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Efficacy Assessment of Treatment Methods against Powdery Mildew Disease of Pea (*Pisum sativum* L.) Caused by *Erysiphe pisi* var. *pisi*

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Abstract The experiment was conducted to find out the efficacy of different treatments against powdery mildew of pea (*Erysiphepisi var. pisi*) in Rampur, Chitwan, Nepal during Dec. 2014 to April 2015. Four treatments viz. Skimmed cow milk (250 ml/plot), Karathane (2 ml/L), *Trichoderma* (10^7condia/ml) and Control (distilled water) were used as foliar spray on plants. Considering Disease severity (%), Disease control (%) and Total Area under Disease Progress Curve, *Trichoderma* was found to be effective than Skimmed cow milk and Control but was at par with Karathane, in controlling over Powdery mildew disease. Number of pods/plant and weight of grains/plant were observed highest in *Trichoderma*which was followed by Karathane. There was no significant difference between treatments in case of weight of pods/plant and dry weight/plant. AUDPC/day value increased in control in different sprayings but AUDPC value/day initially increased and later decreased for *Trichoderma*, Karathane and skimmed cow milk. All the yield and yield attributing characters were negatively correlated with AUDPC but number of pods/plant and weight of pods/plant were positively correlated. Reduction in yield was also caused by infection of Rust (*Uromycespisi*) in field. Four foliar sprays of *Trichoderma* (10^7condia/ml) or Karathane@ 0.2% at interval of 7 days may be the option for the management of powdery mildew in severe condition. *Trichoderma* can be alternative method for farmers to have eco-friendly management of Powdery mildew as Karathane has negative impact on human and plant health.

Keywords: *pea, powdery mildew, Trichoderma, disease control*

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1. Introduction

Pea (*Pisumsativum* L.), also known as Kerau or Matara in local languages, is a key component of Nepalese farming system. It can be grown successfully in terai (<100 m) during winter to high mountain (3000 m) during summer months [9]. The optimum mean monthly temperature for pea is 12.8 0C to 18 0C. Young leaves and green pods are consumed as green vegetable. They are also dried for off-season use as a vegetable and pulse. Fodder is a valuable livestock feed. Green peas are rich in vitamin and proteins. Mature seed contain 10.9 g water 22.9 g protein, 1.4 g fat, 60.7 g carbohydrate, 1.4 g fibre and 2.7 g ash [7]. The area/production/productivity of peas are 17459 ha, 16459 MT, 943 kg/ha respectively in case of Nepal whereas in case of Chitwan condition, area/production/ productivity are 439 ha, 5618 MT, 12MT/ha respectively [11]. The wide gap between the attainable yield potentials and farmers field are due to various biotic, abiotic and socio-economic factors.

Among the various diseases of pea, Powdery mildew caused by *Erysiphe pisi var. pisi* and rust caused by *Uromycespisi* are two major diseases causing severe loss with in short period of time. Powdery mildew appears in epidemic form when the plants are in the pod stage towards the end of January and in February. The disease is characterized by the formation of white, floury patchesinitially on the leaf progressing towards tendrils, pods and stems covering most of the aerial part at advancement of the disease. The losses in yield in a 100% infected crop were estimated to be 21-31% in pod number and 26-47% in pod weight [13]. The pathogen causes up to 50% yield losses and reduces pod quality [6].

Several chemical fungicides and bio-control agents are used to control the disease. Captan @ 2 gL^{-1} gave good control of powdery mildew [12]. Bayletan showed best performance in controlling the disease and increased pea yield by 47-75% [2]. Maneb and Trideimorph were very effective against powdery mildew in pea [18]. Use of botanicals like neem extract, extract of *Reynoutriasachalinensis* and natural product like skimmed cow milk is also found satisfactory. *Trichoderma*species is one of the most effective antagonists used against a wide range of fungal disease [8]. Hence, this experiment was

carried out to find out the efficacy of different treatments against powdery mildew disease of pea.

2. Materials and Methods

The experiment was conducted at Institute of Agriculture and Animal Science (IAAS), Rampur, Chitwan from December 9, 2014 to April 1, 2015 in Simple Randomized Complete Block Design (RCBD) consisting of four treatments with four replications. The treatments were Skimmed Cow milk, Karathane, *Trichoderma* and Control. Seeds of Arkel variety were sown in 16 plots with individual size of 2.4 m x 2.4 m at spacing of 60 cm×60 cm. Four plants were maintained in each 4 rows, and 16 plants in each plots. Five plant samples were taken for the study from each plot with four plants from the centre and one plant from border.

