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Comparative Efficacy of Insecticides

Discovering Thoughts, Inventing Future

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Studies on Kinetics of Nitrogen Uptake in Combination with 2, 4-D in Blue-Green Algae of Rice Fields

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Studies on Kinetics of Nitrogen Uptake in Combination with 2, 4-D in Blue-Green Algae of Rice Fields

Bhagya Lakshmi Jyothi, K [α] & TRK Reddy [σ]

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I. Introduction

In tropical countries such as India, the blue-green algae inhabitated mainly in the paddy fields (Singh,1961;Venkataraman,1972) and play a considerable leading role in the ecosystem of rice agriculture (Watanabe and Brotonegoro,1981). The most important functional characteristics of blue-green algae growing in the soils of paddy-fields are the dinitrogen fixation and reclamination of the soil by enriching with nitrogenous substances and metabolites to obtain more crop yield and reduce the application of nitrogenous chemical fertilizers. To obtain a high yield of grains, farmers employ synthetic chemical fertilizers in the rice-fields, which enrich the nitrogen content and phosphate content. With a view to obtain more crop yield, the farmers have been following the modern agricultural practices such as controlling growth of weeds and pests by employing the pesticides and herbicides irregularly and indiscriminately. As a result, pesticides and herbicides not only pile up and cause environmental hazard in paddy fields but also effect the non targeted beneficial organisms such as blue-green algae and fungi inhabitated in the paddy fields. The coincidence of employing the herbicides to eradicate weeds in the rice-fields while the farmers are simultaneously irrigating the fields with eutrophicated water.

At this juncture the eutrophication promotes the growth and survival of blue-green algae while the pesticides and the herbicides normally suppress the growth of weeds and pests simultaneously affecting the non-targeted beneficial blue-green algae of rice-fields. In fact the blue-green algae should not grow in the rice-fields because of the application of the pesticides and herbicides, but after their application where the irrigated eutrophic water used to stagnate, one could observe the abundance of growth of cyanobacteria which could have been influenced by the environmental factors such as nutrients (nitrogen and phosphate) and physical factors (pH, temperature and light). The intrinsic information acquire from rice-field farmers necessitated to pursue the research on nutrients influence on the herbicidal toxicity in cyanobacteria.

The percentage of nitrogen in cyanobacteria would be 8-10% of the total dry weight and nitrogen becomes an important factor in controlling the luxuriant growth of blue-green algae. The cyanobacterial growth and uptake of nitrogenous substances in rice-fields possibly depended on many physical factors (light, rainfall and temperature) and chemical factors (organic and inorganic nutrients such as nitrogen, carbon, phosphate and pH of the soil).

Various species of blue-green algae exhibited different levels of efficacy in assimilation of high or low levels of nutrients, which becomes a great advantage in nutrient poor waters (Sivasubramanian and Rao, 1988). Normally, cyanobacteria prefers to utilize lower concentrations of nitrogenous fertilizers for optimum growth and nitrogen fixation (Anand, 1990). Ammonium-nitrogen often led to poorer growth of blue-green algae *Anabaena doliolum* than nitrate supplied at comparable levels and the concentrations above 0.4 M were toxic (Singh and Srivastava, 1968). Nitrate nitrogen was evidenced as the most suitable nitrogen source for the growth of *Nostoc muscorum* (Kratz and Myers, 1955), *Anabaena doliolum* (Srivastava and Singh, 1968).

In the present investigation *Nostoc spongiaeforme* was selected for studying the kinetics of

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nitrogen and 2, 4-D uptake. The effect of nitrogen and 2, 4-D on the growth has also been investigated.

II. Materials and Methods

a) Blue-green algae, culture medium and growth and uptake measurement

The filamentous, heterocystous and nitrogen fixing *Nostoc spongiaeforme* isolated from local rice-fields of Andhra Pradesh, India were made unialgal and grown in Chu No.10 medium as modified by Gerloff et al.,(1960). The pH was adjusted to 8.5 and stocks and experimental cultures were maintained in culture chambers at 28±2^{0}C and illuminated with fluorescent light emitting 1600 lux and shaken twice a day. Growth was measured by the estimation of chlorophyll-a and protein. Based on the results of short-term experiments (72 hours incubation period), long-term experiments were conducted to study the effect of various types of nitrogen sources $\left(NO_3^-, NO_2^-, NH_4^+\right)$ alone and in combination with fixed dose of 2,4-D (600 μg per ml) on the growth, uptake and their kinetics in *Nostoc spongiaeforme* up to 25 days of life cycle period with an interval of five days.

The estimation method of nitrate $\left(NO_3^-\right)$ (Brucine sulphanilic method mentioned by Brown et al., 1974), nitrite $\left(NO_2^-,\right)$ (Sulphanilamide and N-(1-naphthyl)- ehtylenediamine - dihydrochloride method, APHA, 1980) and ammonia $\left(NH_4^+\right)$ (Nessler's reagent method, APHA, 1980) were employed in these experiments. 2, 4-D was estimated by hydroxylamine hydrochloride, N, N'-Dicyclohexyl carbodiimide and ethanolic ferric chloride as mentioned by Grzegzolea Klazbita(1979). For chlorophyll estimation, 10 ml algal culture were centrifuged at 5000g for 5 min and O.D. measured to calculate chlorophyll as per the formulae of MacLachlan and Zalik (1963). Proteins were determined by the method of Lowry et al., (1951).

Different concentrations (0.1, 1.0, 1.5 and 2.0 mg per ml) of nitrogen sources i.e. sodium nitrite ($NaNO_2$), potassium nitrate (KNO_3) and ammonium chloride (NH_4Cl) alone and in combination with 2,4-D (600 μg per ml) were supplied in growth medium. Control cultures which do not contain nitrogen source (BM-NO_3; BM-NO_3 +2,4-D) and pH of the growth media were adjusted to 8.5 with 0.1 N NaOH and 0.1 N HCl, were sterilized and inoculated with small vegetative filaments of 2-4 cells (125 x 10^4 per ml which was equivalent to 100 mg fresh weight) of *Nostoc spongiaeforme* grown in NO_3 free basal medium. (For obtaining starved inoculum, the growing cultures were transferred to the required nutrient deficient media and were allowed to grow for one week in that medium. The starvation was observed by the reduction of pigment in the algal cultures). After every five days of inoculation of algae, growth was measured in terms of chlorophyll-a and protein as mentioned earlier. The uptake of nitrogenous substances was measured as the difference between nitrogen content before and after inoculation with algal cells (5 days). The S/V values indicated the rate of uptake of nitrogen/2,4-D herbicide per hour by the algae and it was calculated by dividing the supplemented nitrogen/2,4-D substrate concentration (S) with uptake of nitrogen or the velocity of the nitrogen (V). The Km (Michaelis constant) values of various nitrogen sources were calculated by plotting the velocity of uptake of nitrogen source (S/V) against substrate (S) concentration to deduce the Km values (Plummer,1977).

