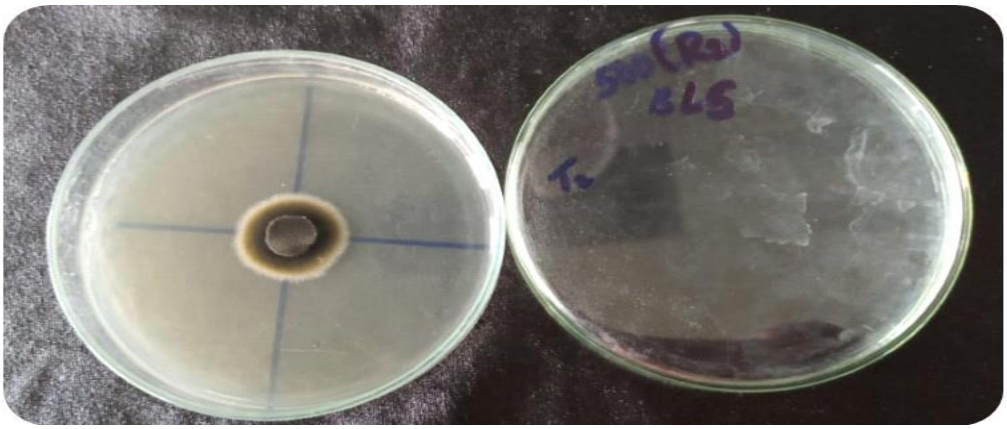


Fig. Inhibition of fungal growth

Different fungicides and their active ingredients

Name of Chemical	Active Ingredient	Company Name
Topsin M	Thiophenate Methyl (70%)	Arysta
Cabriotop	Pyraclostrobin (5%) & Metiram (55 %)	FMC
Forum top	Dimethomorph (9%)+ Metirem (44%)	BASF
Mancozeb	Azoxystrobin (18%) + Difenoconazol (11%)	Smart Pesticide
Excel	Difenoconazol (80%)	Sun crop Pesticides
Kocide	Copper hydro-oxide (54%)	FMC
Control	Distilled water	

Evaluation of chemicals in vivo condition against brown leaf spot:

Citrus plant of 1-year age was collected from research area Institute of Horticulture Sciences (HIS) and planted in the field area under randomized complete block design (RCBD), PxP 1.0m and RxR 1.5m spacing was maintained. Suspension 1×10^6 spore/mL suspension of the test pathogen was prepared was sprayed onto the leaves early in the morning. Five symptomatic plants from each variety were selected for recording observations. From each plant, three leaves from top, middle and bottom portions were observed. Most effective chemicals were applied on plants against pathogen. Concentration was prepared in % form like Excel (10), Fossil (15%) with combination (Excel 10 + Fossil 15%) were mixed with water and sprayed on plants. In plants used as control, only water was sprayed. The disease rating was done by using 0-5 scale and average disease severity.

Scale	Disease incidence (%)	Response
0	No lesions	Immune
1	Few circles	HR
2	1-5%	R
3	6-10%	MR
4	11-25%	MS
5	26-50%	S

(Mohammad and William, 2012)

Data was collected after 7 days' intervals and data was collected through following disease rating scale.

Statistical analysis

Completely Randomized Design (CRD) and Randomized Complete Block Design (DCBD) was used in laboratory and field condition. ANOVA was used to test the significant results of data whereas for the mean value LSD test was used.

Results and Discussion

Evaluation of different chemicals against *Xanthomonas campestris* pv. *fici* under lab conditions

Analysis of variance (ANOVA) indicated that all treatments (T), concentrations (C), days (D) and their interactions between treatments and concentrations (T × C), treatments and days (T × D) expressed significant results while interaction between concentrations and days (C × D), treatments, concentrations and days (T × C × D) expressed non-significant results as indicated in (Table 4.1). Minimum fungal growth was expressed by Mancozeb (5.778mm) followed by, Topsin M (9.019mm), Cabriotop (10.130mm), Excel (18.889 mm), Forum top (27.870mm), kocide (30.593mm), as compared to control indicated in (Table 4.2). In case of interaction between treatments and

concentrations (T x C) indicated that minimum fungal growth was expressed at 200ppm and then at 600ppm and 900ppm by Mancozeb (13.55, 15.05, 17.05) followed by Excel (13.222, 9.500, 7.666), Forum top (21.500 ,27.000, 26.333, Topsin M (21.500,18.556,16.611), Kocide (31.222, 30.444, 30.111), as compared to control shown in (Tale 4.3 and Fig 4.2). The interaction between treatments and days (T × D) expressed that minimum fungal growth was developed after 24hours, 48 and 72 hours by Mancozeb (5.056,5.778,6.500) followed by Topsin M (7.444,9.222 ,10.389), Cabriotop (9.111, 10.389, 10.889), Excel (18.278 ,18.889, 19.500), Forum top (26.944,28.056,28.611), Kocide (29.000,30.889,31.889) as compared to control shown.

Table ANOVA for In-vitro evaluation of different chemicals against *Alternaria alternate*.