Potato Dextrose Agar (PDA) mediawas prepared and mass multiplication of *Trichoderma* was done from pure culture at laboratory.After the first diseases appearance in pea plant, Skimmed Cow milk (250ml/plot), Karathane (2 ml/L water), *Trichoderma* (10^7 conidia/ml of suspension) and Control (Distilled water) were applied as foliar spray. Total four sprays were made at regular interval of a week. Spraying was done in evening hours (after 2 pm) for its effectiveness.

Disease occurrence and progress in the field was assessed by using score scale of 0-9 where 0 = No incidence of disease, 1= 0-20%, 2= 21-30%, 3= 31-40%, 4= 41-50%, 5= 51-60%, 6 = 61-70%, 7 = 71-80%, 8 = 81-90% and 9 = 91-100% of total aerial part of plant infected. Scoring was done in the field before each treatment for each sample plant and ddisease score data was converted intoArea under disease progress curves (AUDPC), Disease severity (%) and Disease control(%) to measure the efficacy of treatments.

Area under Disease Progress Curves (AUDPC) was calculated using the formula given by Das *et al.* (1992) [5].

$$\text{AUDPC} = \sum_{i=1}^{n}\left[\left\{\left(Y_i + Y_{(i+1)}\right)/2\right\}\times\left(t_{(i+1)}-t_i\right)\right]$$

Where, Y_i= disease severity on the i^{th} date

Y_{i+1}= disease severity on the $i+1^{th}$ date and

n= number of dates

The disease severity percent was determined according to Townsend and Heuberger (1943) equation[17].

$$\textit{Disease severity}(\%) = \frac{\textit{Sum of scorings of different ratings}}{\textit{No.ofsample}\times\textit{highest rating}}\times 100$$

Percent disease control of PM was calculated by using the following formulae

$$\textit{Disease control }\% = \frac{\left(\begin{array}{l}\textit{Disease severity in Control}\\ -\textit{Disease severity in treatment}\end{array}\right)}{\textit{Disease Severity in Control}}\times 100$$

The yield traits were recorded as number of pods/plant, weight of pods/plant, weight of grains/plant and dry weight /plant. The obtained data were analysed by using R-stat software package. The means were compared using DMRT(Duncan's Multiple New Range Tests). Daily data on maximum temperature, minimum temperature and total rainfall were taken from National Maize Research Program (NMRP) Rampur, Chitwan, Nepal which lies adjacent to the experimental field.According to the weekly weather data of experimental field during experimental period (Dec. 2014 – Mar. 2015) at IAAS, Rampur, Nepal; temperature during the growth of Pea was favourable for both Powdery mildew and *Trichoderma.*

3. Results and Disc

3.1. Effects of Foliar Spray of Treatments on Disease Development

3.1.1. Effect on Disease Severity (%)

There was no significant difference between treatments against powdery mildew disease severity on 82 DAS and 89 DAS whereas 96 DAS and 103 DAS showed significant difference. In case of 96 DAS, *Trichoderma* treated plots showed the lowest disease severity of powdery mildew (20.00±2.4) between the treatments which was at par with Karathane (24.44±1.5) and Skimmed cow milk (27.78±2.9) and the highest disease severity of Powdery Mildew disease (43.33± 1.4) was observed in untreated control plots (Table 1). Similarly, in case of 103 DAS, *Trichoderma* treated plots showed the lowest disease severity of powdery mildew (20.00±2.4) between the treatments. Karathane and Skimmed cow milk treated plots showed disease severity of (18.89±1.9) & (24.44±3.1). In both 96 DAS and 103 DAS, *Trichoderma* was found to be more effective in comparison to other treatments followed by Karathane and Skimmed cow milk. However, *Trichoderma* was not significantly different with Karathane.