III. Results

a) Long-term effects of nitrogen sources alone and in combination with 2,4-D

The recorded results of short-term experiments conducted on the influence of nitrogen nutrients on growth and kinetics in *Nostoc spongiaeforme* were extremely encouraging which lead to design the following long-term experiments to examine the effects of nitrogen sources i.e. potassium nitrate (KNO_3), sodium nitrite ($NaN0_2$) and ammonium chloride (NH_4Cl) in different doses (0.1, 1.0, 1.5 and 2.0 mg per ml) solely and in combination with a fixed dose of 2, 4-D (600 μg per ml) on the growth, uptake and their kinetics in *Nostoc spongiaeforme* in which recording of results was carried out up to 25 days of life duration with a gap of five days.

Prior to the long term experimental studies on the effects of nitrogen sources alone and in combination with 2, 4-D on growth in *Nostoc sponglaeforme*, the effect of different concentrations (500, 1000, 1500, 2000 and 2200) of the herbicide i.e. 2, 4-D on the growth of *Nostoc sponglaeforme* in terms of chlorophyll-a was examined (Fig. 1a). In these experiments, starved inoculums grown in basal medium which do not contain nitrogen (BM-NO_3) was inoculated in basal medium (BM-NO_3) as control and in basal medium supplemented with different concentrations of 2, 4-D. Among the employed doses, the concentration at 500 μg 2, 4-D per ml was proved as growth promoter since it enhanced the quantity of chlorophyll-a as compared to control and the growth was gradually inhibited with increasing concentrations and completely retarded at 2200 μg per ml dose. The data suggested that the alga *Nostoc spongiaeforme* was intrinsically tolerant to the high doses of 2, 4-D when the growth inhibition of algae was measured in terms of chlorophyll-a, it is evident that the 2, 4-D retarded the biosynthesis of chlorophyll-a in this alga.

In long-term experiments, based on the experience of previous experiments on survival, the sub-lethal dose 600 μg 2, 4-D per ml was selected to examine the toxic effects of 2, 4-D alone and in the

presence of three nitrogenous sources on the growth of *Nostoc spongiaeforme* besides kinetics and uptake of nitrogen sources and 2, 4-D. Nitrogen starved inoculums of *Nostoc spongiaeforme* was inoculated in the basal medium which is normally deficient of nitrogen (BM-NO_3) and in the basal medium supplemented with a fixed dose of 600 μg 2, 4-D per ml (BM-NO_3 + 2, 4-D) as control and nitrogen deficient basal medium supplemented with nitrogen sources individually and in presence of 2, 4-D (BM-NO_3 + nitrogen sources + 600 μg 2, 4-D per ml). Studies on growth, kinetics of nitrogen sources alone and in combination with 2, 4-D in *Nostoc spongiaeforme* were measured upto 25 days of the life cycle duration with an interval of 5 days period (Tables 1 and 2). Table 1 illustrates the effect of nitrogen sources alone on the growth in terms of chlorophyll-a of *Nostoc spongiaeforme*. Among the three nitrogen sources, potassium nitrate (KNO_3) cultures showed better growth than sodium nitrite ($NaN0_2$) and ammonium chloride (NH_4Cl) cultures and control cultures. Normally, chlorophyll-a content was increased with increasing concentrations (0.1, 1.0, 1.5 and 2.0 mg per ml) of potassium nitrate (KNO_3) containing cultures. Sodium nitrite ($NaN0_2$) cultures of *Nostoc spongiaeforme* also showed similar type of growth. The chlorophyll-a quantity was reduced when compared with potassium nitrate (KNO_3) cultures, whereas higher concentrations (1.5 and 2.0 mg per ml) of ammonium chloride (NH_4Cl) showed growth reduction than control cultures (Table 1). Similarly, Table 2 shows the effects of different doses (0.1, 1.0 1.5 and 2.0 mg per ml) of potassium nitrate (KNO_3), sodium nitrite ($NaNO_2$) and ammonium chloride (NH_4Cl) on the growth of *Nostoc spongiaeforme* in relation to quantities of chlorophyll-a and proteins and on the lethality of 600 μg 2, 4-D per ml in *Nostoc sponglaeforme*. Among the three nitrogen-supplemented cultures, potassium nitrate (KNO_3) cultures increased the growth in terms of chlorophyll-a and proteins than the other two nitrogen sources [sodium nitrite ($NaNO_2$) and ammonium chloride (NH_4Cl)]. Potassium nitrate (KNO_3) expressed itself as an efficient protector against the lethality of 2, 4-D on the growth of *Nostoc spongiaeforme* as evidenced in the quantitative enhancement of chlorophyll-a or protein reflects the reduction of toxicity of 2, 4-D. As shown in the Table 2, except at 1.5 mg per ml concentration of sodium nitrite ($NaNO_2$) and ammonium chloride (NH_4Cl) concentrations exhibited higher levels of chlorophyll than the control cultures (BM-NO_3 + 600 μg 2, 4-D per ml) on 5^{th}, 10^{th}, 15^{th}, 20^{th} and 25^{th} days. Likewise, protein content of *Nostoc spongiaeforme* cultures supplemented with nitrogen sources was higher than control cultures except at 1.0, 0.1 and 1.0 mg per ml cultures of ammonium chloride (NH_4Cl) on 5^{th}, 15^{th}, 20^{th} day; at 1.5 and 2.0 mg per ml of sodium nitrite ($NaNO_2$) on 5^{th} and 20^{th} day and at 1.0 mg per ml potassium nitrate (KNO_3) on 20^{th} day respectively. Thus, the experimental results indicated that the alga *Nostoc spongiaeforme* grown in nitrogen supplemented cultures were deemed to be much more protected against the toxicity of 2, 4-D than their respective control cultures (BM-NO_3 + 2, 4-D) as evidenced by the enhancement of chlorophyll-a and proteins. Comparatively, 2, 4-D plus nitrogen supplemented algal cultures showed better growth than nitrogen sources alone containing cultures.

