Agricultural Sciences Journal
Available online at http://asj.mnsuam.net/index.php
ISSN 2707-9716 Print
ISSN 2707-9724 Online

Research Article

Biological Management of Powdery Mildew of Pea (*Pisum Sativum* L)

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ABSTRACT

Pea (Pisum sativum) is leguminous crop generally cultivated around globe. P. sativum is main pulse crop belongs to the family Leguminosae rich in nutritional value. In Pakistan, Pea crop is cultivated on large area mostly in Punjab and in other provinces. The production of Pea in Pakistan is not so good due to the attack of fungal diseases like Powdery mildew, Anthracnose, Rust and Downy mildew. The Powdery mildew, an air borne disease, caused by Erysiphe pisi is distributed globally. An environmental factor like Temperature, Humidity, Rain fall, Wind and Light etc play significant role in occurrence of that disease. When plants are on pod stages it shows epidemic form in Month of March - April. It effects whole green surface of pea initially symptoms are lesion formation and powder like whitish spots on upper and lower surface of leaves. When the pathogen proliferates in all aerial parts; plant became dead. Infected plant seed produced unpleasant smell which decreases the quality of peas. The intensity of the disease can be reduced by increase resistance in host plant by some plant extracts i.e., Neem Extract, Garlic Extract. Pathogenicity of Erysiphe pisi can be supressed by the application of some bio agents i.e., Trichoderma harzianum and Beuveria bassiana. All applications were served as environment safe management and helps to reduce disease severity and increase in numbers and weight of pod/plant which were helping to increase in a yield. From these all treatment most, effective treatment was Garlic Extract which overcome the effect of disease 77% and then Neem Extract 64%. There was also effective result of T. harzianum and B, bassiana overcome the effect of powdery mildew of pea disease was 51% and 38%. The data onto environmental condition was collected which shows with increase in temperature helps to increase the intensity of disease. Decrease in humidity and solar radiation also helps to increase the disease incidence level. Decrease in wind speed and rain fall also increase the disease intensity.

Keywords: *Erysiphe pisi, Trichoderma harzianum, Beuveria bassiana, Neem Extract, Garlic Extract.*

1. INTRODUCTION

Pea *(Pisum sativum)* is third most important legume crop after soybean and common bean in world. It belongs to family *Leguminoceae* (Timmerman-Vaughan *et al*, 2005). Pakistan is on 9th position in top 10 pea producing countries of the world. Pea is also known as Matter in local language with high nutritious value like vitamins, protein and carbohydrates. (Duke and Ayensu, 1984). Pea is cultivated in all provinces of Pakistan (Khokhar, 2014). In Pakistan pea is cultivated in 25204 hectares area with 171511 tons production (FAO, 2019). The various parts like pods and seeds are used for various purposes *via* fresh vegetable. Pea is summer as well as winter crop and mostly grown in hilly areas. As legume crop, pea is

(Received: 18 April 2021, Accepted: 30 June 2021) Cite as: Ahmed. N., Abbas. Z., Riaz. H., Faried. H. N., Mehmood. M. A., Asad. Z., Hamza. Z., Anshan. M. A., Ali. S., Latif. S., Ali. H. R., 2021. Biological management of powdery mildew of pea (Pisum Sativum L). Agric. Sci. J. 3(1): 79-95.

used to increase the soil fertility (Rana and Sharma, 1993).

There are various factors like biotic and abiotic that affect the growth and production of pea in the world especially Pakistan. Among biotic, insect pests, diseases (Anthracnose, Downy Mildew; Powdery Mildew and Rust) and pathogens (*Erysiphe pisi*) are the most important factors that reduced pea production. Powdery mildew is major one among all diseases. The quality and quantity of pea is reduced due to severe attack of powdery mildew (Fondevilla and Rubiales, 2012).

Erysiphe pisi is causing powdery mildew an obligate bio trophic parasite belongs to order Ascomycota. It can cause about 25-50% losses in pea production (Fondevilla and Rubiales, 2012; Fondevilla and Rubiales, 2012). During severe attack of this fungus on pea caused discolouring in seed and white powdery patches appear on both side of leaves. The percentage germination of seed can also reduce due to infection of powdery mildew. The size of white powdery patches on leaves can increased gradually and invade the other parts even the whole plants (Falloon and Viljanen-Rollinson, 2001). The photosynthetic machinery of plant can also affect and even death of plant occurred due attack of powdery mildew.

2. MATERIAL AND METHODS

2.1. Germplasm Collection

Two moderately tolerant varieties were collected from Horticulture Department, MNS-University of Agriculture Multan.

2.2. Site Selection

The trial of pea was sown at 'B' block of MNS-University of Agriculture Multan. Total area of sowing of green pea was two kanals. Experiment was sown with randomise complete block design (RCBD). The experiment was having three replications and thirteen treatments. The experiment was conduct during the December, 2019-2020.

2.3. Sowing time/Germination

When the soil temperature is only 5°C, the pea seeds take more than a month, approximately two weeks at 10°C and from 5 to 10 days at 20°C at 30°C. However, the time of Planting is mainly determined by two factors, that is, the poor performance of plants in high temperature conditions, the frost sensitivity of flowers and pods, especially young pods. Therefore, the timing of planting was choosing so that most of the growth takes place in a suitable cool climate, but only after the danger of frost is overcome, the flowers were begun to bloom. The crop was sown in the month of December and date of sowing 05 December 2019.

2.4. Preparation of Field

Three ploughs were used for the preparation of field. The ground was crushed very finely. During the final tillage period, decomposing corral fertilizers 50kg DAP and 50kg potash per acre were used. After ploughing, the field was levelled for proper distribution water with the help of land leveller. Field was again two-time ploughing. After these exercise preparations of beds was done with the help of planter machine.

2.5. Seed rate

For research purpose two different varieties (i.e., Pencil 2019 and sarsabz 2017) were used at the concentration of approximately 40 kg per acre were used. For two kanals 10kg of pea seed were used containing 5kg of variety pencil 2019 and 5kg of variety sarsabz 2017.