Table 1. Effect of various treatments on severity percentage of powdery mildew (*Erysiphe pisi var. pisi*) of pea in field at IAAS, Rampur, Chitwan, Nepal

Treatments	Disease severity (%)			
	82 DAS	89 DAS	96DAS	103 DAS
Skimmed cow milk@250ml/plot	22.22±0.91	31.11±1.8	27.78±2.9^{b}	24.44±3.1^{b}
Karathane@2ml/L	21.67±1.06	27.23±0.5	24.44±1.5^{b}	18.89±1.9^{bc}
Trichoderma@10^7conidia/ml	19.45±1.67	26.11±1.07	20.00±2.4^{b}	13.89±2.4^{c}
Control	23.33±1.43	31.39±3.02	43.33±1.4^{a}	55.00±2.4^{a}
P-value	0.358	0.174	0.0003***	0.000013***
LSD	Ns	Ns	7.589	9.196
CV (%)	13.668	12.892	16.424	20.49
Grand mean	21.666	28.959	28.889	28.056

Means in a column having same letter(s) do not differ significantly at 5% probability by DMRT. DAS= Days after sowing

Our result was supported by research of Mahmoud, A. H. and Gabr, A. El-Kot (2010) that *Trichodermaharzianum* decreased severity from 87.2 - 7.7% in 2006 season and from 91.5 - 8.1% in 2007 season. Milk and whey, can suppress powdery mildew [3] and [4]. Abd El-Moity, T. H., (1985) observed that *Trichodermaharzianum*inhibit disease by producing some anti-fungal substances, i.e. glio-toxin and some growth regulators[1]. These compounds are amphilic, membrane active surfactants and when sprayed on plant surface, prior infection led to stimulate plant resistant and enforce treated plants to produce some metabolites which depress the pathogen and some growth promoters such as Indols which increased plant growth generally.

Disease severity of powdery mildew was first noticed at the 1st week of March (82 DAS). Gradual increase of disease severity (%) was continued up to 2nd week of March (89 DAS) whereas there was gradual decrement of disease severity % on 96 DAS and 103 DAS in case of various treatments. But, there was remarkable increase of disease severity % up to the last week of March in case of Control (Figure 1).

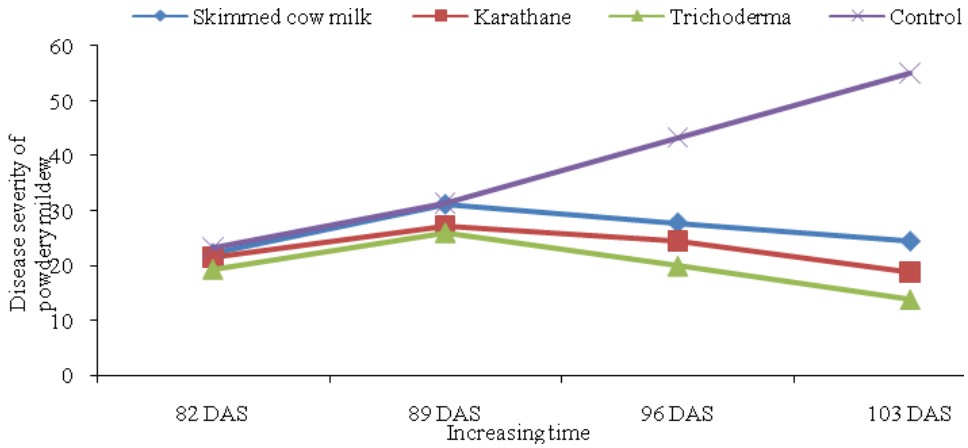

Figure 1. Fluctuation of disease severity of powdery mildew of pea in relation to time with respect to different treatments

3.1.2. Effects on AUDPC (Area under Disease Progress Curve)

There was no significant difference between treatments against powdery mildew disease on AUDPC1 but there was significant variation between the treatments on AUDPC2 and AUDPC3. In case of AUDPC 2, lowest AUDPC was observed in *Trichoderma* (161.39±11.6) treated plot which was at par with Karathane (180.84±5.8) and highest in control plots (261.52±12.6) (Table 2).

Regarding AUDPC 3, *Trichoderma* gave the best result with lowest AUDPC (118.62±16.9) which was at par with Karathane (151.66±12.09). In Skimmed Cow milk, the AUDPC value was found to be (182.77±21.1) and the highest AUDPC was found in Control plots (344.16±13.2).

