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Epidemiology and management of powdery mildew of sunflower

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Abstract

Sunflower *Helianthus annuus* L. is one of the world's most cultivated oil seed crop. And affected by several biotic and abiotic factors. Approximately 13 percent yield is reduced due to powdery mildew disease severity. In the present study, conducive environmental conditions for powdery mildew disease of Sunflower were characterized. All the environmental variables had significant correlate with powdery mildew disease severity. The data on epidemiological failures was analyzed through correlation and regression while the data on management was analyzed through Anova. The treatment means was compared through the help of LSD. Among the six Germplasm lines screened one line G12 was found moderate resistant against powdery mildew disease. Effect of different chemicals that include Topas, Clone, Aerosil, Kumulus and Sulfex Gold was determined in RCBD for controlling of powdery mildew disease of sunflower. In field evaluation of fungicides, three sprays of Kumulus at 0.02 per cent concentration were found superior.

Keywords: Environmental Factors, Fungicide, Powdery mildew, Sunflower.

Introduction

Sunflower (*Helianthus annuus* L.) is originated from southern United States and Mexico. It is a major oilseed crop from the family Asteraceae. It is a rich source of edible oil (40-52%) with anti-cholesterol-properties due to the presence of polyunsaturated fatty acids (55 to 65% linoleic acid and oleic acid 20-30%), which are known to reduce the risk of coronary heart disease Diseases (Joksimovic *et al.*, 2006) [11].

Major cultivating countries are Russia (7.22 m ha), Ukraine (4.71 m ha), Argentina (1.74 m ha), India (0.85 m ha), China (0.97 m ha), USA (0.59 m ha) and Pakistan (0.30 m ha) (FAO, 2011). The World production for sunflower is 40.21 million tons annually and area under harvest is 26.05 million hectares. In Pakistan sunflower crop is grown on an area of 0.30 million hectares and obtained production is 0.40 million tons (FAO, 2011).

The most serious disease of sunflower caused by fungus, including powdery mildew, rust, downy mildew, verticillium wilt, Sclerotinia stem rot and head rot, charcoal rot and the severity of these diseases affect the crop yield (Mukhtar, 2009).

However, in Punjab the pathogens (*E. cichoracearum* and *Sphaerotheca fuliginea*) were identified for the cause of disease in severe form (Bains *et al.*, 1996) [2, 3]. Powdery mildew is the serious pathogen and it is obligate parasite and survives on the leave surface, stems, fruits and a wide range of angiosperm flowers (Takamatsu *et al.*, 1998) [25]. The disease is affected by some factors relating to environment such as relative humidity, light and temperature when rain fall is low then temperature and relative humidity are the main factors to cause disease (Javis *et al.*, 2002) [10].

Sulfex 0.3 per cent was used to spray at an interval of 10 days for one month to control powdery mildew of varieties of *Vignaradiata* (L.) Wilczek. Of the unsprayed varieties tested, 16 had yield losses of greater than 70 per cent maximum loss (85.70%) in Pusa-117. The loss in yield was correlated with the intensity of powdery mildew (Singh *et al.*, 1991) [21]. Different chemicals was used against the powdery mildew disease (*E. polygoni*) of pea were tested i.e. Bayleton, Calixin, Karathane and sulfex were evaluated, among these the Bayleton and calixin were found most effective against powdery mildew and the Karathane and sulfex controlled the disease only to considerable level (Upadhyay and Gupta., 1994) [26].

Paul and Ayres (1986) [16] studied to identify the resistant source and to determine the epidemiological factors favouring the powdery mildew disease.

In current studies, a positive correlation between powdery mildew disease and maximum temperature, minimum temperature, relative humidity, wind velocity were observed in all the six sunflower varieties/lines. It was observed that all varieties were significantly correlated with environmental factors and disease severity increased with increase in environment factors.

Materials & Methods

The present investigations were carried out at two different locations i.e., Research area of Plant Pathology at University of Agriculture Faisalabad and PARAS during the year 2012. The materials and methods followed during the course of investigation are described here under.

2.1 Sources of Seed.

Seed of Sunflower lines/varieties were obtained from the Department of Plant Breeding and Genetics University of Agriculture, Faisalabad and from the AARI, Faisalabad.

$$\text{Percent Disease Index (PDI)} = \frac{\text{Sum of the individual disease ratings}}{\text{Total number of leaves observed} \times \text{Maximum grade}} \times 100$$

Per cent disease index was determined by using this formula proposed by (Wheeler, 1969) [27].