SOV	DF	SS	MS	F	P
Treatment (T)	6	35280.3	5880.05	2171.63	0.0000*
Concentration(C)	2	554.7	277.33	102.43	0.0000*
Days (D)	2	184.9	92.43	34.14	0.0000*
T ✕ C	12	125.3	10.44	3.86	0.0001*
T ✕ D	12	74.2	6.18	2.28	0.0017*
C ✕ D	4	29.6	7.39	2.73	0.0021*
T × C ✕ D	24	105.6	4.40	1.63	0.0054*
Error	126	341.2	2.71		
Total	188	36695.6			

(*) = Significant at P< 0.05 by LSD test. (Ns) = Non-significant at P> 0.05 by LSD test.

Table In-vitro evaluation of different chemicals against *Alternaria alternata*

Treatment	Fungal growth (mm)
Kocide	30.593
Forum top	27.870
Excel	18.889
Cabriotop	10.130
Topsin M	9.019
Mancozeb	5.778
Control	0.0000h
LSD	**0.2820**

Table Impact of interaction between treatments and concentration against *Alternaria alternata* under lab conditions

Treatment	Fungal growth (mm)		
	C1	C2	C3
Kocide	31.222	30.444	30.111
Forum top	21.500	27.000	26.333
Excel	13.222	9.500	7.667
Cabriotop	12.056	8.444	6.556
Topsin M	21.500	18.556	16.611
Mancozeb	7.444	5.778	4.111
Control	48.556	48.556	48.556
LSD	**1.5351**		

C1= 200ppm, **C2**= 600ppm, **C3**= 900ppm

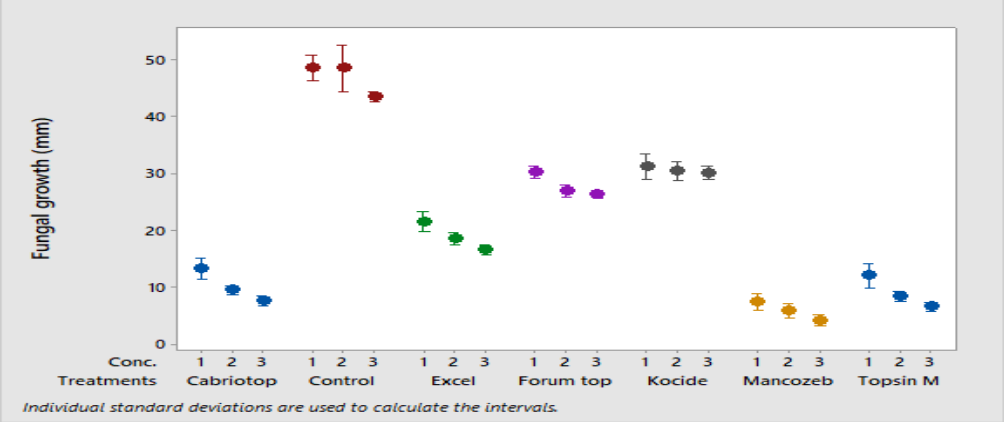


Fig Impact of interaction between treatments and concentration against *Alternaria alternata* under lab conditions

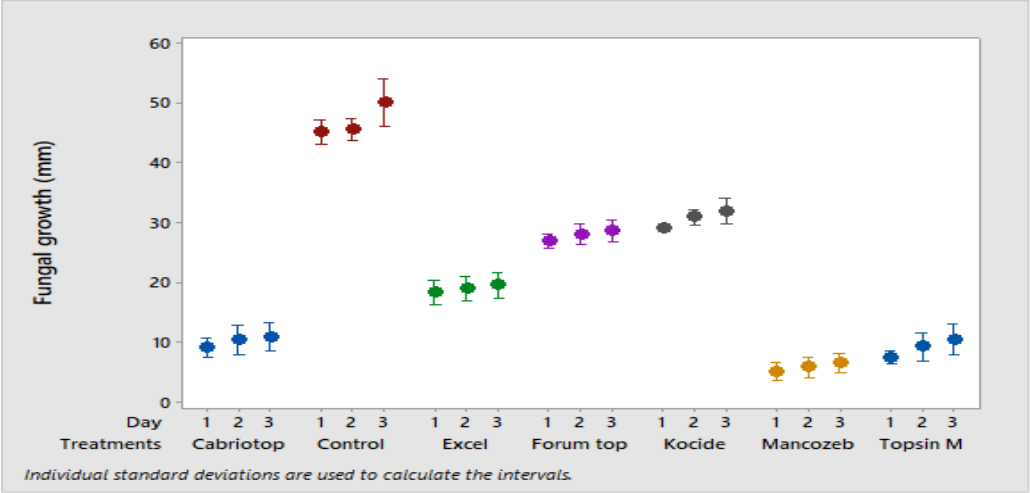


Fig Impact of interaction between treatments and Day against *Alternaria alternata* under lab conditions

Table ANOVA for In-vitro evaluation of different Plant activators against *Alternaria alternate*.