Table 2. Effect of various treatments on AUDPC of powdery mildew (*Erysiphe pisi var. pisi*) of pea in field at IAAS, Rampur, Chitwan, Nepal

Treatments	AUDPC 1	AUDPC 2	AUDPC 3	Mean AUDPC	Total AUDPC
Cow skimmed milk@250ml/plot	186.66±8.39	206.12±15.72^{b}	182.77±21.1^{b}	191.85±14.2^{b}	575.55±42.7^{b}
Karathane@2ml/L	171.12±5.4	180.84±5.83^{bc}	151.66±12.1^{bc}	167.87±5.93^{bc}	503.62±17.78^{bc}
Trichoderma@10^{7} conidia/ml	159.44±8.09	161.39±11.62^{c}	118.62±16.91^{c}	146.49±1.04^{c}	439.46±36.13^{c}
Control	191.52±15.1	261.52±12.65^{a}	344.16±13.24^{a}	265.73±10.41^{a}	797.20±31.22^{a}
P-value	0.204	0.00171**	0.00004***	0.0003**	0.0003***
LSD	Ns	40.148	58.248	38.729	116.187
CV (%)	12.099	12.397	18.271	12.546	12.546
Grand mean	177.185	202.466	199.305	192.986	578.957

Means in a column having same letter(s) do not differ significantly at 5% probability by DMRT.
LSD = Least Significant Difference.

There was no statistical variation between treatments in Mean AUDPC and Total AUDPC value. Mean AUDPC and Total AUDPC value was found to be lowest for *Trichoderma* treated plots which were 146.49±1.04 and 439.46±36.1 respectively and was found highest in Control which were 265.73±10.4 and 797.20±31.2 resp. *Trichoderma* was at par with Karathane for which values of mean AUDPC and total AUDPC were 167.87±5.9 and 503.62±17.7. Mean and total AUDPC value for skimmed cow milk was 191.85±14.2 and 575.55±42.7 respectively. AUDPC value was low in case of *Trichoderma* treated plots due to lower powdery mildew disease severity value(Table 1).

3.1.3. Effect on AUDPC per day

Powdery mildew disease progress/day was higher in case of Control followed by Skimmed cow milk, Karathane and *Trichoderma*. AUDPC/day value increased in control in different sprayings but AUDPC value/day increased initially and later decreased for *Trichoderma*, Karathane and skimmed cow milk. The rate of decrement in AUDPC/day was found more in *Trichoderma* followed by Karathane and Skimmed cow milk (Figure 2). Fluctuation of AUDPC/day between different treatments was found highest in AUDPC 3/day and least at AUDPC 1/day.

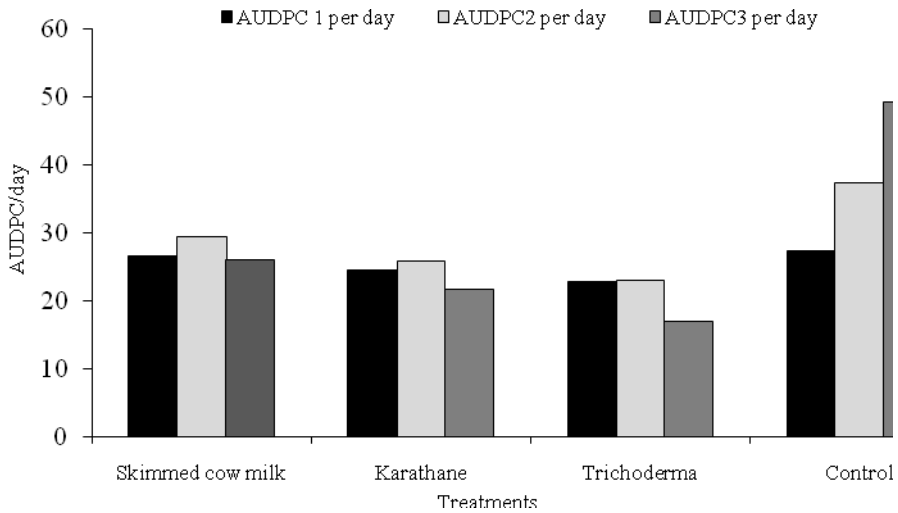

Figure 2. AUDPC/day values with respect to various treatments

3.1.4. Effects on Disease Control by Various Treatments with Respect to Control

In both 96 DAS and 103 DAS, *Trichoderma* was statistically different with Skimmed cow milk but it was at par with Karathane in percentage disease control. However, *Trichoderma* showed better control over disease with respect to Control which were 53.51±6.18 & 74.62±4.6 in 96 DAS and 103 DAS res. Karathane showed disease control by 43.22±4.8 & 65.28±4.2. Least disease control was observed in Skimmed cow milk which was 35.33±8.4 & 54.68±7.8 in 96 DAS and 103 DAS respectively (Table 3).