2.6. Plant population and spacing

In each replication 60 plants were sown, and every variety were having three replications. Total plants of one variety were 1080. Row to Row distance of the experiment was 1.5ft and plant to plant distance was 1ft. Propagation of pea was done by the seed.

2.7. Fertilizer Application

At the time of filed preparation one bag(50kg) of Diammonium Phosphate (DAP) and one bag(50kg) of Potassium (K) was used and after the plantation when the germination was started then half bag of urea was applied per acre. Half bag of urea was also applied randomly with other irrigation.

2.8. Irrigation Application

First irrigation was applied with the sowing of pea crop. Second irrigation was applied four days after first irrigation. Other all irrigation was applied with 7 days interval. Total 17 irrigation were applied in the duration of four month.

2.9. Weed Eradication

Hand weeds eradication was done four time during crop duration of four month. First weeds eradication exercise was done after third application of water second weed eradication exercise was done after the seventh application of water, third weed eradication was done with the eleventh application of water and fourth hand weed eradication was done after fifteenth application of irrigation.

2.10. Cultural Practices

Pea were Planting away from other legumes crop. Providing of proper irrigation and proper fertilization were help crops get too large against defoliation. Cultural practices were done at the time, when necessary, which was helping to enhance the growth of peas plants. i.e., disease free seed, rouging, sanitation, proper planting time, Land preparation, irrigation and application of fertilizers etc.

2.11. Appearance of Powdery mildew

The most common pea disease was powdery mildew, manifesting as areas of white powdery mass at upper surface of leaves, stems, and pods. Powdery mildew was damaging to appearance of pods and production of peas. Places which are warm and dry having cool nights with dew were founded better for disease progression. If in cool, damp conditions (including frequent sprinkler irrigation), powdery mildew is nearly invisible. However, the large amount of water in the crops was favoring in the development of downy mildew.

2.12. Harvesting and Yield

Five-time harvesting was done with different intervals. First time harvesting was done 5 February of 2020. The total yield of all harvesting was almost 320 kg of two kanals which was almost 1280kg per acre.

2.13. Collection of Disease Data

Disease incidence was observed by using percentage disease index (PDI) for this purpose data was recorded from first symptom to disease appearance.

$$\text{Disease incidence \%} = \frac{\text{Total no. of infected plants}}{\text{Total no. of plants}} \times 100$$

2.14. Disease rating scale

Powdery mildew disease was confirmed through symptoms of the plants and disease incidence-based data was recorded by applying of disease rating scale (Table 1).

Table no 1: Disease rating scale

Scale	Disease Incidence %age	Disease Status
0	(0%) No disease symptoms	(HR) High resistant
1	0.1-10% plant showing disease symptoms.	(R) Resistant
2	10.1-25% plant showing disease symptoms.	(MR) Moderately resistant
3	25.1-50% plant showing disease symptoms.	(MS) Moderately susceptible
4	50.1-75% plant showing disease symptoms.	(S) Susceptible
5	75.1-100% plant showing disease symptoms.	(HS) Highly susceptible

(Patil and K.P 2017).

2.15. Environmental Data Collection

Data onto environmental condition including minimum and maximum temperature, RH, velocity of wind and rainfall measurement was collected from the Website of MNSUAM (https://mnsuam.edu.pk) because these environmental factors was directly promoted the establishment of pathogen.

2.16. Statistical analysis

Data about the evaluation of treatments for powdery mildew of pea incidence was recorded before and after the

use of treatment. All data onto powdery mildew disease as influenced by the treatment was statistically analysed, analysis of variance of all treatments was determined through ANOVA technique and these treatments were compared with HSD test at five present level of probability.

3. RESULTS AND DISCUSSION

The results indicated that before the spray of Botanicals and Bio control agents, all sprays recorded least percent disease index (PDI) the disease was not controlled by zero spray (28.846) which was on par with bio agents like., Trichoderma (44.038) and Beauverria (54.295), followed by botanicals like., garlic extract (65.385) which was on par with Neem extract (75.096). The PDI of control plot T0 (28.846) were recorded. The maximum PDI (75.096) was observed in spray no 4 followed by other spray and control plot. Before application of treatment in the field, there was higher disease incidence in most of the plots where treatments were supposed to be applied. The result indicated that after application of treatments, in controlled plot maximum disease was recorded which was sprayed by distilled water. Minimum disease was recorded in that plot which was sprayed by garlic extract. After garlic extract, Neem extract was found most effective against powdery mildew of pea. *Trichoderma harzianum* and *Beuveria bassiana* as a biological control agent was gives valuable result to overcome the effect of Erysiphi pisi. In present study, results after last spray revealed that all the four treatments were significantly superior over control in managing the powdery mildew disease without T0 which was controlled. Among all spray T4 and T3 was significantly superior over other all treatments. But these all treatments were superior to T0. The lowest mean per cent disease intensity was recorded from the plots receiving the sprays Garlic and neem respectively. These finding are in agreement with the results of Singh and Prithiviraj (1997), Ravikumar (1998), Sindhan *et al*., (1999) and Rettinassbabady *et al*., (2000), Sharmila (2006) similar with those reported earlier by Sudha and Lakshmanan (2007), Surwase *et al*., (2009), Kacchot *et al*., (2011), Dinesh *et al*., (2011) and Khalikar *et al*., (2011). After taking the results of botanical neem extract which was found effective against powdery mildew disease. Same result was observed by Surwase *et al*., 2009, Akhileshwari *et al*., 2012, Parasad and Dwivedi, (2007), Jagtap and Khalikar, (2012) and Suryawanshi *et al*., (2009). *Trichoderma harzianum* overcome the severity of disease and can suppress powdery mildew (Bettiol *et al*., 1999 and Bettiol *et al*., 2008). Abd El-Moity, (1985) observed that by the producing of some anti-fungal substances *Trichoderma harzianum* can inhibit disease. Same result was observed by Surwase *et al*., 2009, Akhileshwari *et al*., 2012, Parasad and Dwivedi (2007), Jagtap and Khalikar, (2012) and Suryawanshi *et al*., (2009). *Beuveria bassiana* were least effective over powdery mildew disease. Same results have been reported by Rettinassababady *et al*., 2000, Deora and Sawant (2004), Ahmad *et al*., (2005) and Kiran and Ahmad (2005), Vikas and Ratnoo, 2011. Induction of plant extracts in early stage was good sign these are antifungal (Singh *et al*., 2002).