SOV	DF	SS	MS	F	P
Treatment (T)	6	35668.2	5944.70	2733.69	0.0000
Concentration(C)	2	453.6	226.78	104.28	0.0000
Days (D)	2	129.4	64.71	29.76	0.0000
T ✕ C	12	75.6	6.30	2.90	0.0004
T ✕ D	12	374.7	31.22	14.36	0.0000
C ✕ D	4	12.0	3.00	1.38	0.0441
T × C ✕ D	24	101.6	4.23	1.95	0.0000
Error	126	274.0	2.17		
Total	188	37089.0			

(*) = Significant at P< 0.05 by LSD test. (Ns) = Non-significant at P> 0.05 by LSD test.

Table 4.6 In-vitro evaluation of different plant activators against *Alternaria alternate*

Plant Activators	Fungal Growth
KH2PO4	42.093
Benzoic acid	30.685
Salicylic acid	24.593
K2HPO4	20.167
Citric acid	14.037
CaCl2	12.778
Control	52.815
LSD	0.7943

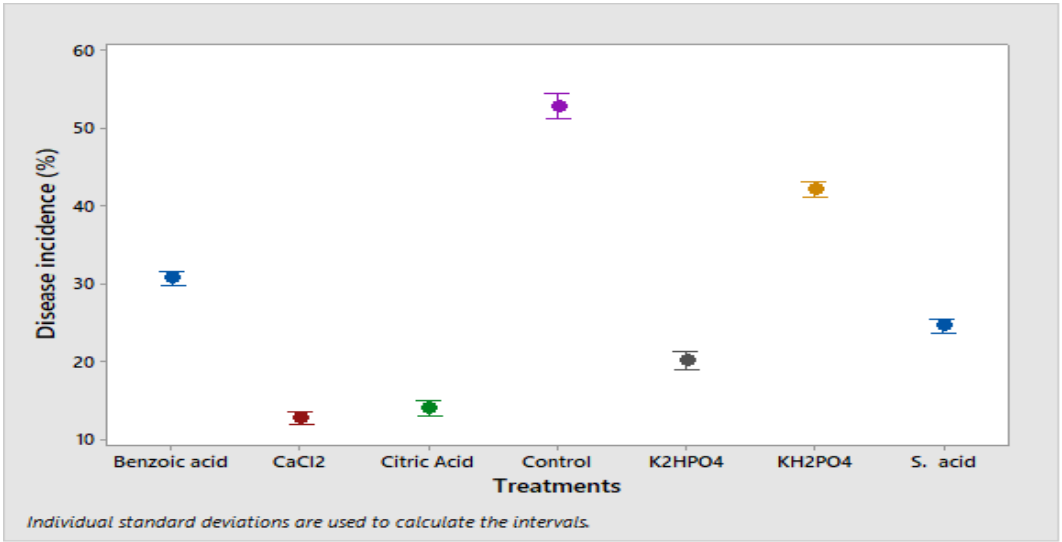


Fig In-vitro evaluation of different Plant activators against *Alternaria alternate*

Table Impact of interaction between treatments and concentration against *Alternaria alternata* under lab conditions

Treatment	Fungal growth (mm)		
	C1	**C2**	**C3**
KH2PO4	43.944	42.167	40.167
Benzoic acid	32.333	30.000	29.722
Salicylic acid	26.167	24.500	23.111
K2HPO4	22.833	19.500	18.167
Citric acid	16.000	14.000	12.111
CaCl2	14.444	14.000	12.111
Control	54.556	54.556	49.333
LSD	1.3757		

C1= 200ppm, **C2**= 600ppm, **C3**= 900ppm

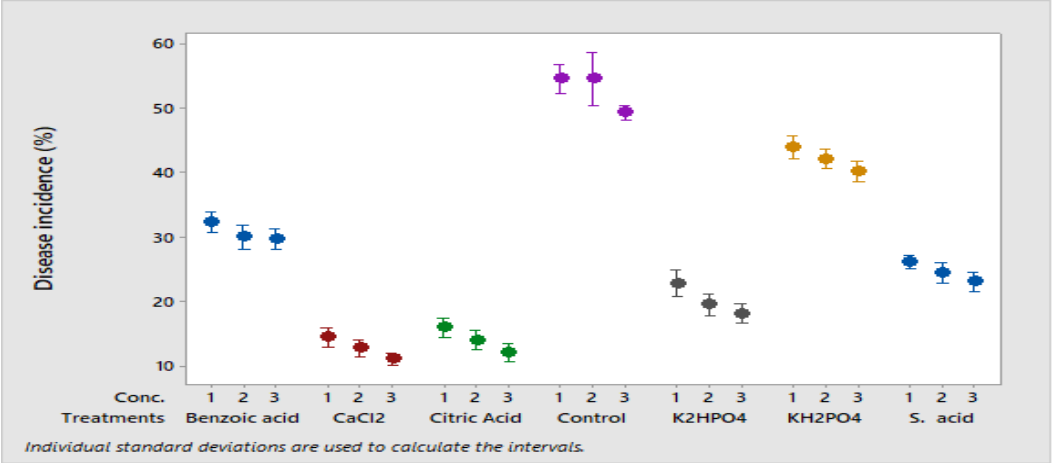


Fig Impact of interaction between treatments and concentration against *Alternaria alternata* under lab conditions

Table Impact of interaction between treatments and days against *Alternaria alternata* under lab conditions