Table 1: Disease Rating Scale

Grade	Percent disease severity	Level of Resistance/Susceptibility
0	No disease	HR (Highly Resistant)
1	1-10	R (Resistant)
2	11-25	MR (Moderately Resistant)
3	26-50	MS (Moderately Susceptible)
4	51-75	S (Susceptible)
5	>75	HS (Highly Susceptible)

2.3 Correlation of Environmental factors and Disease development

Meteorological data was taken from crop physiology department of UAF and for second field the data was taken

Table 2: List of Treatments with their Trade name, Company name and Active Ingredients

Treatments	Trade Name	Active Ingredient	Company Name
T ₁	Clone	Cymoxanil (8% w/w), Mencozeb (64% w/w)	4 Brothers
T ₂	Kumulus	Sulfur 80% w/w	FMC
T ₃	Sulfex Gold	Sulfex Gold (80 WDG)	--
T ₄	Aerosil	Thiophanate methyl (70% w/w)	4 Brothers
T ₅	Topas	Panconazol (100 EC)	Syngenta
T ₆	Control	--	--

Results

3.1 Correlation of Environmental Factors with Powdery Mildew Disease Severity.

Data showing the correlation of environmental factors with powdery mildew disease severity has given in the table 1, lines/varieties (A18, G12, G36, G55, G57, Hysun-33) having statistically significant correlation with maximum temperature and statistically significant correlation with minimum temperature. These lines/varieties also showed statistically significant correlation with relative humidity and wind speed. The relationship of temperature (maximum temperature and minimum temperature) with disease severity for all the varieties was best explained by linear regression. The effect of maximum temperature with disease severity was positive. With the increase of maximum temperature from 36.6 to 44.7,

2.2 Screening of Sunflower varieties/lines in Field

A field experiment was conducted to find out the resistance source to sunflower powdery mildew. In first field sowing of Sunflower Hysun-33 variety was done on 10 March 2012. And in second field sowing was done on 14 March 2012.

Plots remained open to natural epidemics in the course of the growing season. Two bags of Urea and two bags of DAP fertilizer per acre were applied. Urea was applied in two splits, i.e., half urea was applied at the time of bed preparation and the remaining half urea was used with 2nd irrigation.

The experiment was conducted in RCBD by sowing six varieties/ lines i.e. A18, G12, G36, G55, G57 and Hysun-33 in blocks with three replications. Disease incidence level was calculated with the help of following formula. Based on their reaction (0, 1-10, 11-25, 26-50, 51-75, >75 percent disease index) varieties/ lines were categorized into immune, resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible respectively.

from Regional Agro-Meteorological Centre that is located in AARI Faisalabad. Data including temperature, relative humidity, wind speed and average rain fall was processed for weekly averages. Whole data of disease incidence on Sunflower was subject to analysis of variance. The effect of environment and disease was determined by correlation Steel *et al.*, 1997.

2.4 Evaluation of Fungicides against Powdery Mildew of Sunflower (in-vivo)

Five fungicides were used to manage the powdery mildew of Sunflower disease. Three replications were used in each chemical treatment. Hysun-33 variety was sown in first field and Sunflower lines A18, G12, G36, G55 and G57 was sown in second field at PARAS. The experiment was conducted in RCBD.

disease severity increased.

The correlation of minimum temperature with disease severity was significant. The effect of minimum temperature was also positive. When minimum temperature increased from 22.6 to 29.1 degree the disease severity increased. The relationship was explained by linear regression model as indicated by very higher value (0.81) on the five varieties/ lines in minimum temperature graph.

The correlation of relative humidity with disease severity was also significant on all the varieties and relationship was best explained by linear regression model as indicated by =0.70, 0.74, 0.74, 0.73 and 0.72 r values respectively. As the relative humidity increased from 26.1 to 44.3 the disease severity was also increased. Show in relative humidity graph.

The correlation of rainfall with disease severity was non-

significant because as the rainfall increased from 0 to 0 there was no effect on disease incidence. The relationship was best explained by linear regression model as indicated by higher value ($r=0.65$) show in rain fall graph.

The correlation of wind speed with disease severity was significant on all the varieties and relationship was best explained by linear regression model as indicated by 0.90, 0.53, 0.56, 0.56 and 0.54 r values respectively show in wind speed graph.

Table 3. Correlation of Environmental Variables with Powdery Mildew Disease Severity

PARS Field					
Variety	Temperature (°C)		R.H (%)	Rain Fall(mm)	Wind Speed (km/hr)
	Max.	Min.			
V1	0.72190.0670	0.82390.0227	0.66560.1027	-	0.51190.2402
V2	0.75170.0513	0.84410.0169	0.62380.1344	-	0.51640.2354
V3	0.70460.0771	0.81140.0267	0.67930.0933	-	0.51760.2341
V4	0.70560.0765	0.80910.0275	0.67090.0990	-	0.51090.2412
V5	0.73400.0603	0.83170.0203	0.63690.1240	-	0.52580.2255
Department Field					
Variety	Temperature (°C)		R.H (%)	Rain Fall(mm)	Wind Speed (km/hr)
	Max.	Min.			
Hysen-33	0.80730.0281	0.87920.0091	0.70070.0795	0.64880.1149	0.90720.0048