The uptake of nitrogen sources (V) in *Nostoc sponglaeforme* was shown in figs.1 to 15 indicates that the uptake of ammonium chloride (NH_4Cl) was higher at all concentrations during the life cycle intervals (5^{th}, 10^{th}, 15^{th}, 20^{th} and 25^{th} day) than the other two nitrogen sources. When the cultures of *Nostoc spongiaeforme* were supplemented with different concentrations of nitrogen sources alone (0.1,1.0,1.5 & 2.0) and with a fixed dose of 2, 4-D (600 μg per ml), the uptake of nitrogen was progressively decreased in all the observed intervals. Comparatively uptake of nitrogen by *Nostoc spongiaeforme* was higher in nitrogen solely containing cultures than in nitrogen plus 2,4-D containing cultures. The S/V values denote the rate of uptake of nitrogen sources by *Nostoc spongiaeforme* at different concentrations solely and in association with 600 μg per ml 2,4-D. The Km values signify the affinity of the nitrogen substrate (S) and its rate of nitrogen uptake velocity S/V in nitrogen solely cultures and in combination with 2,4-D (600 μg per ml) containing cultures. At the beginning of the life cycle, *Nostoc spongiaeforme* exhibited low Km values which indicated the high uptake of nitrogen. Later, increasing Km values at every successive period of the growing stages of algae indicate the reduction in uptake of nitrogen from the medium suggesting that the algal cells might have absorbed sufficient nitrogen.

Table 1: Kinetics and effect of Nitrogen sources alone on the growth of *Nostoc spongiaeforme*

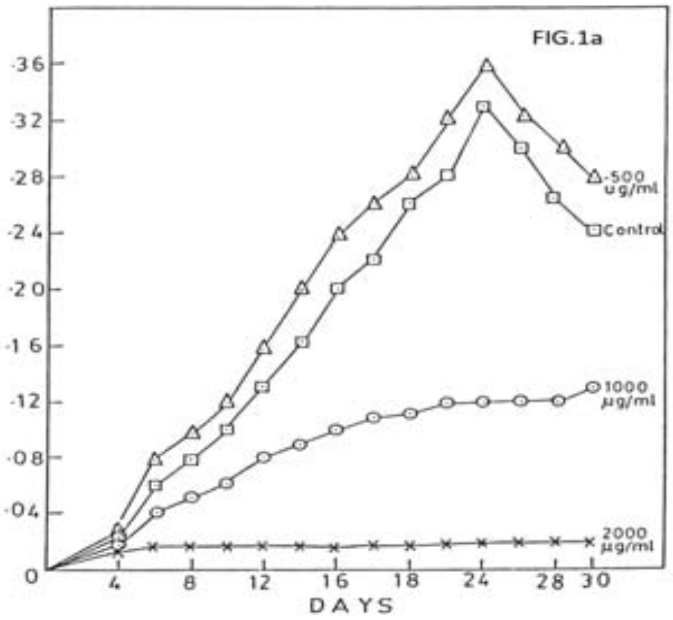
	5th day	10th day	15th day	20th day	25th day
Concentration (mg per ml)	Chlorophyll-a	Chlorophyll-a	Chlorophyll-a	Chlorophyll-a	Chlorophyll-a
BM – NO_3	0.0905	0.0235	0.0035	0.0048	0.0040
BM-NO_3+ the following nitrogen sources					
NH_4Cl					
0.1	0.0185	0.1132	0.0112	0.2068	0.0243
1.0	0.0329	0.0847	0.0216	0.0038	0.0421
1.5	0.0553	0.0010	0.0034	0.0254	0.0356
2.0	0.0208	0.1054	0.0028	0.0153	0.0258
$NaNO_2$					
0.1	0.0300	0.0936	0.0016	0.0085	0.0285
1.0	0.0650	0.0872	0.0020	0.0046	0.0264
1.5	0.0748	0.0128	0.0028	0.0048	0.0238
2.0	0.0862	0.0735	0.0042	0.0092	0.1041
KNO_3					
0.1	0.0785	0.3068	0.0325	0.0246	0.0398
1.0	0.0852	0.3217	0.0127	0.0165	0.0146
1.5	0.0898	0.1815	0.0156	0.0258	0.3015
2.0	0.0943	0.4816	0.0242	0.0324	0.1228

NH_4 Cl = Ammonium Chloride; $NaNO_2$ = Sodium nitrite; KNO_3 = Potassium nitrate;

Table 2: Kinetics and effect of Nitrogen sources and 2, 4-D on the growth of *Nostoc spongiaeforme*

Concen-tration (mg/ml)	5th day		10th day		15th day		20th day		25th day	
	Chlorophyll -a mg/g	Protein (µg/ 100mg fw)	Chlor-ophyll-a mg/g	Protein (µg/ 100mg fw)	Chlorophyll-a mg/g	Protein (µg/ 100mg fw)	Chloro-phyll-a mg/g	Protein (µg/ 100mg fw)	Chlorophyll-a mg/g	Protein (µg/ 100mg fw)
BM – NO_3	0.0920	72.50	0.0256	40.16	0.0042	45.66	0.0055	21.68	0.038	
Bm-NO_3+2,3-D (600 µg/ml)	0.0105	20.50	0.0158	25.83	0.0015	30.50	0.0030	14.18	0.020	20.32
BM – Nitrogen + 600 µg 2, 4-D per ml + the following nitrogen:										10.15
NH_4Cl										
0.1	0.0198	16.66	0.1282	61.66	0.0135	1.66	0.234	71.06	0.0340	100.00
1.0	0.0421	93.33	0.1049	74.16	0.0228	42.5	0.0040	12.5	0.0499	23.33
1.5	0.0605	73.33	0.0013	40.83	0.0049	-	0.0271	26.58	-	-
2.0	0.0292	44.16	0.1162	88.33	0.0049	-	0.0178	25.10	-	-
$NaNO_2$										
0.1	0.0358	35.52	0.1025	28.33	0.0018	57.50	0.0098	15.83	0.0320	130.83
1.0	0.0985	40.21	0.0924	40.83	0.0025	56.66	0.0052	16.68	0.0264	80.00
1.5	0.1032	40.18	0.0248	45.83	0.0032	27.50	0.0056	28.33	0.0246	52.50
2.0	0.1548	42.12	0.0875	75.00	0.0043	41.50	0.0105	8.33	0.1153	55.00
KNO_3										
0.1	0.0948	37.50	0.3234	175.00	0.0379	150.00	0.0284	24.18	0.0469	166.66
1.0	0.1129	45.83	0.3970	130.83	0.0435	225.00	0.0191	13.33	0.0161	216.25
1.5	0.2499	65.00	0.2092	146.66	0.0178	123.33	0.0254	15.83	0.3239	166.78
2.0	0.3562	46.66	0.5077	168.33	0.0357	298.33	0.0391	21.68	0.1454	250.00