	Fungal growth (mm)		
Treatment	**D1**	**D2**	**D3**
KH2PO4	44.111	42.111	40.056
Benzoic acid	32.944	30.722	28.389
Salicylic acid	26.389	24.556	22.833
K2HPO4	22.278	20.111	18.111
Citric acid	16.000	14.000	12.111
CaCl2	12.056	12.778	13.500
Control	51.056	51.556	55.833
LSD	**1.3757**		

D1= 24 hrs, **D2**= 48hrs, **D3**= 72 hrs

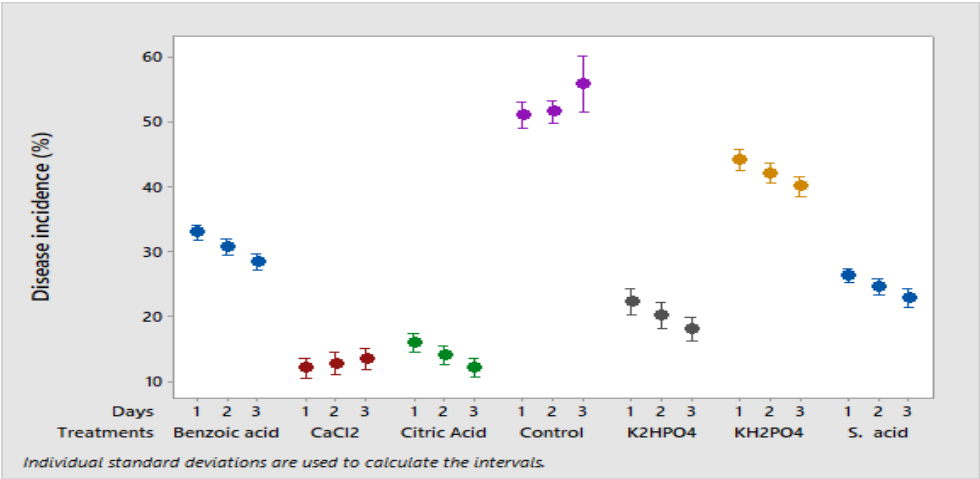


Fig Impact of interaction between treatments and days against *Alternaria alternata* under lab conditions

Table Correlation of environmental factors with citrus melanose disease on different citrus varieties

Varieties	Temperature		Rainfall (mm)	Sunshine (Cambel stokes)	Relative Humidity (%)
	Max.T (ºC)	Min.T (ºC)			
Desi lemon	0 0.975* 0.000	0.984* 0.000	0.975* 0.000	0.986* 0.000	0.974* 0.000
Desi Khattii	0.986* 0.000	0.975* 0.000	0.961* 0.000	0.984* 0.000	0.974* 0.000
Sweet Lemon	0.988* 0.000	0.980* 0.000	0.957* 0.000	0.991* 0.000	0.971* 0.000
Musami	0.967* 0.000	0.990* 0.000	0.966* 0.000	0.982* 0.000	0.973* 0.000
Grapefruit	0.988* 0.000	0.979* 0.000	0.942* 0.000	0.994* 0.000	0.979* 0.000
Kinnow	0.971* 0.000	0.962* 0.000	0.982* 0.000	0.977* 0.000	0.958* 0.000

Assessment of disease predictive model by comparing the dependent variable with regression coefficient

Analysis of variance of regression articulated that T (Max. and Min.), RH, RF and SS significant contribution towards development of disease. The R^2 value 98.45 expressed the model to be statistically suitable under the given environment conditions. Variables coefficients of regression model for citrus are given in table.

Table Regression model's coefficients of variables for *Alternaria* brown leaf spot of citrus

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	5	1410.83	282.166	533.11	0.000*
Max T	1	6.58	6.580	12.43	0.001*
Min T	1	5.79	5.794	10.95	0.002*
RF	1	7.80	7.801	14.74	0.000*
SS	1	0.42	0.423	0.80	0.376(NS)
RH	1	1.79	1.789	3.38	0.073(NS)

***=Significant at P<0.05**

Estimation of model on the basis of predicted and observed values:

For assessing the reliability of model value differences of observed and predicted data points were estimated. Among the observed values 14 data points were beyond reference line (standard error =0.695167) and created an error in experiment. According to the graph, maximum prediction has differences were consolidated between 95% interval of confidence (C.I) and 95% of interval predictive (P.I) which show that there were a good fit in between predictive and observed values.