3.1. Analysis of Variance

Data of disease incidence was recorded and the formula of RCBD was applied with the software of statistics 8.1 which shows the following results. If the P value of sources were below 0.05, this were considered as significant. The value of spray, variety, treatment individually observed was 0, 0, and 0, showed their significant result. There was significant interaction between spray and variety was 0 and significant interaction between spray and treatment which was 0.0135. There was non-significant interaction between variety and treatment which was 0.0001. When we compare Spray and variety and treatment there were also non-significant interaction between them was 0.7719. Total grand man was 53.532 and CV was 9.93 (Table 4.1).

Table No. 4.1 ANOVA table for disease

Source	DF	SS	MS	F	P
Replication	2	808	404.1		
Spray	4	132062	33015.6	1169.22	0
Variety	12	22292	1916	67.85	0
treatment	1	901	901.4	31.92	0
Spray and Variety	48	7127	148.5	5.26	0
Spray and treatment	4	361	90.3	3.2	0.0135
Variety and treatment	12	1124	93.7	3.332	0.0001
Spray, Variety and treatment	48	1133	23.6	0.84	0.7719
Error	336	9488	28.2		
Total	467	808	404.1		

3.2. Tukey HSD all paired analysis tests

3.2.1. Pairwise comparison test of disease for spray

Tukey HSD All-Paired analysis of Disease for Spray is shown in fig no.4.1 which shows that spray no 4 gave the mean of 77, spray no.3 gave the mean of 64, Spray no 2 almost gave the mean of 51, Spray no 1 gave the mean of 38 and spray 0 gave the mean of almost 22. The means of all 5 treatments was significantly different from one another having Alpha 0.05, Standard error for comparison was 1.6432 to 1.7776, Critical Q value was 3.857 and critical value for comparison was 4.4810 to 4.8476. This result supported the result of Sharma, (2000) Rajapan *et al.*, (2000), Mahdy, (2006), Gaber, (2010), Biswas and Ray, (1958), Spencer *et al.*, (1980).

3.2.2. Pairwise comparison test for Disease and Variety

Fig. 4.2 shows that all pairwise analysis of disease for variety gave almost similar results against the powdery mildew of pea. There are no significant pairwise differences among the means having alpha value of 0.05, Standard Error for Comparison value 1.0910, Critical Q value of 2.772 and Critical Value for Comparison is 2.1384. Result of research of the comparison of varieties was similar with Prithiviraj *et al.*, (1997), Singh *et al.*, (2002).