NH_4 Cl = Ammonium Chloride; $NaNO_2$ = Sodium nitrite; KNO_3 = Potassium nitrate; 2, 4-D = Dichlorophenoxyacetic acid; mM = Milli Molar; = f.w., = fresh weight



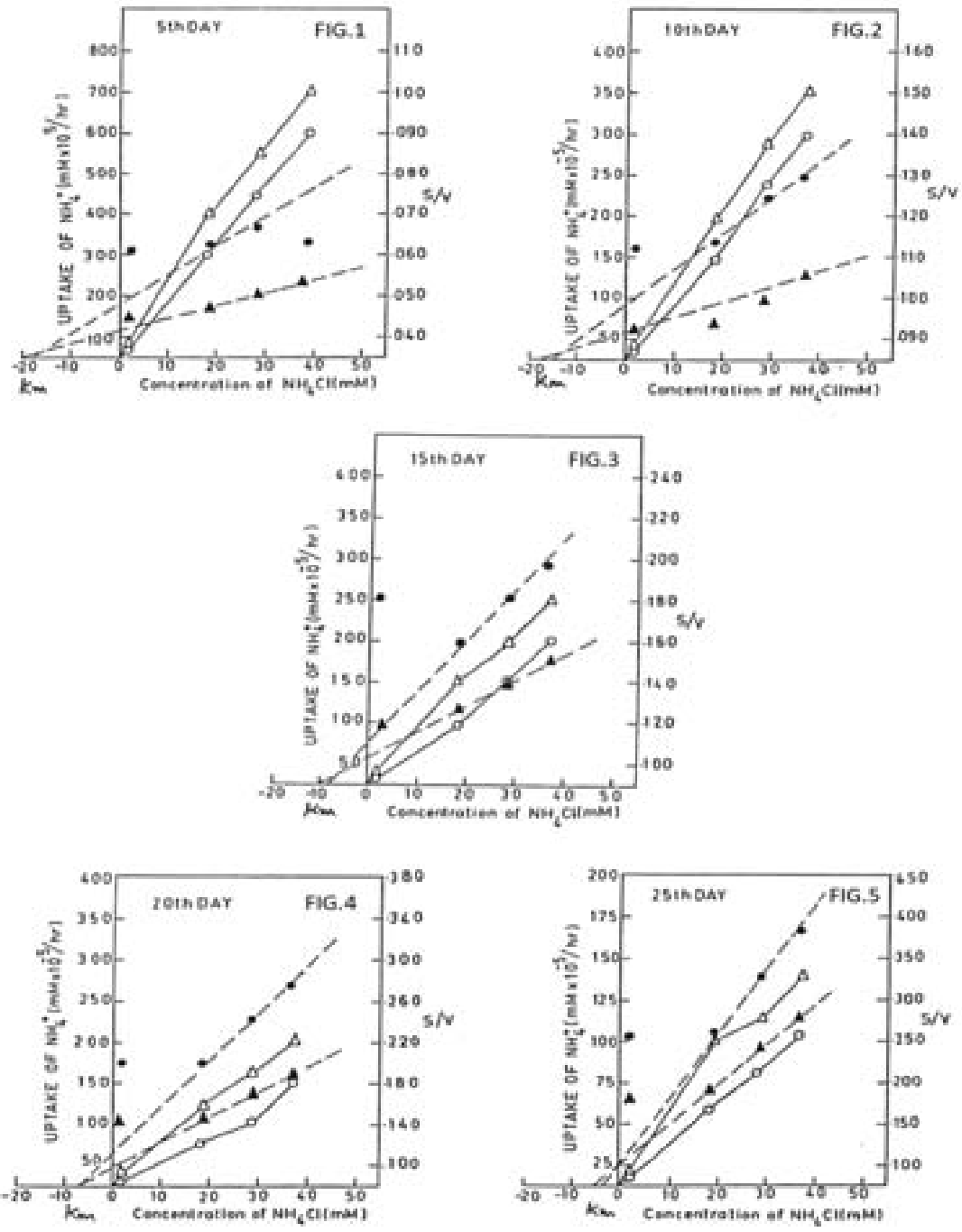
□ - □ : Control

△ - △ : 500 μg per ml

○ - ○ : 1000 μg per ml

x - x : 2000 μg per ml

Figure 1: Growth of *Nostoc spongiaeforme* in nitrogen depleted basal media supplemented with graded concentrations of 2, 4-D with normal inoculums by optical density