Upper values indicated Pearson’s correlation coefficient; Lower values indicated level of significance at 5% probability. * = Significant ($P<0.05$); ** = Highly significant ($P<0.01$)

Correlation of Environmental Factors with Powdery Mildew Disease Severity at PARS

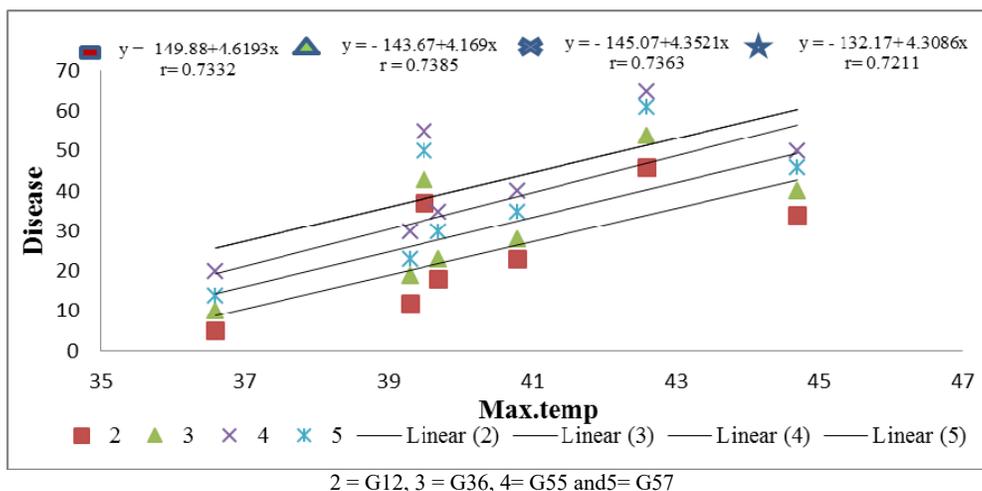


Fig 1.1: Relationship of maximum temperature with powdery mildew disease severity

The graph showed the relationship between maximum temperatures and powdery mildew disease severity. The lines/varieties (G12, G36, G55 and G57) which were sown at PARS field showed significant reaction with increase in

maximum temperature 36.6-44.7 °C. In this disease severity increase as the rate of maximum temperature increase. Therefore, a positive interaction exists between maximum temperature and disease severity.

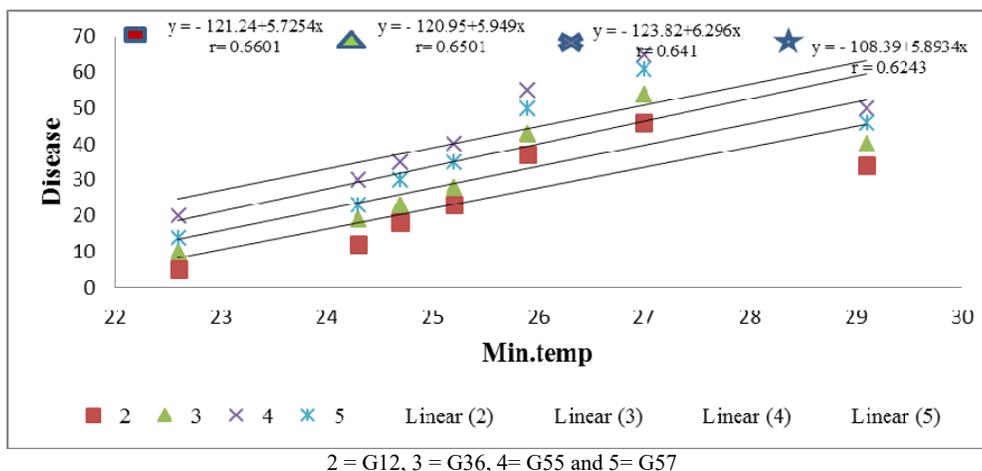


Fig 1.2: Relationship of minimum temperature with powdery mildew disease severity

The graph showed the relationship between minimum temperatures and powdery mildew disease severity. The lines/verities (G12, G36, G55 and G57) which were sown at PARS field showed significant reaction with increase in

minimum temperature 22.6-29.1 °C. In this disease severity increase as the rate of minimum temperature increase. Therefore, a positive interaction exists between minimum temperature and disease severity.