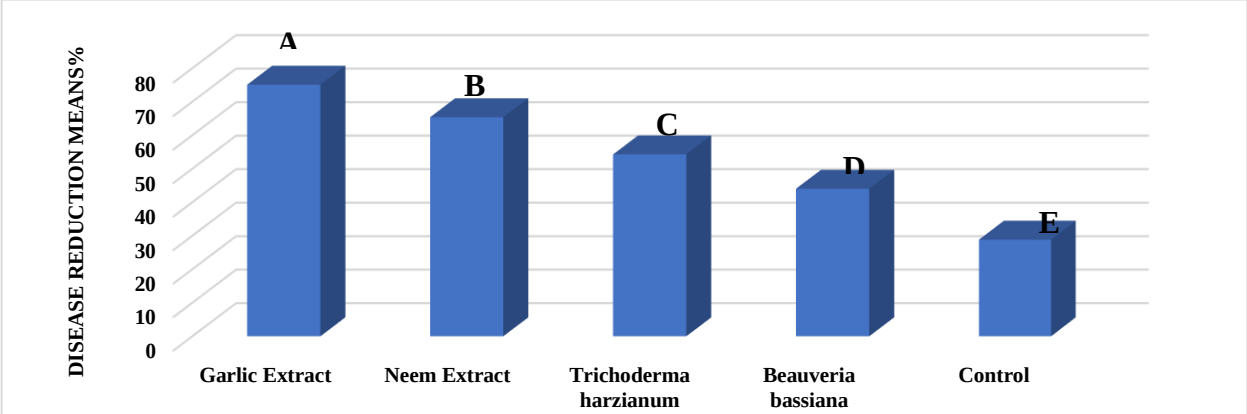


Fig 4.1 Pairwise comparison test of disease with spray

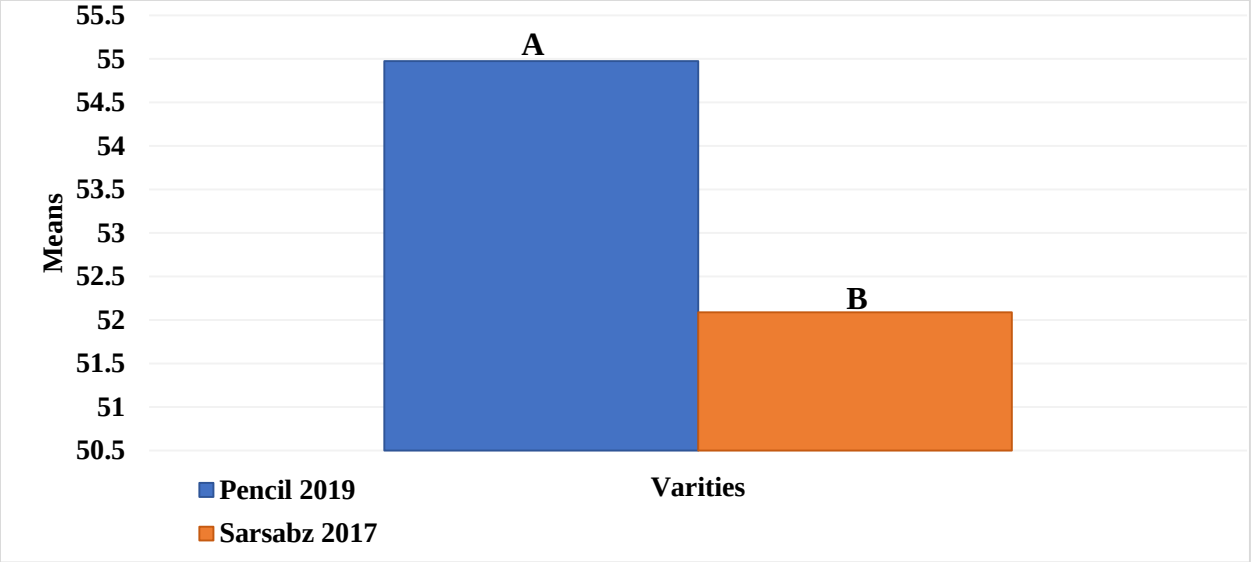


Fig 4.2 All-Pairwise Comparisons Test of Disease for Variety

3.2.3. Pairwise comparison test for disease and treatment

In all pairwise comparison test of disease for treatment different treatments gave different means. The treatment T0, T1, T2 respectively T12 gave the different mean value showed in fig 4.3. There are different groups in which the means are significantly different from one another. Having alpha value of 0.05, Standard Error for Comparison from 1.8728 to 1.8980, Critical Q Value of 4.029 and Critical Value for Comparison 5.3354 to 5.4070 (Fig 4.3). Similar results were reported by Varma (1986), Bakr and Rahman (1998), Singh and Tripathi, (2012), Rahman *et al.*, (1984), Rahman *et al.*, (2005), Ahmed *et al.*, (2006), Singh (2007), and Barnwal (2009).

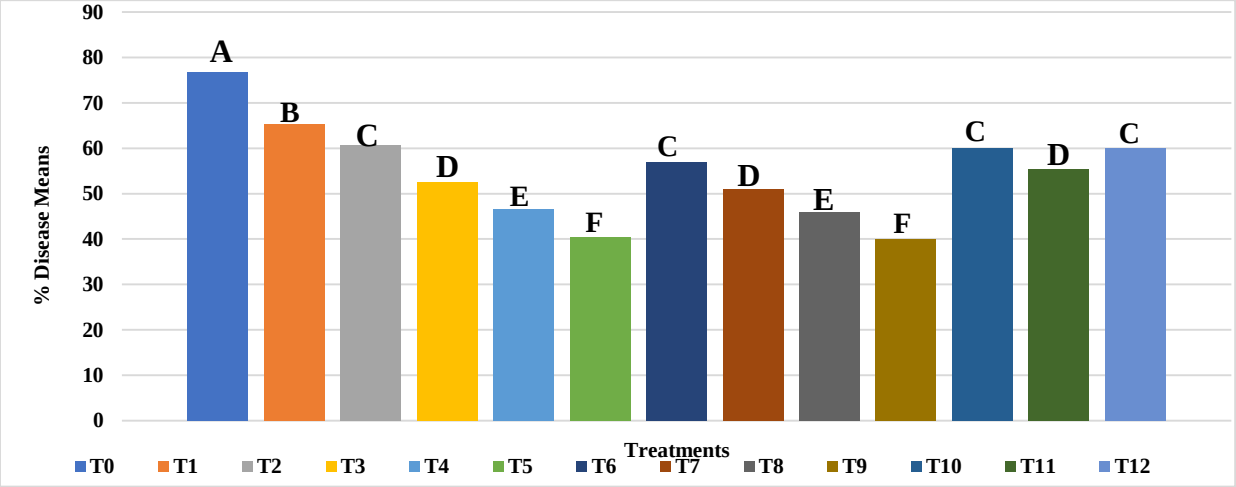


Fig 4.3 Comparisons Test of Disease for Treatment

3.2.4. Pairwise comparison test of disease for spray and variety

HSD All-Pairwise Comparisons Test of Disease for Spray and Variety gave different means result. Pairwise means of disease for spray and variety is shown in fig. There are different groups (A, B, etc.) in which the means are significantly different from one another. The Alpha value was 0.05, Standard Error for Comparison was

2.1167 to 2.5139, Critical Q Value was 4.470 and Critical Value for Comparison 6.6896 to 7.9450 (Fig 4.4). Same result was observed with different treatments gave different means value. The means of pairwise comparison test of disease for spray and

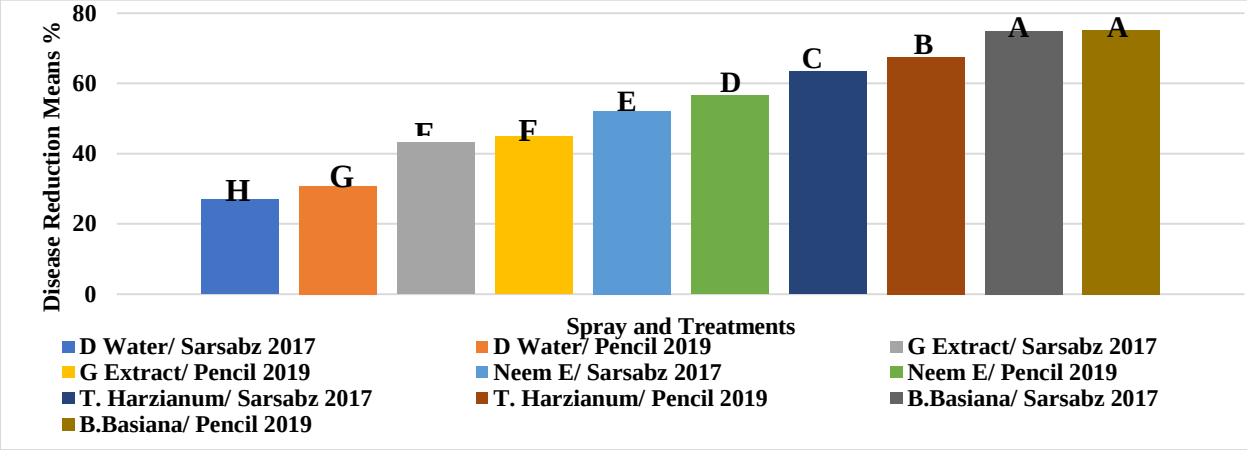


Fig 4.4 Pairwise comparison test of disease for spray and variety

by Singh *et al.*, (1994) and Baker, (1918). Colhoun, (1973), Rotem, (1978), Cochen and Rotem, (1970), and Bashi and Rotem, (1976).