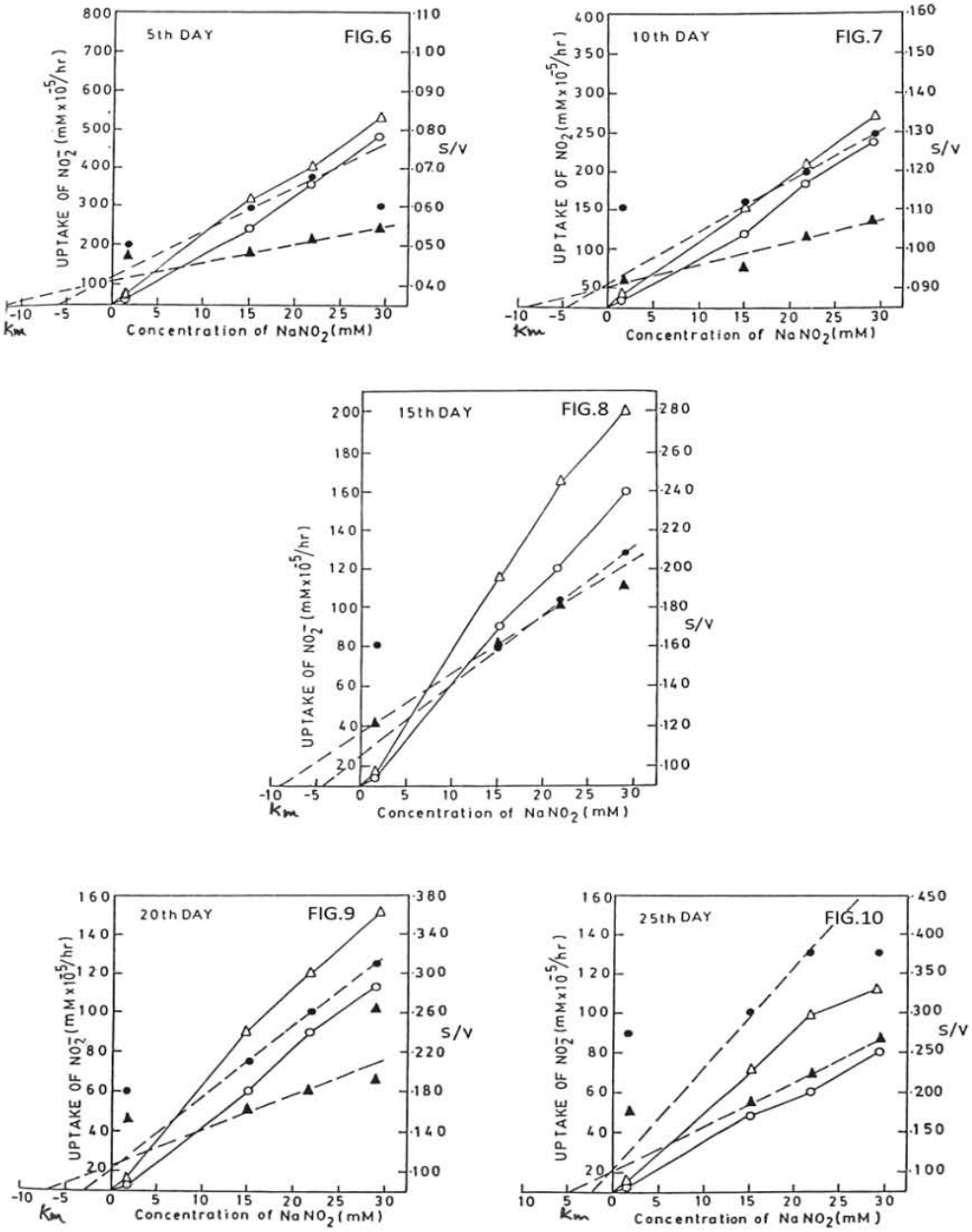
○-○ : Uptake of NH_4^+ (m M x 10^{-5} / hr.) in NH_4CL cultures.

△-△ : Uptake of NH_4^+ (m M x 10^{-5} / hr.) in NH_4CL + 2, 4-D cultures.

●-● : S/V (rate of uptake) of NH_4^+ in NH_4CL cultures.

▲-▲ : S/V (rate of uptake) of NH_4^+ in NH_4CL + 2, 4-D cultures.

Figure 2: The uptake of NH_4^+ (mM x 10^{-5} / hr.) in different concentrations (0.1, 1.0, 1.5, and 2.0 mg per ml) of NH_4CL alone and in association with fixed dose of 2, 4-D (600 μg per ml) containing cultures of *Nostoc spongiaeforme* on 5^{th}, 10^{th}, 15^{th}, 20^{th} and 25^{th} day



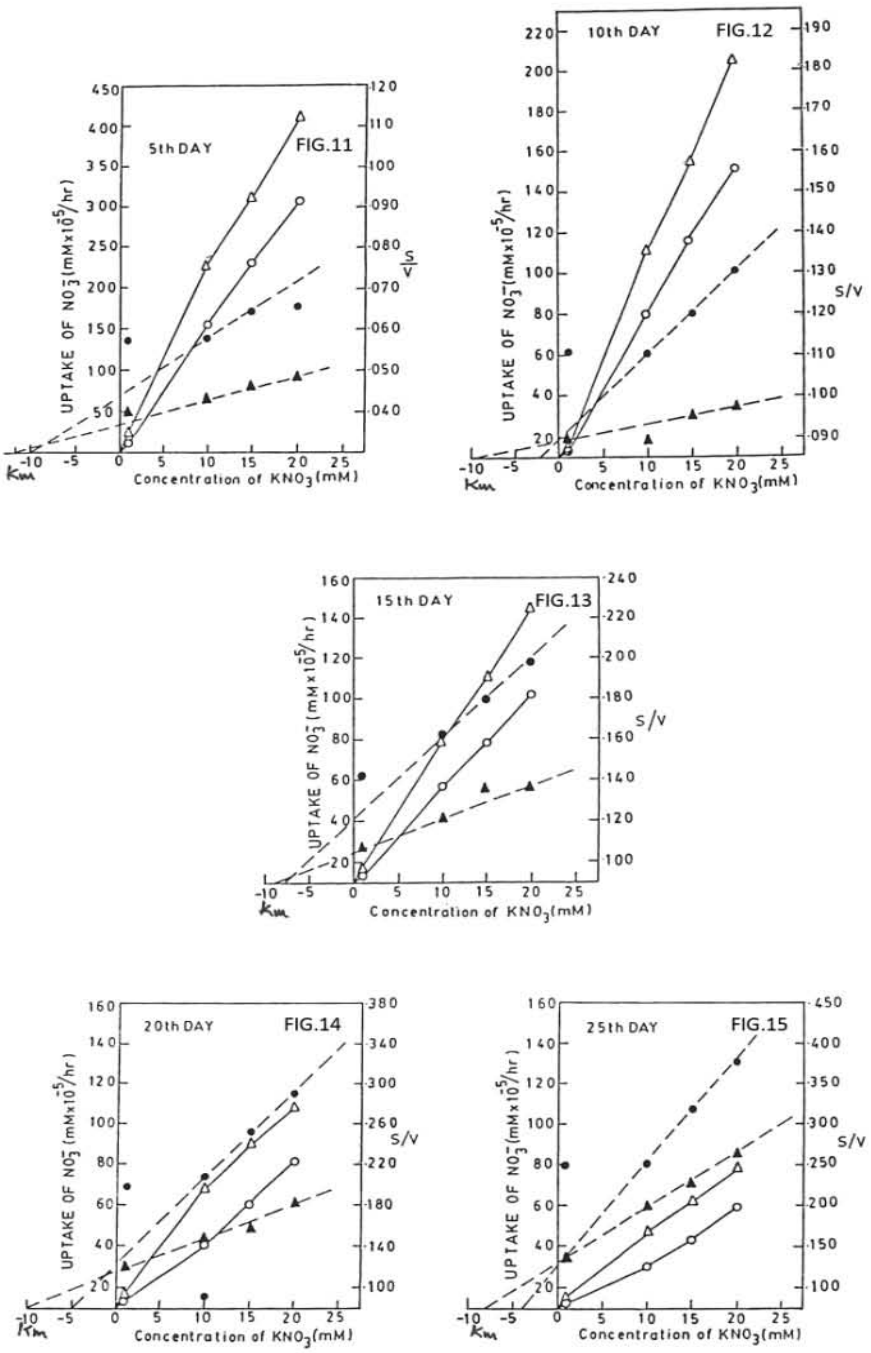
○-○ : Uptake of NO_2^- (mM x 10^{-5} / hr.) in $NaNO_2$ cultures.