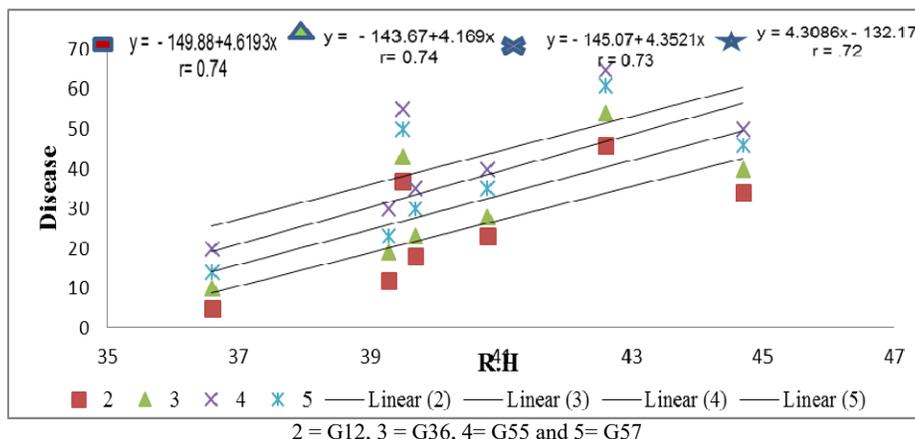


Fig 1.3: Relationship of relative humidity with powdery mildew disease severity

The graph showed the relationship between relative humidity and powdery mildew disease severity. The lines/verities (G12, G36, G55 and G57) which were sown at PARS field showed significant reaction with increase in relative humidity 26.1-

44.3% in this disease severity increase as the rate of relative humidity. Therefore, a positive interaction exists between relative humidity and disease severity.

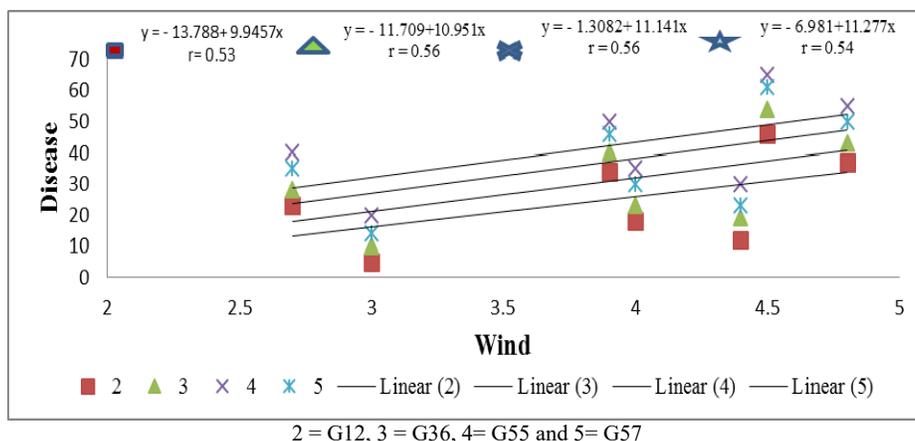


Fig 1.4: Relationship of wind speed with powdery mildew disease severity

The graph showed the relationship between wind speed and powdery mildew disease severity. The lines/verities (G12, G36, G55 and G57) which were sown at PARS field showed significant reaction with increase in wind speed 2.7-4.8Kmh. in this disease severity increase as the rate of wind speed.

Therefore, a positive interaction exists between wind speed and disease severity.

Correlation of Environmental Factors with Powdery Mildew Disease Severity at Department Field.

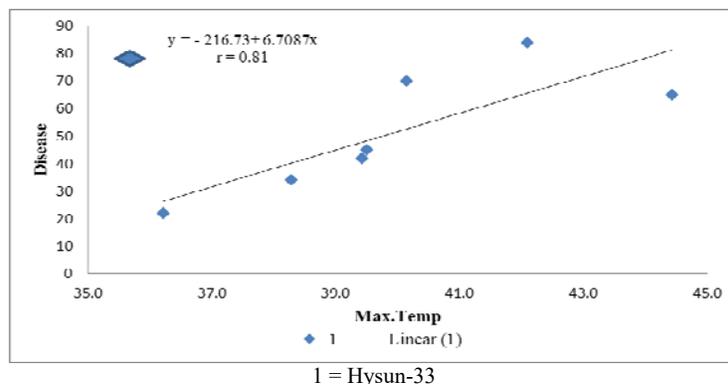


Fig 2.1: Relationship of maximum temperature with powdery mildew disease severity

The graph showed the relationship between maximum temperatures and powdery mildew disease severity. The line/verity (Hysun-33) which was sown at Department field showed significant reaction with increase in maximum temperature 36.2-44.4 °C. In this disease severity increase as the rate of maximum temperature increase. Therefore, a positive interaction exists between maximum temperature and disease severity.