3.2.5. Pairwise comparison test of disease for spray and treatment

The fig 4.5, 4.6, 4.7, 4.8 showed that HSD All-Pairwise Comparisons Test of Disease for Spray one, two, three and four treatment is shown in figs, Having alpha value of 0.05 and simultaneous 95% confidence intervals of means. Result was resembled with the result of Khairi and Preece, (1979), Singh, (1994), Singh (2009), Alexopoulos *et al.*, (2000) and Ostfeld *et al.*, (2005).

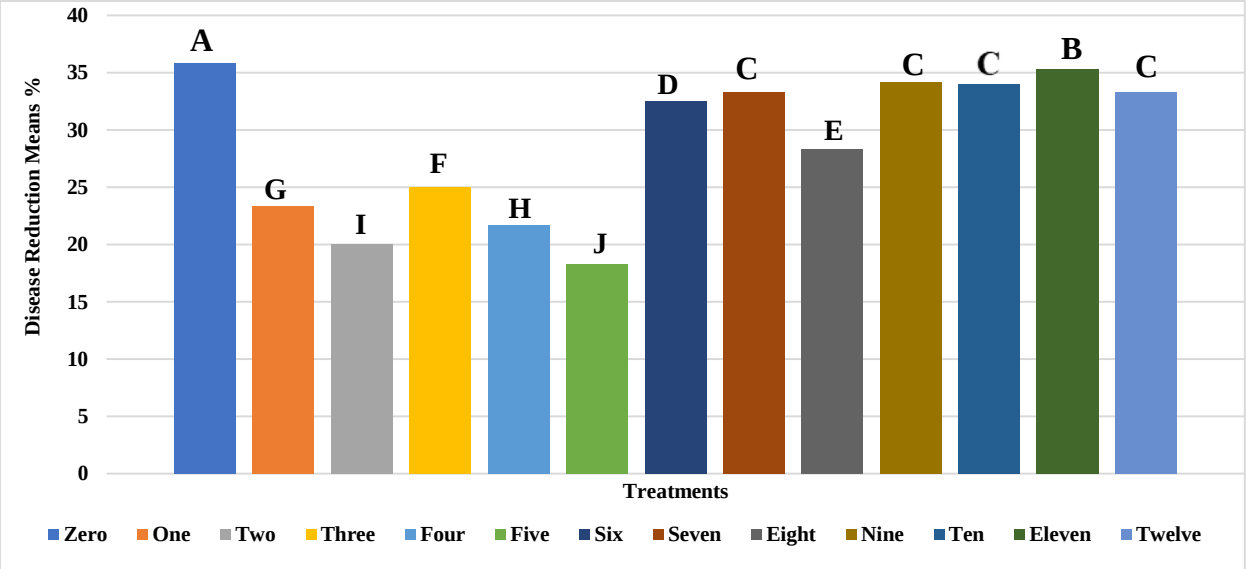


Fig.4.5 Tukey HSD All-Pairwise Comparisons Test of Disease for Spray One

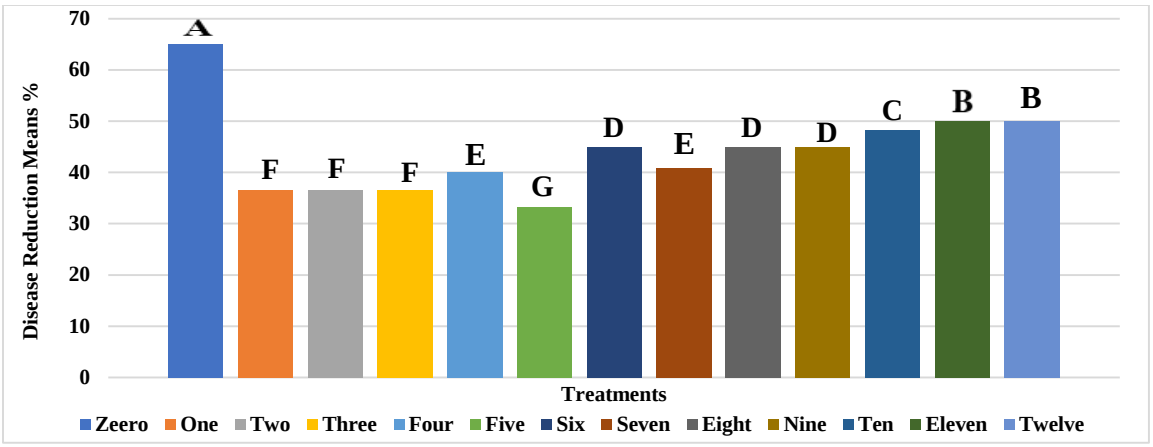


Fig.4.6 Tukey HSD All-Pairwise Comparisons Test of Disease for Spray Two