△-△ : Uptake of NO_2^- (mM x 10^{-5} / hr.) in $NaNO_2$ + 2, 4-D cultures.

● - ● : S/V (rate of uptake) of NO_2^- in $NaNO_2$ cultures.

▲ - ▲ : S/V (rate of uptake) of NO_2^- in $NaNO_2$ + 2, 4-D cultures.

Figure 3: The uptake of NO_2^- (mM x 10^{-5} / hr.) in different concentrations (0.1, 1.0, 1.5, and 2.0 mg per ml) of $NaNO_2$ alone and in association with fixed dose of 2, 4-D (600 μg per ml) containing cultures of *Nostoc spongiaeforme* on 5th, 10th, 15th, 20th and 25th day



○ - ○ : Uptake of NO_3^- (mM x 10^{-5} / hr.) in KNO_3 cultures.

△ - △ : Uptake of NO_3^- (mM x 10^{-5} / hr.) in KNO_3 + 2, 4-D cultures.

● - ● : S/V (rate of uptake) of NO_3^- in KNO_3 cultures.

▲ - ▲ : S/V (rate of uptake) of NO_3^- in KNO_3 + 2, 4-D cultures.

Figure 4: The uptake of NO_3^- (mM x 10^{-5} / hr.) in different concentrations (0.1, 1.0, 1.5, and 2.0 mg per ml) of KNO_3^- alone and in association with fixed dose of 2, 4-D (600 μg per ml) containing cultures of *Nostoc spongiaeforme* on 5^{th}, 10^{th}, 15^{th}, 20^{th} and 25^{th} day.

IV. Discussion

Farmers have been employing nitrogen fertilizers and pesticides simultaneously to eradicate the weeds in the rice-fields and pests on the rice plants with a view to get more crop yield. 2, 4-Dichlorophenoxyacetic acid (2, 4-D) is a harmone type herbicide used for control of many seasonally annual broad leafed weeds in rice-fields where the Cyanobacteria have been reported to be distributed and became resistant to pesticides doses than the doses of pesticides recommended for pest and weed control in rice-field. Tiwari and Pandey (1981) reported the herbicide resistant mutants of *Anacystis nidulans* in the form of filaments at 2 mg per ml concentration of 2, 4-D cultures. Depending upon the nitrogen, phosphorus and carbon sources, the toxicity of 2, 4-D was modified in *Nostoc calcicola*, *Synechococcus aeruginosus* and *Scenedesmus incrassatulus* and the mechanism of antagonistic action involved in these studies was not understood by mere laboratory experiments. Sivasubramanian and Rao (1988) studied the kinetics of nitrogen uptake in the presence of metabolic inhibitors (KCN, PCMB, DCMU) in diatoms and concluded that uptake of NO_3^- and NH_4^+ was evidenced by the active absorption by utilizing energy produced in photosynthesis and respiration and partially by a passive diffusion whereas NO_2^- is taken up only through photosynthesis mediated active uptake. Similarly, Hii et. al., (2011) observed the interactive affect of ammonia and nitrate on the nitrogen uptake by *Nannochloropsis* sp. as well as by conducting the short-term experiments Jyothi and Reddy (2017) studied the nitrogen uptake and kinetics in blue-green algae *Nostoc spongiaeforme*.

As evidenced by the above studies, currently it is of great interest to understand the relationship between kinetics of uptake of nutrients by Cyanobacteria and nutrient levels. The assimilation of nitrate by blue-green algae involving nitrate uptake and reduction of intracellular nitrate to ammonium occurred through a transport system having a high affinity for nitrate (Flores et al., 1980) and even low concentrations of nitrate found in their natural aquatic environments induced the nitrate reductase to function. The inorganic nitrogenous substances were considered as significant metabolites for cellular growth and cell constituents. Magee and Burris (1954) studied the nitrogen metabolism in diazotrophic cyanobacteria by incubating with $^{15}N_2$, $^{15}NH_4$, and $^{15}NO_3^-$, and concluded that the amino acid composition of the proteins were the same irrespective of the nitrogen source and incorporated into cellular proteins and cell wall material. Probably the nitrogen substances absorbed by active uptake through a carrier system as mentioned in *Anacystis nidulans* by Flores et al. (1983) and Meeks et al. (1983) and Tischner and Schmidt (1984) or simple diffusion process into cytoplasm where they preceded the nitrogen assimilation pathway, and incorporated into amino acids, proteins and cell materials and thereby increased the biomass and protein content of the algae as reported by Thomas et al. (1977), Wohlhueter et al. (1973) and Ingraham et al. (1983).

The present experimental studies suggested that, comparatively growth of *Nostoc spongiaeforme* was augmented in nitrogen plus 2, 4-D containing cultures than nitrogen alone supplemented cultures. It indicated that these three nitrogen sources significantly increased the growth of algae by antagonizing the 2, 4-D lethality and enhanced the biomass which was evident from the increased levels of chlorophyll-a, and proteins of *Nostoc spongiaeforme* (Tables 1&2). However, among the three nitrogen sources, potassium nitrate (KNO_3) was found to be a better protector against 2, 4-D toxicity than sodium nitrite ($NaNO_2$) and ammonium chloride (NH_4Cl) in *Nostoc spongiaeforme* cultures as evidenced by augmentation of biomass, chlorophyll-a and proteins.

V. Conclusion

The present study reveals that the nitrogen uptake capacity of *Nostoc spongiaeforme* was greater in ammonium chloride (NH_4Cl) among the employed nitrogen sources. Nitrogen uptake was very high in NH_4Cl supplemented cultures. So that *Nostoc spongiaeforme* is well suited as a phycoremediation organism for NH_4 removal from waste waters and effluents.