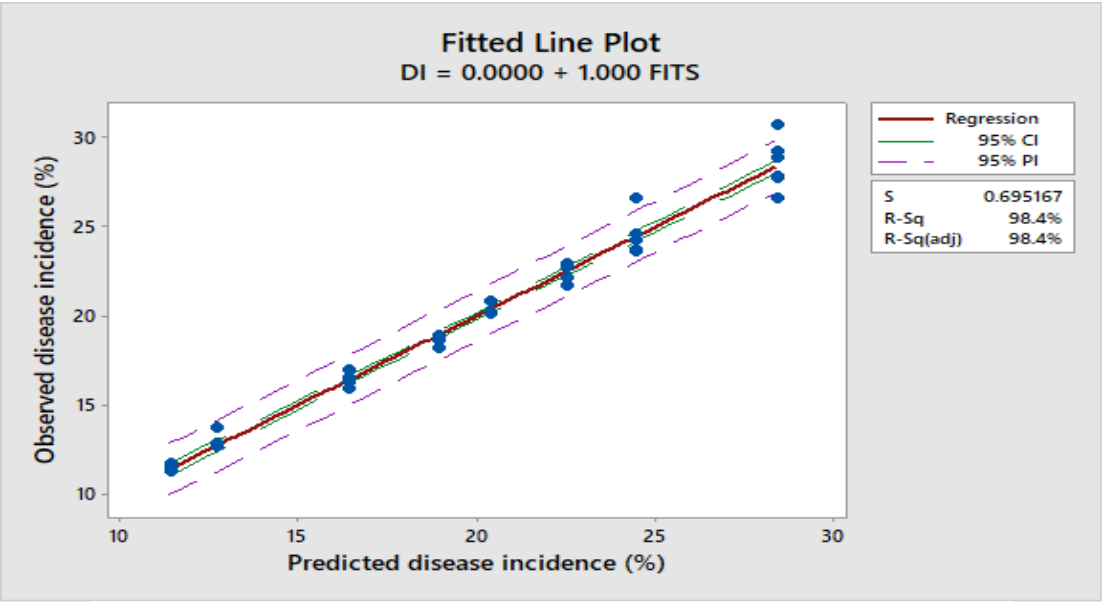
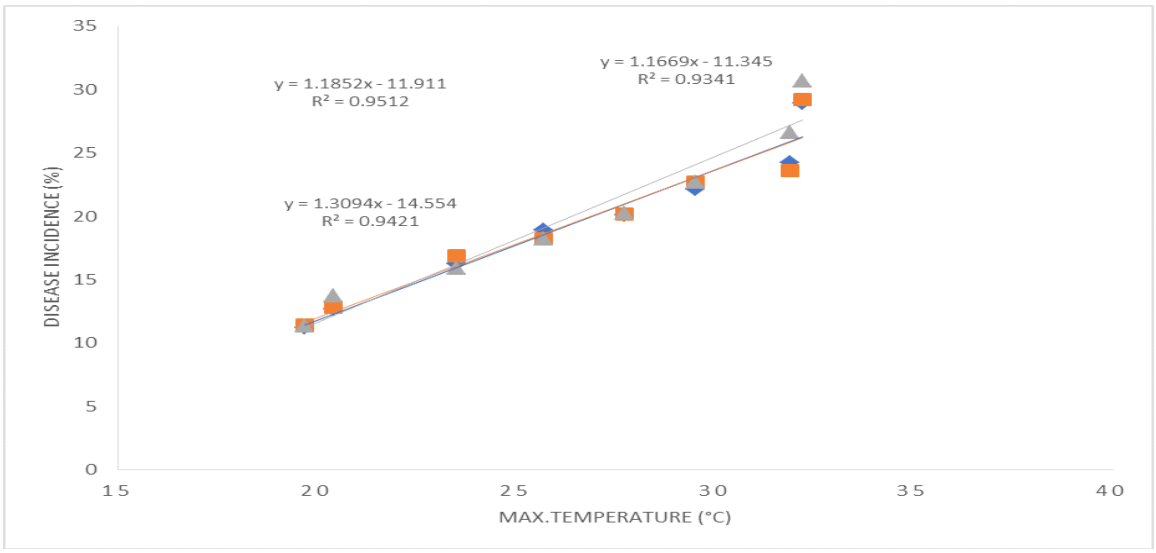


Fig A fitted line plot for Brown leaf spot with observed and predicted data points



Relationship between maximum temperature (Max T) and disease incidence of *Alternaria alternate* viz, Kinno, Mosambi and Desi Lemon.