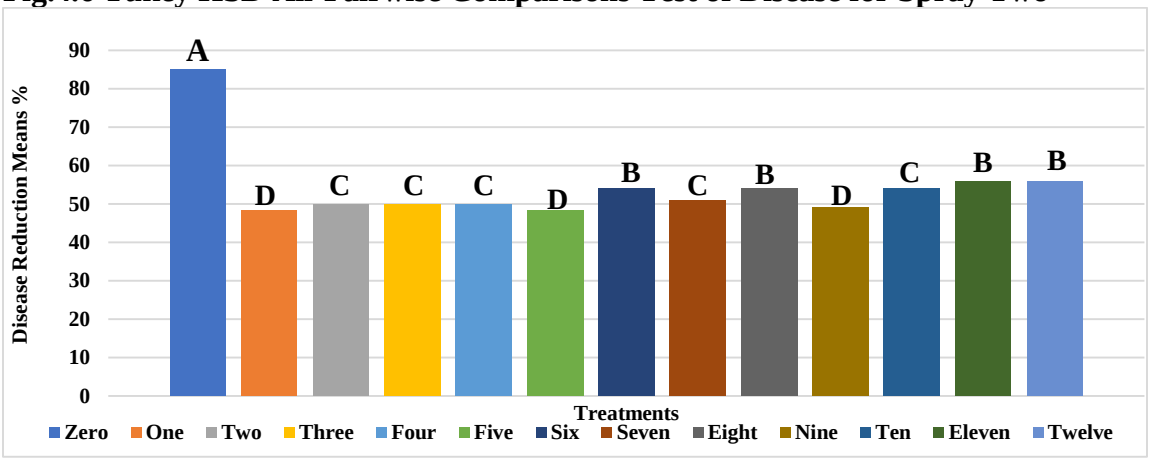


Fig.4.7 Tukey HSD All-Pairwise Comparisons Test of Disease for Spray Three

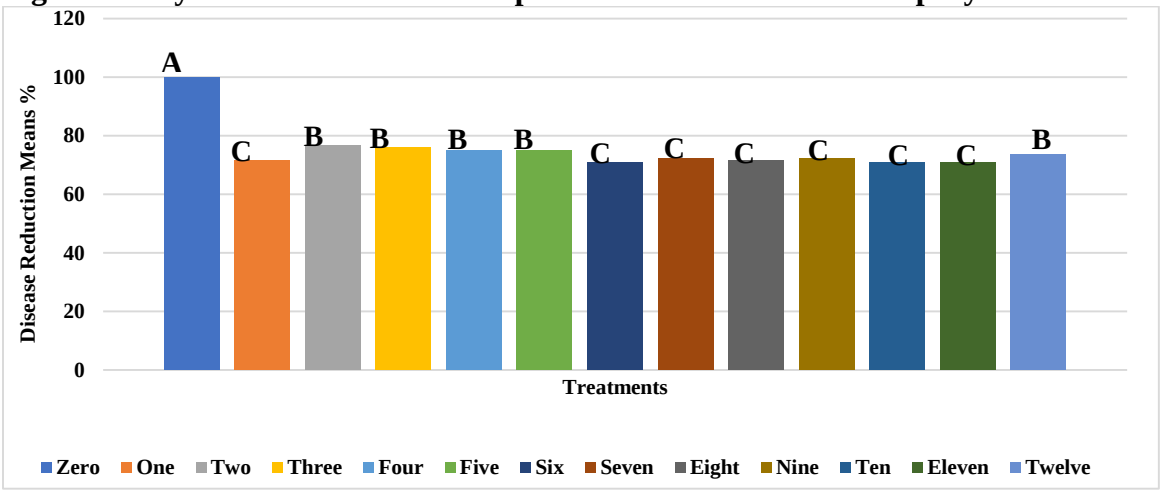


Fig.4.8 Tukey HSD All-Pairwise Comparisons Test of Disease for Spray Four

3.2.6. Tukey HSD All-Pairwise Comparisons Test of Disease for Variety and treatment

The fig.4.9 shows that HSD All-Pairwise Comparisons Test of Disease for Variety and treatment. The means of variety and treatment for disease gave the different

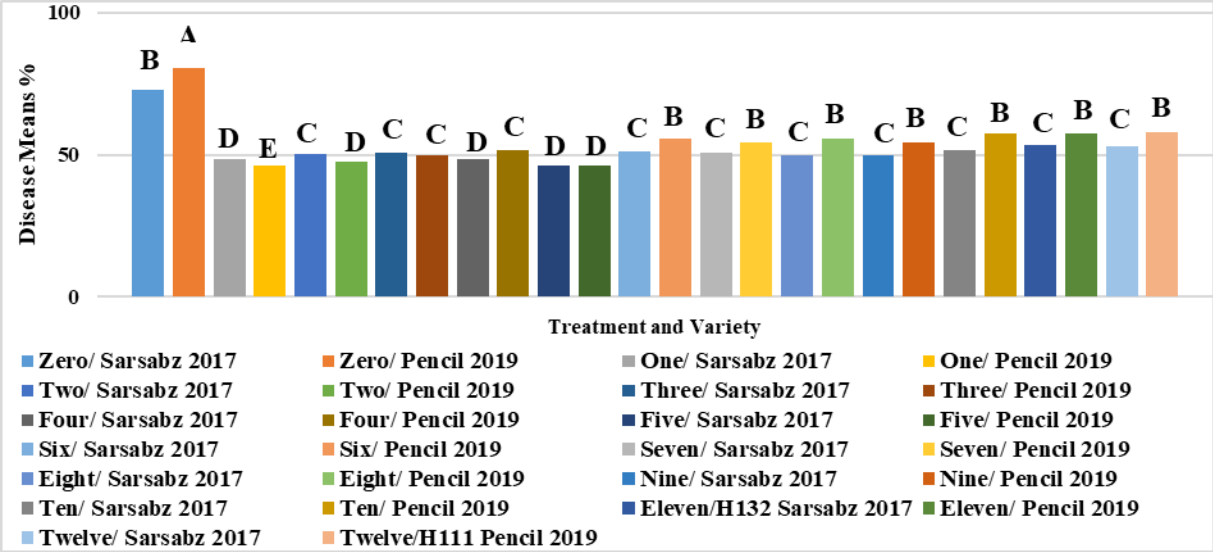


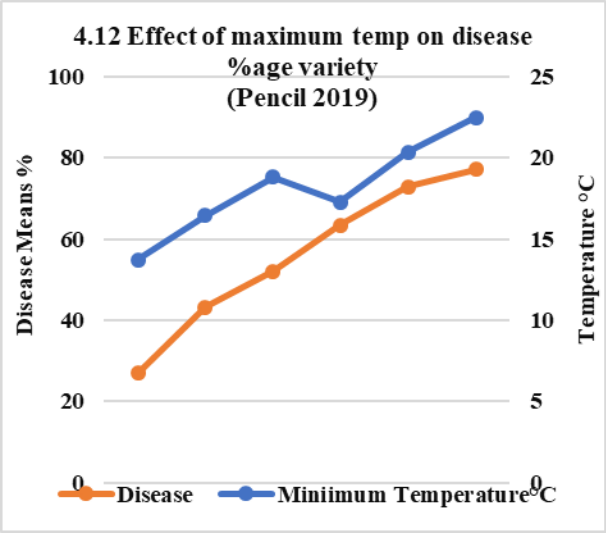
Fig 4.9 Comparisons Test of Disease for Variety and treatment