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Comparative Efficacy of Insecticides and Plant Extracts for Management of ChiLCV Disease in Relation to Epidemiology

By Maryam Iftikhar, M. Aslam Khan & Sajjad Haider

Abstract- Three Chilli varieties/lines including 7-ph, Biaddy and Tatapuri were sown to check the comparative efficacy of different insecticides and plant extracts. Three insecticides including Imidacloprid, Bifenthrin and acetameprid and three different plant extracts including onion extract, Garlic extract and parthenium were evaluated against Chilli leaf curl virus (ChiLCV) and whitefly. Bifenthrin was very much effective in reducing whitefly population while Acetameprid was least effective as compared to control.Garlic extract at 5% concentration was very much effective in reducing whitefly population while parthenium extract at 5% concentration was least effective compared to control. Correlation of environ- mental factors (maximum and minimum temperature, relative humidity and rainfall) chiLCV disease incidence % was also determined. There was a significant correlation of environmental variables with ChiLCV disease incidence %. The use of Bifenthrin (10%EC) proves to be significant option in case of epidemiological occurrence of environmental variables followed by acetamaprid (20%SL) and imidacloprid (25%WP) respectively.

Keywords: *chilli leaf curl virus, (chiLCV), correlation, insecticides, plant extracts and disease incidence.*

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Abstract- Three Chilli varieties/lines including 7-ph, Biaddy and Tatapuri were sown to check the comparative efficacy of different insecticides and plant extracts. Three insecticides including Imidacloprid, Bifenthrin and acetameprid and three different plant extracts including onion extract, Garlic extract and parthenium were evaluated against Chilli leaf curl virus (ChiLCV) and whitefly. Bifenthrin was very much effective in reducing whitefly population while Acetameprid was least effective as compared to control.Garlic extract at 5% concentration was very much effective in reducing whitefly population while parthenium extract at 5% concentration was least effective compared to control. Correlation of environmental factors (maximum and minimum temperature, relative humidity and rainfall) chiLCV disease incidence % was also determined. There was a significant correlation of environmental variables with ChiLCV disease incidence %. The use of Bifenthrin (10%EC) proves to be significant option in case of epidemiological occurrence of environmental variables followed by acetamaprid (20%SL) and imidacloprid (25%WP) respectively.

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I. Introduction

Chilli pepper (*capsicum annum L.*) belongs to solaneaous family and it is grown in Pakistan ranked at third position after potato and tomato (Iqbal *et al*., 2012). Chilli has good nutritional value because it is excellent source of Vitamins A, B, C, E and P. Fresh green chilli peppers contain more vitamin C and A than citrus fruits and carrots (Osuna-Garciaet *et al*.,1998; Marinet *et al*.,2004). Sindh is one of the main chili growing area in Pakistan, and approximately 85% of chilli pepper area and production is accomplished especially from lower regions of Sindh province including Kunri, New Koat, Umerkot, Mirpurkhas, and some other towns because these are main source of Chilli.

In Pakistan, chilies are grown on an area of 64.2 thousand hectares with production of 142.6 thousand tones, with 2.1% change in production (GOP, 2015-2016).Suzuki and Mori, (2003) observed Chilli (*capsicum annum L.*) production is affected by viruses which are the most important group of pathogens and cause huge economic losses by reducing its yield. Plant viruChilli pepper is more susceptible to biotic factors including fungi, bacteria and viruses. Viral infection is the most important threat to cultivated pepper (Venkataiah *et al*., 2003). Ochoa-Alejo and Ramírez-Malagón, (2001) described that abiotic factors such as temperature, moisture, light, nutrients, pH and others significantly diminish the yield and quality of peppers. Weerarathne and Yap, (2002) reported that low yield of Chilli crop is mainly occurred due to biotic and abiotic factors are the main cause of losses in temperate regions of the world (Hull and Davies, 1992).

Chilies are affected by a number of insect pests including whitefly, aphids, jassids etc. Whitefly plays very important role in the transmission of chili leaf curl virus disease (ChiLCV). The insecticides have been used for the management of whitefly population. Environmental factors also play very important role in the development of ChiLCV disease and whitefly population. The correct time of application of insecticides can be very helpful to manage the whitefly population. The main objective of this study was to find relationship of different environmental factors with ChiLCV disease incidence and to find the effect of chemicals on ChiLCV and whitefly population.

II. Materials and Methods

a) Collection of Chilli Varieties and Sowing

Germplasm of Chilies was obtained from Ayub AgricultureResearch Institute, Faisalabad. Experiment was conducted during 2016, in the experimental area of Department of Plant Pathology, University ofAgriculture Faisalabad. The eight varieties/lines viz 9-patayla, Hot shot, 5-Glory, 7-PH, Biaddy, tatapuri, Maha and Hot Shot were cultivated. Chilli nursery was sown at 60 cm row to row and 30 cm plant to plant distance on ridges.After three rows of test line single line of local susceptible check variety is grown to serve asspreader. The experiment was conducted according to Randomized Complete Block Design, with three replications.

b) Evaluation of chemicals and plant extracts for the management of whitefly and ChiLCV disease

Three chemicals including Bifenthrin, Megamos and Imidacloprid and three plant extracts (Garlic, onion and parthenium extract) were used as separately. There were total seven treatment including one as control.

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Each treatment was replicated three times. The experiment was performed with randomized complete block design. Spraying was repeated fortnightly. And plant extracts were applied at 5% concentration. Data regarding the appearance of disease symptoms, disease severity and whitefly density were recorded before and after treatment and subjected to analysis of variance and individual comparison between treatments was done by Turkey's honestly significant difference test at 5% level of significance.

c) Collection of Environmental data

Environmental data like temperature, humidity and rainfall was taken from meteorological station of Department of Crop Physiology, University of Agriculture Faisalabad. Relationship of epidemiological factors with percent disease incidence of ChiLCV and whitefly density through correlation and regression was determined. Effect of treatments on the yield of ChiLCV was Determined through ANOVA and LSD test.

III. Results

a) Response of Chilli varieties/lines to ChiLCV

None of the varieties/lines showed immune to ChiLCV and *Bemisiatabaci.* 9-Patyala, Hot Queen show moderately resistance response and Hot Shot, Maha, 5-Glory showed moderately susceptible response, 7-PH, Biaddy were susceptible and tatapuri was highly susceptible.

b) Effects of treatments on Chilli leaf curl disease incidence (ChiLCV)

The effect of all the treatments was significant on ChiLCV disease infection. Mean number of the infected plants by ChiLCV was significantly higher in untreated control followed by Acetamaprid and imidacloprid. The most effective treatment was bifenthrin.

c) Effect of treatments on whitefly population

All the treatments reduced the whitefly population. The whitefly population was high at untreated control while, it was low where bifenthrin was applied.