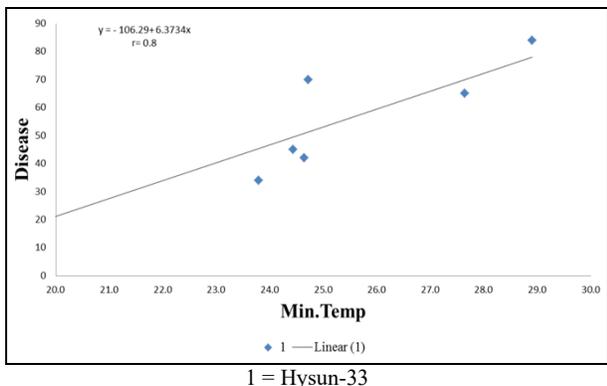


Fig 2.2: Relationship of minimum temperature with powdery mildew disease severity.

The graph showed the relationship between minimum temperatures and powdery mildew disease severity. The line/verity (Hysun-33) which was sown at Department field showed significant reaction with increase in minimum temperature 19.4-28.9 °C. In this disease severity increase as the rate of maximum temperature increase. Therefore, a positive interaction exists between minimum temperature and disease severity.

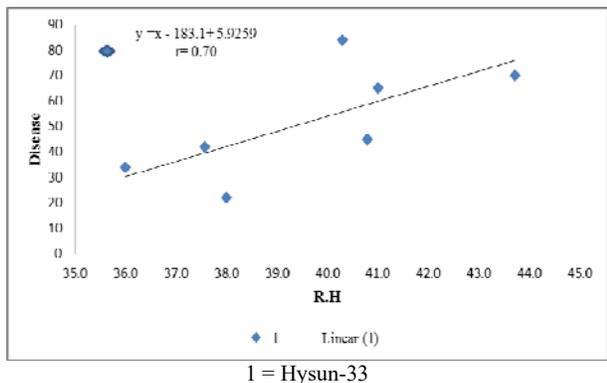


Fig 2.3: Relationship of Relative Humidity with Powdery Mildew Disease Severity.

The graph showed the relationship between relative humidity and powdery mildew disease severity. The line/verity (Hysun-33) which was sown at Department field showed significant reaction with increase in relative humidity 36-43.7%. In this disease severity increase as the rate of relative humidity increase. Therefore, a positive interaction exists between relative humidity and disease severity.

The graph showed the relationship between wind speed and powdery mildew disease severity. The line/verity (Hysun-33) which was sown at Department field showed significant reaction with increase in wind speed 3-6.2Km/h. In this disease severity increase as the rate of wind speed increase. Therefore, a positive interaction exists between maximum temperature and disease severity.

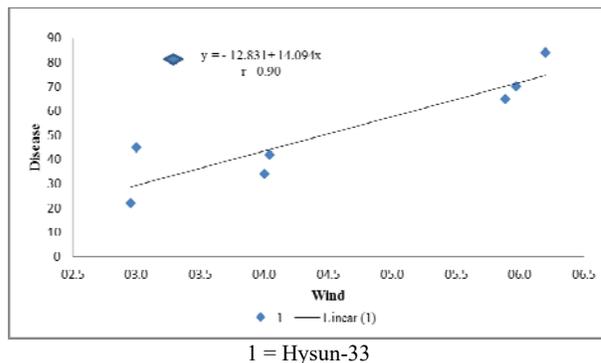


Fig 2.4: Relationship of Wind speed with Powdery Mildew Disease Severity.

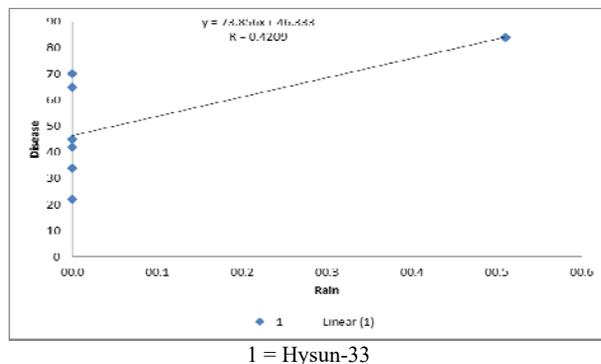


Fig 2.5: Relationship of rain with Powdery Mildew Disease Severity

The graph showed the relationship between rain and powdery mildew disease severity. The line/verity (Hysun-33) which was sown at Department field showed less significant reaction with increase in rain 0-0.5mm. In this disease severity increase as the rate of rain increase. Therefore, a positive interaction exists between rain and disease severity.