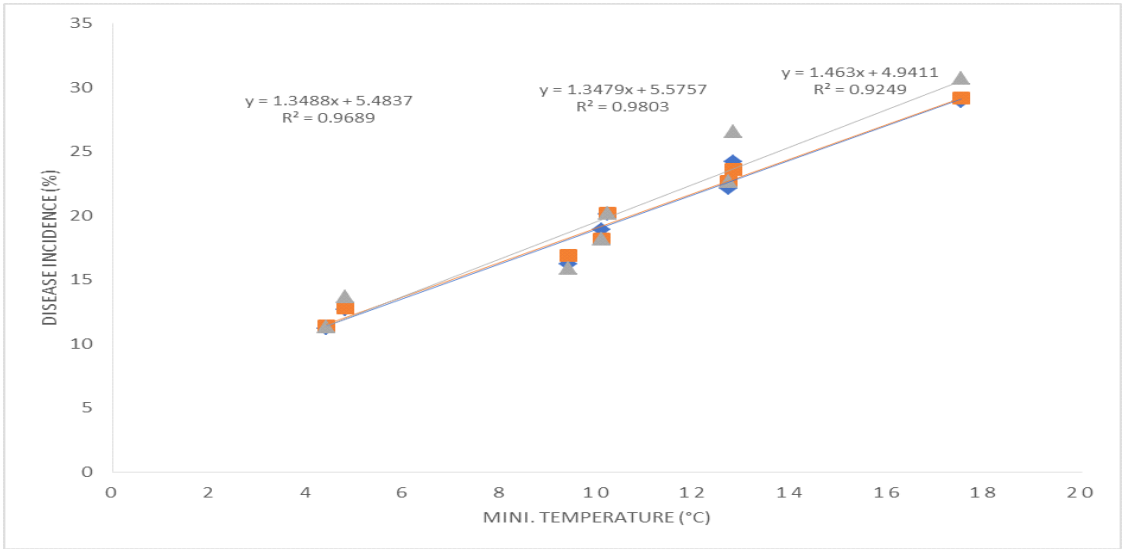


Fig Relationship between minimum temperature (Min T) and disease incidence of *Alternaria alternate* viz, Kinno, Mosambi and Desi Lemon.

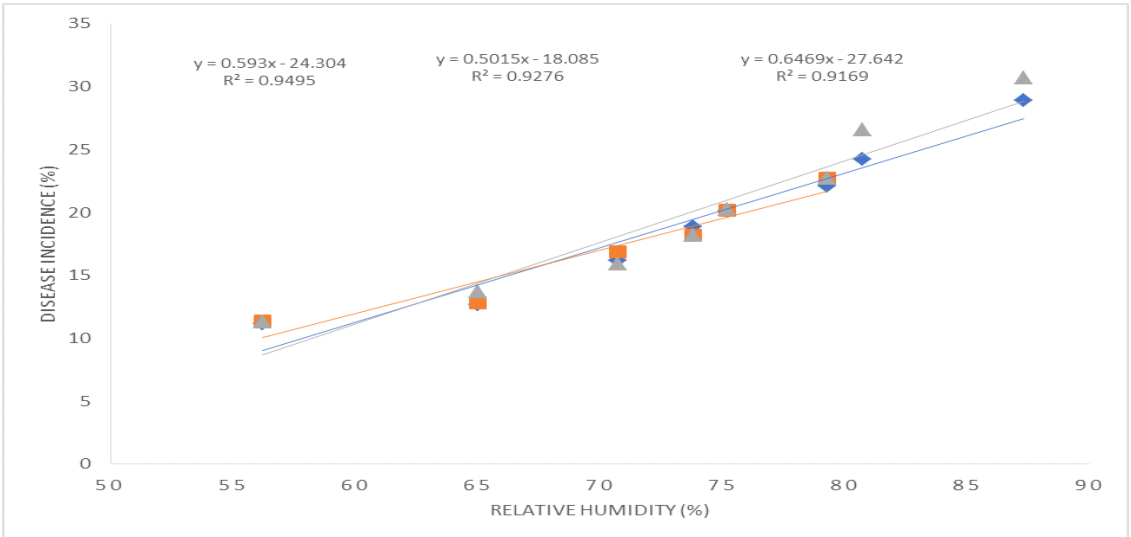


Fig Relationship between Relative humidity (RH) and disease incidence of *Alternaria alternate* viz, Kinno, Mosambi and Desi Lemon.

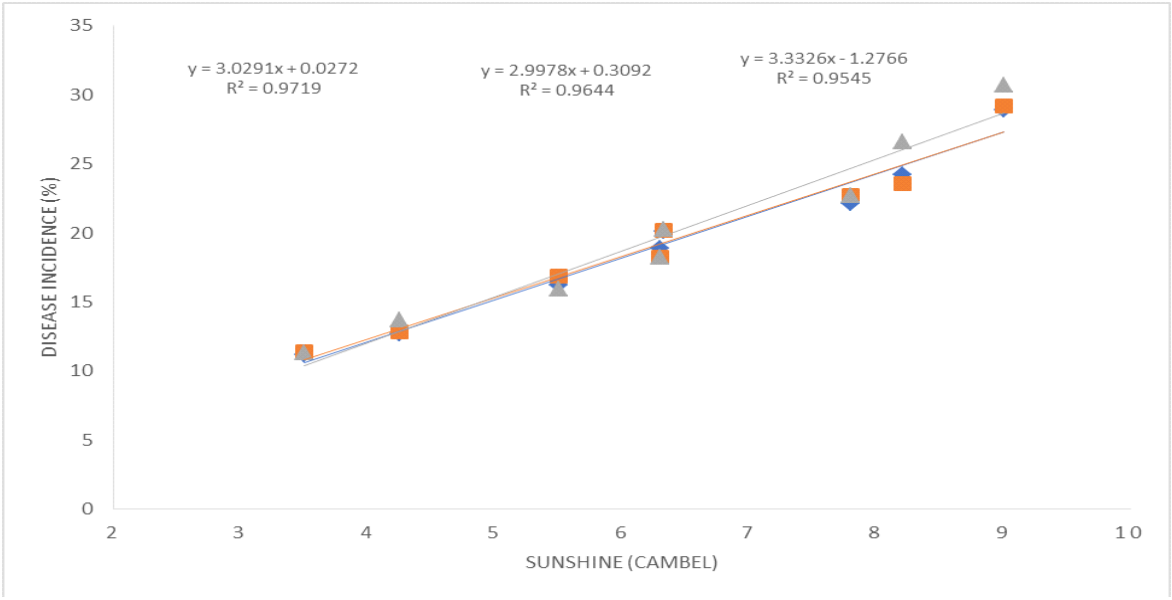


Fig Relationship between Sunshine (SS) and disease incidence of *Alternaria alternate* viz, Kinno, Mosambi and Desi Lemon.