Table 3. Effect of different treatments on disease control of powdery mildew (*E. pisi var. pisi*) of pea at Rampur, Chitwan, Nepalwith respect to control treatment

Treatments	Disease control (%)	
	96 DAS	103 DAS
Skimmed cow milk (250 ml/plot)	35.33±8.45b	54.68±7.83b
Karathane @ 2 ml/L	43.22±4.87ab	65.28±4.27ab
Trichoderma@107 conidia/ml	53.51±6.18a	74.62±4.6a
Control	0	0
P-value	0.000305***	0.000008***
LSD	17.006	15.60
CV (%)	32.204	20.055
Grand mean	33.014	48.641

Means in a column having same letter(s) do not differ significantly at 5% probability by DMRT. LSD = Least Significant Difference.

This result supported the result of Sharma [16], (2000) that four sprays of Karathane (0.1%) at weekly interval gave effective control of powdery mildew. Using *Trichoderma* reduced 47.20% in powdery mildew disease severity of cucumber compared to control [10].In an experiment conducted on Tamil Nadu, India, Karathane was found to be superior over *Trichoderma* [14]. However, contrast result was found in our research where *Trichoderma* showed lowest severity followed by Karathane. Furthermore, temperature was favourable (25-300C) for growth during application (Figure 9) which helped in increasing antagonistic effect of *Trichoderma*. As well as better concentration of *Trichoderma* reduced the competition between them and thus increased its effectiveness.

3.2. Effects on Yield and Yield Attributing Characters

There was no significant difference between treatments in case of weight of pods/plant and dry weight/plant. But significant difference was found in case of number of pods/plant and weight of grains/plant.

Number of pods/plant was observed highest in *Trichoderma* (11.95±1.8) which was followed by Karathane (7.80±2.3). Least number of pods/plant was observed in Control (5.55±0.5). Skimmed cow milk (6.85±0.9) and Control was at par with Karathane.

Highest weight of grains/plant was found in *Trichoderma* (13.59±2.5) and minimum weight of grains/plant was found in Control plots (5.66±0.9). *Trichoderma* was found to be statistically different from other treatments for weight of grains/plant (13.59±2.5) but there was no significant difference between Karathane (9.50±2.9), skimmed cow milk (6.44±1.29) and control (5.66±0.99) (Table 4).

Table 4. Effect of various treatments on yield of powdery mildew (*Erysiphe pisi var. pisi*) of pea in field at IAAS, Rampur, Chitwan, Nepal

Treatments	Yield			
	No. of pods/plant	Weight of pods/plant	Weight of grains/plant	Dry weight /plant
Skimmed Cow milk @250 ml/plot	6.85±0.91^{b}	10.87±2.08	6.44±1.29^{b}	11.48±1.45
Karathane@2ml/L	7.80±2.35^{b}	12.50±3.37	9.50±2.92^{b}	17.95±5.13
Trichoderma@10^{7} conidia/ml.	11.95±1.82^{a}	15.59±2.99	13.59±2.59^{a}	21.35±5.65
Control	5.55±0.57^{b}	8.07±1.08	5.66±0.99^{b}	8.3±0.39
P-value	0.0237*	ns	0.0124*	Ns
LSD	3.887	5.822	4.517	10.891
CV (%)	30.239	30.958	32.095	46.103
Grand mean	8.038	11.756	8.799	14.769

Means in a column having same letter(s) do not differ significantly at 5% probability by DMRT.

Maximum yield of Arkel variety of Pea in Chitwan condition was 1.835 t/ha and seed yield/plant was 10.089g [15]. But in our case obtained yield (avg.) was 0.244 t/ha. Reduction in yield might be due to occurrence of Powdery mildew in field along with heavy infestation of plants by Rust (*Uromycespisi*) at reproductive stage of crop. Higher disease severity led to decrement in photosynthetic area of leaf and thus plant failed to assimilate food products leading to decrement in yield quantity.

However, yield in *Trichoderma* treated plots was higher than other treatments which were due to low severity of Powdery mildew on plants (Table 1). Higher yield in *Trichoderma* treated plots might be due to increased hormonal effects of *Trichoderma*.