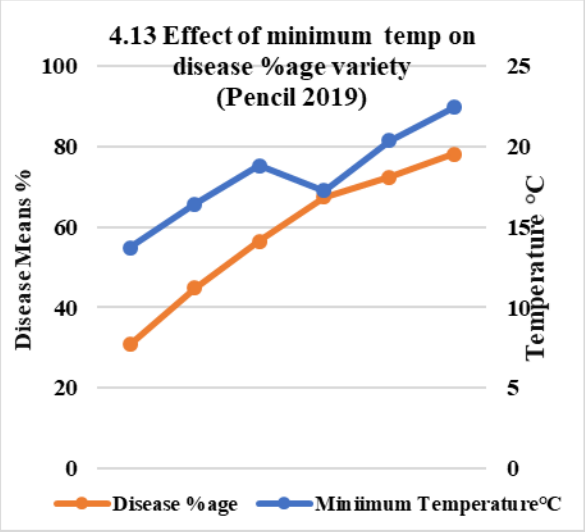
means showing in graph with the alpha value of 0.05 and simultaneous 95% confidence intervals of mean - largest of other means. There are different groups in which the means are not significantly different from one another. Result of research was similar as the result obtained by Boesewinkel, (1979), Royle, (1978), Day and Scott, (1973), Beckett and Read, (1986), Martin and Gay, (1983).

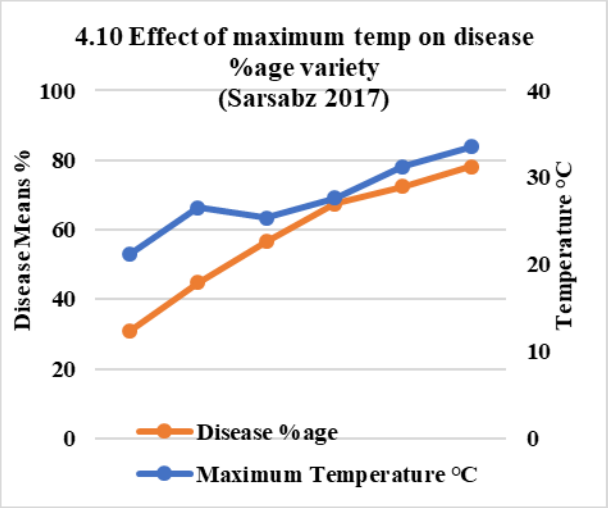
3.3. Environmental Condition Data

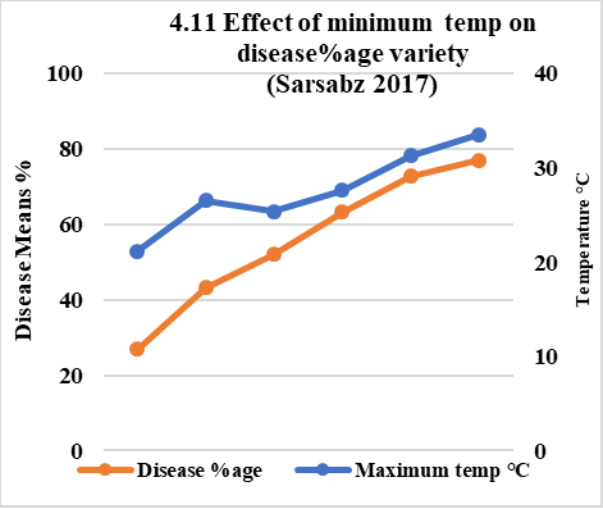
3.3.1. Effect of maximum and minimum temperature on disease %age on variety 1 (Sarsabz 2017) and 2(Pencil 2019)

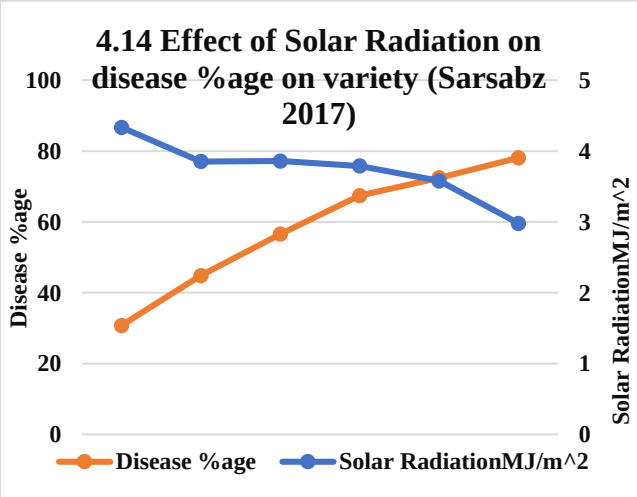
Data on to environmental condition of the month of March and April 2020 was collected from the website of MNSUAM. Effect of maximum and minimum temperature on both varieties 1 and 2 is shown in fig.4.10, 4.11, 4.12 ,4.13 which showed that with the increase in temperature the effect of disease is also increases on both varieties.