Development and evaluation of Brown leaf spot predictive model based on 4 months

$Y=5.58+0.520X^1+0.447X^2+1.388X^3+0.549X^4+0.1164X^5$ (Y= Disease incidence X^1=maximum temperature, X^2=Minimum temperature, X^3=rainfall, X^4=sunshine, X^5=relative humidity).

The R^2 value 90.32% that model was statistically appropriate under prescribed environmental conditions. Some data points deviated from the reference line according to the normal probability graph (Fig.). While most values were scattered equally around the residual line in case of residual c. fit model which showed better fit of regression model.

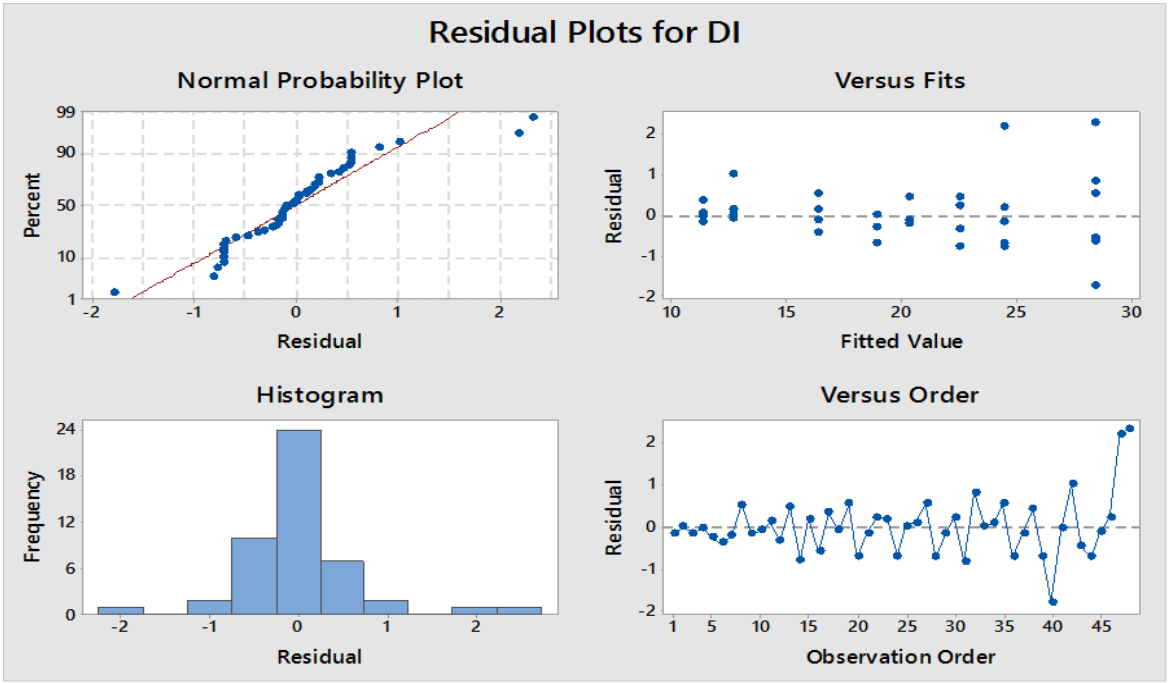


Fig Residual plots for disease incidence of Brown leaf spot predictive model based on 4 months

Expression of symptoms is the true picture of disease development. Appearance of small brown or black spots, surrounded by yellowish halos is the characteristic symptoms of Brown leaf spot. With time, the necrotic spots enlarged, became irregular, and often coalesced blighting down the leaf. Brown to black lesions appears on fruit, corky eruptions will sometimes develop and dislodge, pockmark can also be observed on the surface. A number of management strategies are available for disease control but screening of germplasm through conventional breeding for resistance/susceptibility is an accessible approach to farmers.it considered as the best as cheapest way to manage disease (Adhikari *et al.*, 2017). Huang *et al.* (2018) screened different varieties of citrus against brown leaf spot to find out source of resistance and concluded that it was the best use of resistance varieties against brown leaf spot. So, in present

study 10 varieties were evaluated against brown leaf spot and its observed that three varieties (V3, V5, V-10) show the highly resistant response. The resistant varieties in the present screening, can be proceeded as resistant genotype against brown leaf spot disease. When disease appeared in the field epidemically then for quick control of disease farmer have no option except to use chemicals. The uses of chemicals have been reported to have positive effect and are believed to be the most effective way to control the disease (khadka *et al.*, 2020). There were six different chemicals along with the control like (Topsin M, Cabriotop, Forum top, Mancozeb, Excel and Kocide) evaluated at against *Alternaria alternata*. Mancozeb found to be highly effective.