3.3. Correlation between Total AUDPC with Yield Contributing Characters

No. of pods/plant (r= -0.64), weight of pod/plant (r= -0.60), weight of grains/plant (r= -0.61), and dry weight/plant (r= -0.68) were negatively correlated with AUDPC (Figure 3, Figure 4, Figure 5 & Figure 6). Maximum number of pods/plant, weight of pod/plant, weight of grains/plant, and dry weight/plant was obtained with minimum AUDPC and vice versa.

Total AUDPC value contributed 40.8% loss in no. of pods/plant, 35.6% in weight of pods/plant, 36.8% weight of grains/plant and 46% in dry weight/plant.

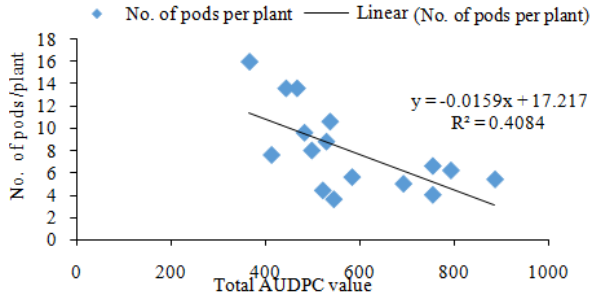

Figure 3. Correlation between total AUDPC and number of pod/plant

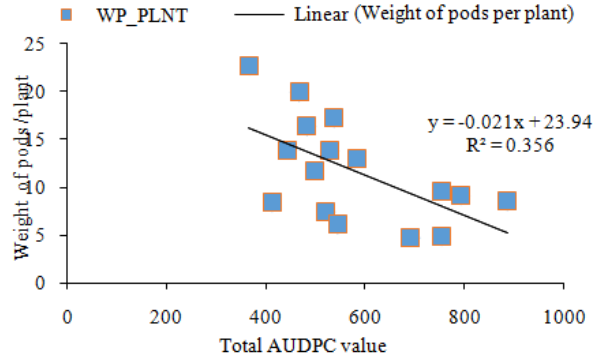

Figure 4. Correlation between total AUDPC and weight of pod/plant

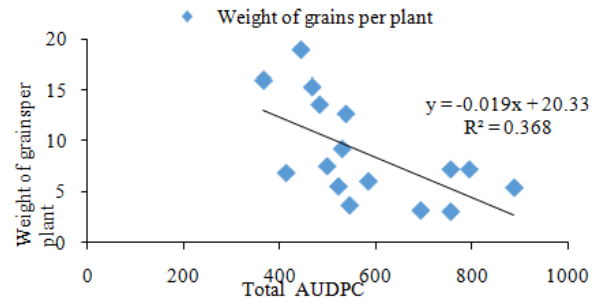

Figure 5. Correlation between total AUDPC and weight of grains/plant

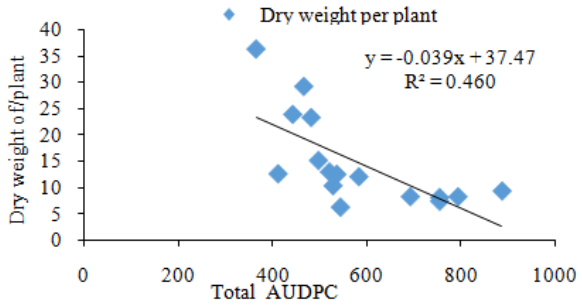

Figure 6. Correlation between total AUDPC and dry weight/plant

3.4. Correlation between Number of Pod/Plant and Weight of Pod/Plant

Number of pods/plant was positively correlated with weight of pods/plant (r= 0.90). Maximum weight of pods/plant was obtained with maximum number of pods/plant. Number of pods/plant contributes 81.4% in increasing weight of pods/plant (Figure 7).