Table 3, which is given in start clearly defines the relationship of all six varieties/lines sown for the study with epidemiological factors. In these, five varieties/lines were sown at PARS field and one was sown at Department field. The correlation was an essential component of results description as it describes the resistivity, immunity and susceptibility responses of every variety/line as affected by several environmental parameters. More specifically, table indicates the solution of our main objective of the research i.e. to find out the resistant sources for powdery mildew in relation to the epidemiological factors. Amongst these five varieties/lines used for the present study of powdery mildew, two lines/verities were highly susceptible to the powdery mildew disease, so it's show linear correlation with all five epidemiological factors under the research. However discussing relationship of epidemiological factors, one line is not included in each case of epidemiological factors. If we discussed relation of maximum temperature and powdery mildew disease, it was concluded that all five varieties/lines showed significant result for powdery mildew disease severity and form a linear relationship with maximum temperature. In contrast to maximum temperature, minimum temperature showed more susceptibility for the increasing of powdery mildew disease severity. All five varieties/advance lines showed significant interaction with powdery mildew disease severity. Another epidemiological parameter relative humidity was studied and showed significant effect on all five varieties/lines that with increase in relative humidity disease severity also increased in both PARS and Department field. Rainfall

did not show any positive interaction with disease severity because at the time of data recording during research there was no rainfall at PARS field but little rain was recorded at Department field which cause no significant effect on disease severity and that showed no relationship of rainfall with powdery mildew disease severity. The graph also describes the same objects as described in the above mentioned paragraph.

On the basis of above discussions of the correlation table of powdery mildew, five varieties/advance lines (G12, G36, G55, G57 and Hysun-33) were selected to show the relationship between powdery mildew and epidemiological parameters under research to show linearity in relation on graphical explanations. All of these five varieties/lines had significant relationship with all the epidemiological factors.

4. Reaction of Sunflower varieties/lines against Powdery Mildew

The screening results of sunflower varieties/lines against powdery mildew showed that no line was highly resistant or resistant against powdery mildew at both locations (Department field and PARS field) A18 and Hysun-33 were found susceptible while G12 was observed as moderately resistant line. G36, G55 and G57 were found moderately susceptible lines. In these varieties/lines only Hysun-33 was sowed in Department field and others five were sown in PARS field.

Table 4. Analysis of variance of sunflower varieties/lines against powdery mildew

Source	DF	SS	MS	F	P
Replication	2	6.97	3.484		
Variety	5	3174.81	634.962	460.21	0.0000
Error	10	13.80	1.380	--	--
Total	17	3195.57	--	--	--

Table 5. Response of different lines/varieties against powdery mildew disease severity.

Variety	Grade	Reaction	varieties/lines
A18	0	Immune (0%)	Nil
G12	1	Resistant (1-10%)	Nil
G36	2	Moderately resistant (11-25%)	G12
G55	3	Moderately susceptible (26-50%)	G36, G55, G57
G57	4	Susceptible (51-75%)	A18, Hysun-33
Hysun-33	5	Highly susceptible (>75%)	Nil

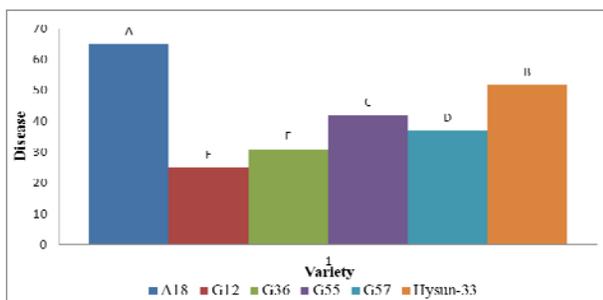


Fig 3.1: Response of different Cultivars against Disease Severity

In this graph disease severity of all six varieties/lines are shown which were sown in both fields (PARS field and Department). Hysun-33 variety was sown in Department field and others five were sown in PARS field. A18 show the maximum disease severity then Hysun-33, G55, G57, G36 and G12.

4.1 Comparison of the Both Field

Powdery mildew disease severity appeared on both locations at same time and disease severity increased on both locations as time passed. Five varieties/lines i.e. A18, G12, G36, G55 and G57 were sown on PARS field and one variety Hysun-33 was grown on Department field. At PARS field maximum disease severity at the end of disease rating time appeared on A18 line compared to Department field.

5.1 In Vivo Evaluation of Chemicals against Powdery Mildew

Field experiment was conducted to evaluate the relative efficacy of fungicides against powdery mildew of sunflower during March to July 2012. Five lines/varieties (A18, G12, G36, G55 and G57) were sown in PARAS field and one variety Hysun-33 was sown in Department field. Five different fungicides and one control were used for evaluation named as Topas, Clone, Aerosil, Kumulus and Sulfex Gold as described in "Material and Methods. In this experiment three foliar sprays of fungicides were applied.

Table 6: Analysis of variance of disease management

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	12	17490.27	1457.52	655.09	<.0001
Error	41	91.22	2.22	--	--
Corrected Total	53	17581.50	--	--	--

List of treatments and their trade name, active ingredient and company name was given in in the table:

Table 7: Treatments and their descriptions.