Plant activators help the plants to produce defensive responses that can prevent or delay pathogen infection, and some of them have been shown to increase yield. These activators improve the defence system of plant by involving in defense mechanism. Plant activators represent to be environment friendly compounds capable of inducing resistance against many plant pathogens. Earlier studies showed that foliar spray of plant defense inducers could slow down Alternaria brown leaf spot disease progress (Rahman *et al.*, 2019) Hu *et al.* (2018). Six Plant activators (Salicylic acid, Potassium dihydrogen phosphate, Benzoic acid, Di-potassium hydrogen phosphate, Calcium Chloride and Citric Acid) were applied under RCBD design to evaluate the efficacy of plant activators against brown leaf spot. Salicylic acid gives the best result against brown leaf spot. Khan *et al.*, (2018) observed that Salicylic acid, naphthalic acid, copper hydroxide and Flare + salicylic acid were evaluated under greenhouse conditions. Maximum reduction (4.091%) in disease was expressed by the combination of Flare + salicylic acid (4.09) followed by Flare (4.60), salicylic acid (5.05), naphthalene acetic acid (5.495) and copper hydroxide (5.94)% as compared to control .In the interaction between treatments and days naphtalic acid expressed 6.10, 5.95 and 5.78, salicylic acid 5.636, 5.500 and 5.350, copper hydroxide 8.34, 8.19 and 8.04 while combination of Flare + salicylic acid exhibited minimum disease incidence of 4.240, 4.086 and 3.946% after 5,10 and 15 days of application .

References

1. Aiello, D., Guarnaccia, V. Azzaro, A. and Polizzi, G., 2020. Alternaria brown spot on new clones of sweet orange and lemon in Italy. *Phytopathologia Mediterranea*, 59(1), 131-145.
2. Arlotta, C., Ciacciulli, A. Strano, M. C. Cafaro, V. Salonia, F. Caruso, P. and Caruso, M., 2020. Disease resistant citrus breeding using newly developed high resolution melting and CAPS protocols for Alternaria brown spot marker assisted selection. *Agronomy*, 10(9), 1368.
3. Chhikara, N., Kour, R. Jaglan, S. Gupta, P. Gat, Y. and Panghal, A., 2018. Citrus medica: nutritional, phytochemical composition and health benefits–a review. *Food and Function*, 9(4), 1978-1992.
4. FAOSTAT. Food and Agriculture Organization of the United Nations. 2021. Available online: http://www.fao.org/faostat/en/ #home (accessed on 24 November 2020).
5. Farooq, M., Ilyas, N. Ilyas, N. Khan, I. Saboor, A. Khan, S. Khan, M.N. Qayum, A. and Bakhtiar, M., 2018. Antifungal activity of plant extracts and silver Nano particles against Citrus brown spot pathogen (*Alternaria* citri). *International Journal of Environmental Research*, 4(4), 118-125.
6. Hu, J., Jiang, J. and Wang, N., 2018. Control of citrus Huanglongbing via trunk injection of plant defense activators and antibiotics. *Phytopathology*, 108(2), 186-195.
7. Huang, F., Udayanga, D. Wang, X. Hou, X. Mei, X. Fu, Y. Hyde, K.D. and Li, H., 2018. Endophytic Diaporthe associated with Citrus: A phylogenetic reassessment with seven new species from China. *Fungal Biology*, 119, 331–347.
8. Inglese, Paolo, and Giuseppe Sortino."Citrus history, taxonomy, breeding, and fruit quality." *Oxford Research Encyclopedia of Environmental Science*, 2019.
9. Khadka, L., 2020. Effect of Hexanal Formulation on Host Recognition and Oviposition Behaviour of Spotted-Wing Drosophila (*Drosophila suzukii*) (*Matsumura*). Doctoral dissertation, University of Guelph.
10. Khan, A., Iram, S. and Rasool, A., 2015. Pathogens identification and characterization that compromised citrus fruit quality in selected orchards of Sargodha. *International Journal of Environmental Science and Toxicology Research*, 3(4), 54-59.
11. Liaquat, F., Qunlu, L. Arif, S. Haroon, U. Saqib, S. Zaman, W. and Munis, M. F. H., 2021. Isolation and characterization of pathogen causing brown rot in lemon and its control by using ecofriendly botanicals. *Physiological and Molecular Plant Pathology*, 114: 101639.
12. Memon, N. A., and KASBIT, D., 2017. Citrus fruit (Kino): Punjab produced 98% of production. *Exclusive on Kino*, 29-31.
13. Miles, A. K., Willingham, S. L. and Cooke, A. W., 2004."Field evaluation of strobilurins and a plant activator for the control of citrus black spot." *Australasian Plant Pathology*, 33, 371-378.
14. Rahman, M. M., Jahan, F. I. and Mim, S. A., 2019. A brief phytochemical investigation and pharmacological uses of citrus seed–a review. *Pharmacology Online*, 1, 94-103.
15. Talat, H., Shafqat, W. Qureshi, M. A. Sharif, N. Raza, M. K. Ud Din, S. and Jaskani, M. J., 2020. Effect of gibberellic acid on fruit quality of Kinnow mandarin. Journal of Global Innovation Agricultural Science, 8(2), 59-63.