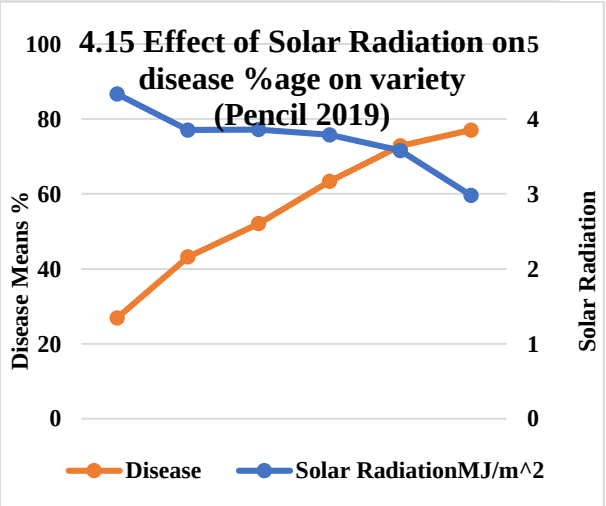

4.12 Effect of maximum temp on disease %age variety (Pencil 2019)
Disease Means %
Temperature °C
100
80
60
40
20
0
25
20
15
10
5
0
Disease
Miniimum Temperature°C

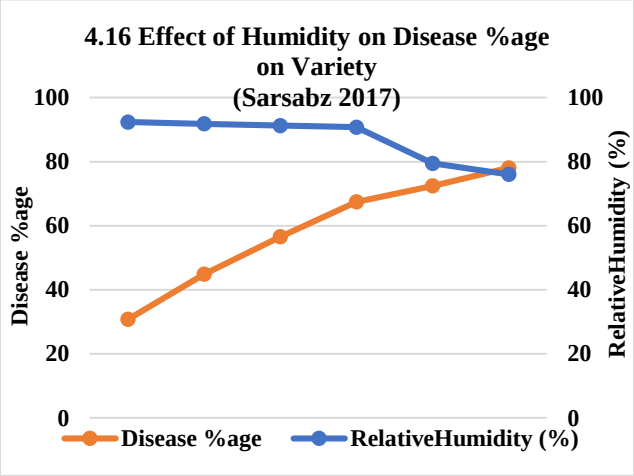

4.13 Effect of minimum temp on disease %age variety (Pencil 2019)
Disease Means %
Temperature °C
100
80
60
40
20
0
25
20
15
10
5
0
Disease %age
Miniimum Temperature°C

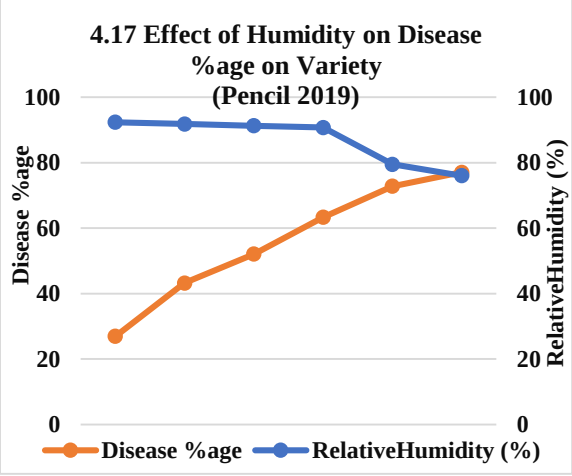

4.10 Effect of maximum temp on disease %age variety (Sarsabz 2017)
Disease Means %
Temperature °C
100
80
60
40
20
0
40
30
20
10
0
Disease %age
Maximum Temperature °C

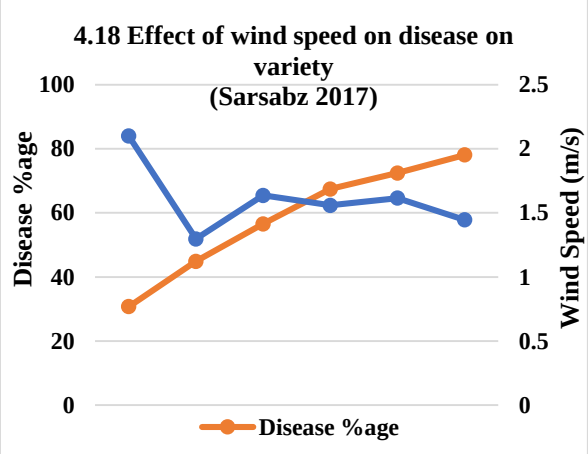

4.11 Effect of minimum temp on disease%age variety (Sarsabz 2017)
Disease Means %
Temperature °C
100
80
60
40
20
0
40
30
20
10
0
Disease %age
Maximum temp °C

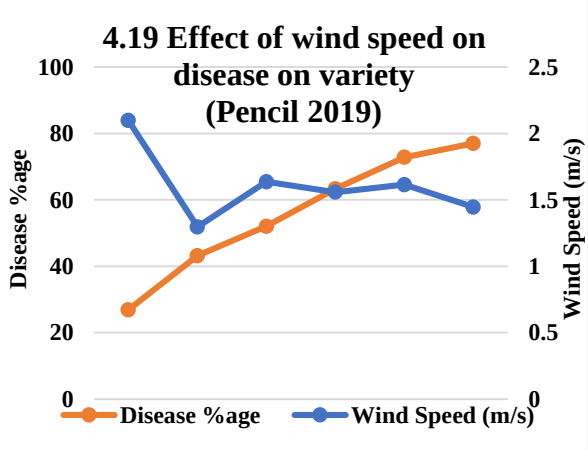

4.14 Effect of Solar Radiation on disease %age on variety (Sarsabz 2017)
Disease %age
Solar RadiationMJ/m^2
100
80
60
40
20
0
5
4
3
2
1
0
Disease %age
Solar RadiationMJ/m^2


4.15 Effect of Solar Radiation on disease %age on variety (Pencil 2019)
Disease Means %
Solar Radiation
100
80
60
40
20
0
5
4
3
2
1
0
Disease
Solar RadiationMJ/m^2









3.3.2. Effect of Solar radiation on disease %age on Variety 1 and 2

Effect of solar radiation in the occurrence of disease on both varieties is shown in fig. 4.14, 4.15 which showed that with the decrease of solar radiation the effect of disease on both varieties increases.

3.3.3. Effect of Humidity level on occurrence of disease on variety 1 and 2

Effect of humidity level in the occurrence of disease on both varieties is shown in fig. 4.16 and 4.17 which showed that with the decrease in the humidity level the effect of disease on both varieties was not infect further.

3.3.4. Effect of Wind speed on occurrence of disease on variety 1 and 2

Effect of wind speed in the occurrence of disease on both varieties is shown in fig. 4.18 and 4.19 which showed that with the decrease in the wind speed the effect of disease on both varieties increases.

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