Treatments	Trade Name	Active Ingredient	Company Name
T1	Clone	Cymoxanil (8% w/w), Mencozeb (64% w/w)	4 Brothers
T2	Kumulus	Sulfur 80% w/w	FMC
T3	Sulfex Gold	Sulfex Gold (80 WDG)	--
T4	Aerosil	Thiophanate methyl (70% w/w)	4 Brothers
T5	Topas	Panconazol (100 EC)	Syngenta
T6	Control	--	--

Three replications of each treatment were applied and one remained control where no fungicide was applied. The data was collected before applying of treatment and after application of treatments. The all fungicides that are used for research experiment were recommended and also available in the market. The doses of fungicides which were used in the experiment were also recommended. On control no chemical was applied and finally data was collected to check the efficacy of chemicals.

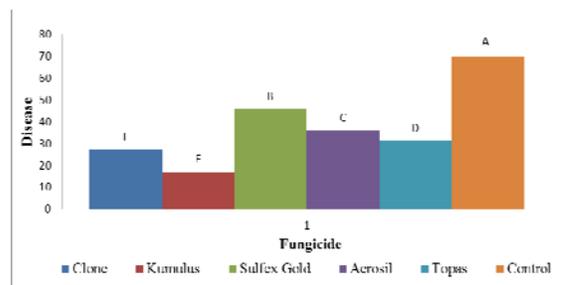


Fig 4.1: Interaction between disease incidence and fungicidal effect at Department field

As in this graph an interaction between mean disease severity and chemical efficacy is shown. All treatments significantly reduced the disease severity as compared with the control. But the best result is shown by the Kumulus then Clone, Topas, Aerosil and Sulfex Gold. And control shows no result and disease severity was maximum on control.

The per cent disease reduction over control was calculated with the given formula as under.

$$\text{Disease reduction over control} = \frac{\text{control-treatment}}{\text{control}} \times 100$$

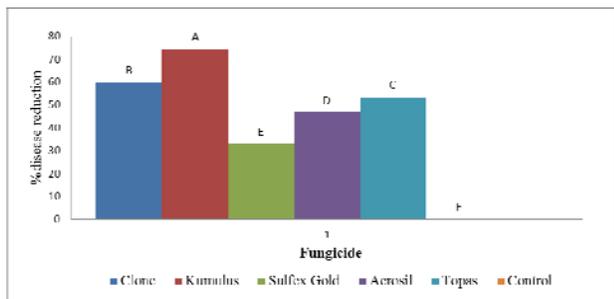


Fig 4.2. Interaction between treatments and per cent disease reduction over control at Department field.

In this graph treatment 2 (Kumulus) showed the best result and control the disease at maximum extant as compared with control and then Clone, Topas, Aerosil and Sulfex Gold showed best result after treatment 2. And control showed no result because the disease was not controlled on it.

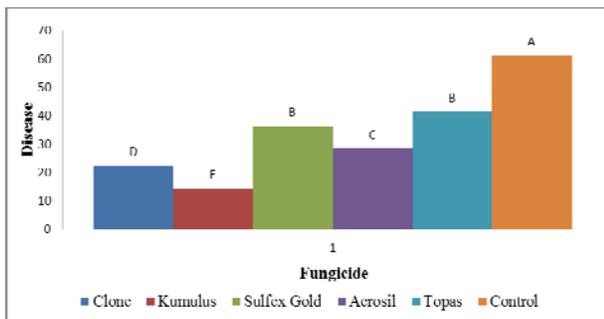


Fig 4.3. Interaction between disease incidence and fungicidal effect at PARS Field

As in this graph an interaction between disease severity and chemical efficacy is shown. All treatments significantly reduced the disease severity as compared with the control. But the best result is shown by the Kumulus then Clone, Aerosil, Sulfex Gold and Topas. And control shows no result and disease severity was maximum on control.

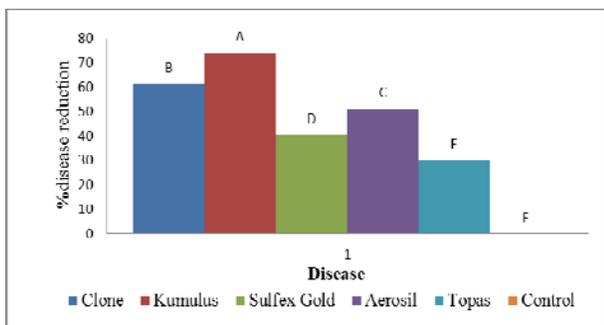


Fig 4.4: Interaction between treatments and per cent disease reduction over control at PARS

In this graph treatment 2 (Kumulus) showed the best result and control the disease at maximum extant as compared with control and then Clone, Aerosil, Sulfex Gold and Topas showed best result after treatment 2. And control showed no result because the disease was not controlled on it.