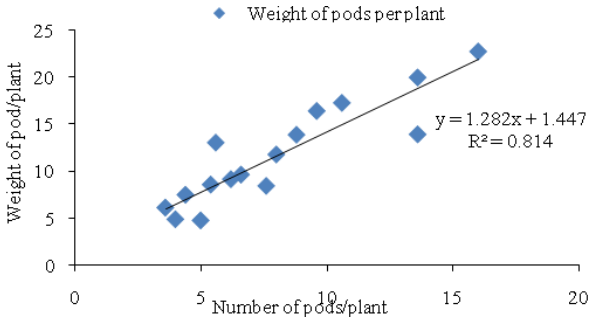

Figure 7. Correlation between number of pod/plant and weight of pod/plant

3.5. Effect of Rust (*Uromyces pisi*) Severity over Increasing Time

Number of pods/plant (r= -0.57) was negatively correlated with total AUDPC (Figure 8). Total AUDPC value contributed 33.57% loss in no. of pods/plant and thus led in reduction of total yield of pea in combination with Powdery mildew.

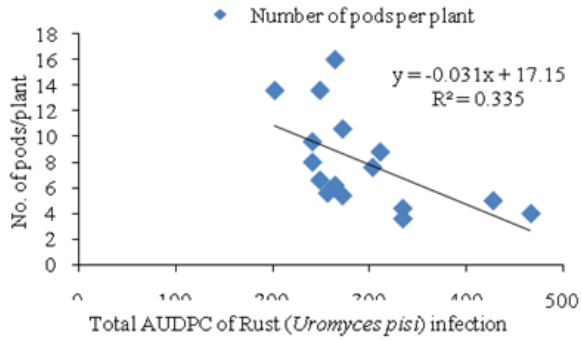

Figure 8. Correlation of number of plants with total AUDPC by rust infection

4. Conclusion

From results and fore going discussion it may be concluded that disease symptom was first observed in the 1st week of March (82 DAS) which increased up to 2nd

week of March (89 DAS) followed by gradual decrement of disease severity % on 96 DAS and 103 DAS. Treatment should be applied just after the appearance of disease symptoms. High disease severity and AUDPC directly reduced the yield. *Trichoderma* was found to be effective for controlling disease which was at par with Karathane. Four foliar sprays of *Trichoderma*(@10^7conidia/ml) or Karathane (@ 0.2%) at interval of 7 days may be the option for the management of powdery mildew in severe condition. However, use of Karathane is harmful for beneficial organisms, plants and human health. So, *Trichoderma* can be alternative method for farmers for management of powdery mildew.

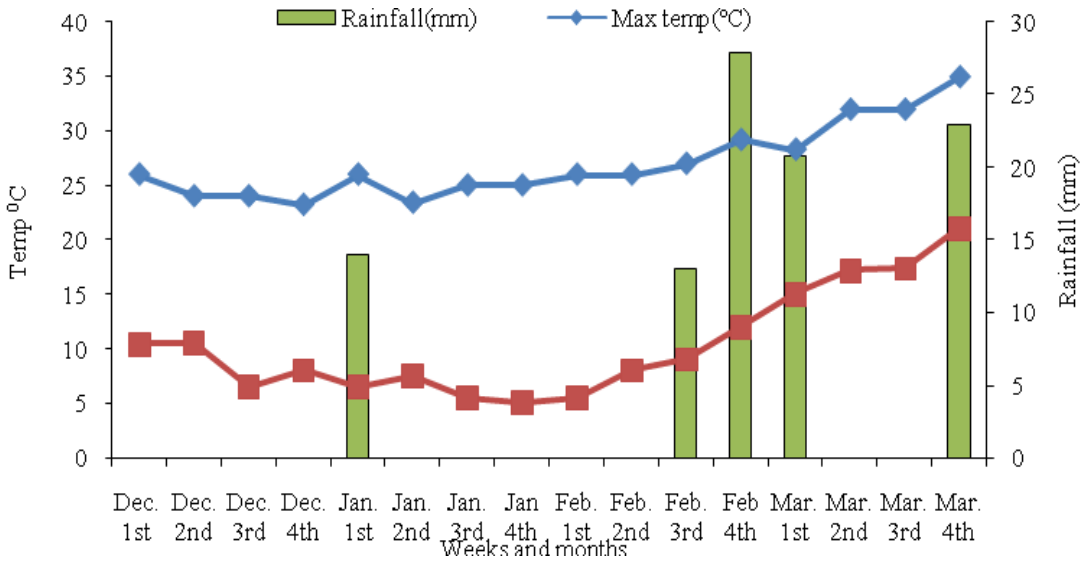

Figure 9. Graph showing weekly weather data of experimental field during experimental period (Dec. 2014 to Mar 2015) at IAAS, Rampur

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