5.2 Comparison of the Both Field

Powdery mildew disease severity appeared on both locations and for the management of the powdery mildew disease five fungicides i.e. Topas, Clone, Aerosil, Kumulus and Sulfex Gold were applied at both locations. Three sprays were applied with same concentration in both fields and disease severity reduced significantly in both fields. Kumulus gave the best results in both location and reduced the disease severity near about 80 per cent at both locations.

Discussion

Crop exploited due to several biotic and abiotic stresses. The crop suffers from many fungal diseases, among them foliar diseases takes a heavy toll by reducing the yield to considerable extent. Among the foliar diseases powdery mildew caused by Erysiphe cichoracearum DC is a potential destructive disease in recent years causing severe yield loss. The disease is prevalent in all sunflower growing states of Pakistan and in all the countries of the world wherever it is cultivated (Bains *et al.*, 1996) [2, 3].

Study of environmental parameters with powdery mildew disease helps out to calculate powdery mildew disease epidemics, so that precautionary measures should be taken to minimize the yield losses. In the current study, focus was to identify the resistant source and to determine the epidemiological factors favouring the powdery mildew disease. In current studies, a positive correlation between powdery mildew disease and maximum temperature, minimum temperature, relative humidity, wind velocity were observed in all the six sunflower varieties/lines. It was observed that all varieties were significantly correlated with environmental factors and disease severity increased with increase in environment factors. Matching results after evaluation were found by Paul and Ayres (1986) [16].

The management of the disease through host plants resistance has been the best choice in all the crop improvement programmes. Utilization of resistant cultivars in farming system is the most simple, effective and economical method in the management of disease. (Puscasu and Iuoras, 1987, Ivancia *et al.*, 1992 and Eva, 2002) [17, 9, 6]. Though the germplasm lines are resistance source to the breeders, they have to be used in breeding programme for the development of new hybrids for the benefit of farmers.

Use of chemicals to manage the disease is an age old practice in plant protection in the absence of resistant varieties or where there is break down of resistance in commercial varieties by evolution of virulent strain of a pathogen. Hence, efficacy of fungicide including new generation molecules will help in reducing the loss due to powdery mildew epidemics.

In the present investigation, a field experiment was conducted during 2012 in Faisalabad at two different locations. Five fungicides along with unprotected control were evaluated for their efficacy in disease control under natural epiphytotic condition. The results after three sprays revealed that, among the five fungicides Kumulus at 0.02 per cent concentration gave the best result. The results were agree with several workers (Begum, 1989, Upadhyay, Gupta, 1994, Kapoor, Sugha, 1995, Rathore, 1995, Dhruj *et al.*, 1996, Malani, 1998, Naik, Nagaraja, 2000, Singh *et al.* 2000, Khunti, 2002,

Saxena, Moly Saxena, 2002, Sharmila 2004, Shivanna 2006, Ashtaputre 2007, Gupta, Amit Kumar 2008) [4, 26, 5, 13, 15, 23, 12, 18, 19, 20, 1, 8] while working with powdery mildew disease of various crops.

Summary

Sunflower (*Helianthus annuus* L.) is an important oilseed crop of Pakistan. It is grown over an area of 2.4 million hectares with a production of 1.44 million tonnes. The crop is being affected by major biotic and abiotic stresses which cause drastic reduction in yield. One of the major biotic stresses is powdery mildew caused by *Erysiphechichoracearum* which heavily affect the yields.

Keeping in all these factors the present investigations were carried out at two different locations, 1st in Research area of Plant pathology at University of Agricultural Faisalabad and 2nd in PARA Sin Randomized Complete Block Design RCBD. There were five treatments and one untreated control, having three replications each. The observations were recorded from each plot after 48 hours of the spray. All the treatments gave significant reduction in disease severity. And to determine the most conducive environment for the application of fungicides at the right time. And also determined the correlation between environmental factors and disease severity.

Nearly all environmental variables had significant correlation with disease severity. It was observed that with increase in maximum and minimum temperature disease severity was increased. Relative humidity, rainfall and wind speed also effect the disease incidence.

The plants of 50 days old were highly susceptible to disease. The plants of age 10 and 20 were not shown any disease infection. Among six Germplasm lines screened against *E. cichoracearum* no one germplasm was found resistant. One germplasm line G12 was found to be moderately resistant. Three germplasm lines G36, G55, G57 were found moderately susceptible.

For the management of powdery mildew of sunflower, new molecules of fungicides were evaluated in field. Among the five chemicals tested Topas, Clone, Aerosil, Kumulus and Sulfex Gold at 0.02 per cent concentration found superior as they recorded the lowest disease index.

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