Table 1: Evaluation of chemicals against whitefly population recorded on various Chilli varieties

Chemicals	7-PH	Biaddy	Tatapuri	Mean
Imidacloprid	30.09i	31.02h	32.1g	31.07C
Megamos(Acetamaprid)	33.17f	34.23e	35.4d	34.27B
Bifenthrin	22.83l	23.89k	25.09j	23.94D
Control	82.24c	84.88b	87.77a	84.96A
Mean	42.08C	43.51B	45.09A	LSD =2.510

Table 2: Evaluation of chemicals against ChiLCV disease incidence recorded on various Chilli varieties

Chemicals	7-PH	Biaddy	Tatapuri	Mean
Imidacloprid	30.09i	31.02h	32.1g	31.07D
Megamos	33.17f	34.23e	35.4d	34.27C
Bifenthrin	22.83l	23.89k	25.09j	23.94B
Control	82.24c	84.88b	87.77a	84.96A
Mean	42.08C	43.51B	45.09A	LSD=1.12

Table 3: Evaluation of plant extracts against whitefly population recorded on various Chilli varieties

Plant Extracts	9-Patyla	5-Glory	Hot queen	Mean
Onion	5.07h	5.43g	5.4g	5.3C
Garlic	2.4k	2.7j	2.97i	2.69D
Parthenium	7.17f	7.87d	7.7e	7.58B
Control	12.63c	13.72b	14.88a	13.74A
Mean	6.82C	7.43B	7.74A	LSD=1.134

Table 4: Evaluation of plant extracts against ChiLCV recorded on various Chilli varieties

Treatments	Varieties			
Plant Extracts	9-patyla	5-Glory	Hot queen	Mean
Onion	36.21h	37.39g	38.53f	37.38C
Garlic	36.61k	33.26j	34.3i	33.06D
Parthenium	42.46d	41.49e	42.39d	42.11B
Control	82.87c	85.17b	88.13a	85.39A
Mean	48.29C	49.33B	50.83A	1.14

d) Correlation of Environmental factors with percent disease incidence by ChiLCV

All varieties responded differently to temperature (maximum/minimum), relative humidity and rainfall. The relationship of these environmental parameters with percent disease incidence by ChiLCV on most varieties was positive except for rainfall and wind speed it was negative.

IV. Discussion

Chilli varieties were affected by the disease and whitefly in early growth stages. The experiment was conducted for screening of Chilli germplasm for ChiLCV infection. None of the cultivar evaluated was found to be immune or highly resistant to ChiLCV disease. Evaluation of Chilli (*capsicum annum* L.) varieties/lines consisting of eight lines/varietiesagainst Chilli leaf curl virus. Begomovirus (ChiLCV) under natural field conditions conducive for development of disease and whitefly virus vector population. Whitefly population, ranged between 1.5-8 adults/plant with an average of 4 adults. ChiLCV virus occurred over a wide range of climatic conditions in summer. None of the lines appeared to be resistant of any category, 2 lines/varieties were classified as moderately resistance and 3 as moderately susceptible and two were susceptible and one was highly susceptible. All varieties responded differently to temperature (maximum /minimum), relative humidity and rainfall. The relationship of these environmental parameters with percent plant infection by ChiLCV on most varieties was positive. Studied was done on the effect of epidemiological factors on the incidence of ChiLCV.

V. Conclusion

According to observed results we can conclude that the increasing rate of maximum temperature range and increasing rate of minimum temperature and relative humidity range cause increase in disease incidence, while increase in the rain fall and wind speed cause decrease in plant infections by decreasing pathogen population. While, for management of pathogen vector (*Bemisiatabaci*) and ChiLCV the treatments of Bifenthrin was proved to be most effective followed by, megamos and imidacloprid respectively. It was also concluded that with the increasing temperature, relative humidity the disease may cause economic losses and in these epidemiological conditions the use of bifenthrin was best option in Chilies. These chemicals were sprayed thrice with an interval of 7 days. The results concluded that Bifenthrin showed effective results in the reduction of ChiLCV disease incidence while Acetameprid showed less significant results as compared to control. Bifenthrin was effective after three sprays of one week interval on all varieties i.e. 7-PH, biaddy and tatapuri. Similarly, Bifenthrin was very much effective in reducing whitefly population while Acetameprid was least significant as compared to control.

Bifenthrin showed minimum insect population after three sprays on all varieties i.e. 7-PH, biaddy and tatapuri. The results concluded that garlic extract at 5% concentration showed effective results in the reduction of ChiLCV disease incidence while parthenium extract at 5% concentration showed less significant results as compared to control. Garlic extract was significant after three sprays of one week interval on all varieties i.e. 7-PH, biaddy and tatapuri. Similarly, garlic extract at 5% concentration was very much significant in reducing whitefly population while parthenium extract at 5% concentration was least significant as compared to control. Garlic showed minimum insect population after three sprays on all varieties i.e. 7-PH, biaddy and tatapuri.

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Lichens used in the Traditional Medicine by the Pankararu Indigenous Community, Pernambuco-Brazil

By P. A. Londoño-Castañeda, M. L. L. Buril, I. P. Rego-Cunha, N. H. Silva, N. K. Honda, E. C. Pereira & L. H. C. Andrade

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Introduction- Traditional knowledge refers to the knowledge accumulated over the years and transmitted through generations over time(Martin, 2005). For some communities the only resource available for health disorders is the traditional phytotherapy (Forero, 2004).

Ethnobiology has grown increasingly the scientific knowledge about organisms popularly used as medicinal; it made the researchers be aware of the substances that were found in order to produce new drugs(Posey, 1992). In this regard, lichens have been extensively studied in temperate countries, in the Euro-Asiatic axis or on the USA, with particular emphasis on survey work conducted by Sylvia Sharnoff (Brodo et al., 2001; Sharnoff, 2015). In Neotropical countries, however, its study is scarce; there are just a few cases related to Brazil, used as dyes (Mors, 1966),or for tingling and sneezing when sniffed (Prance, 1972).It is known that Brazilian lichen flora is highly diverse (Cáceres, 2007; Eliasaro and Adler, 2000; Fleig and Grüninger, 2008; Marcelli, 2003), showing great pharmacological potential (Pereira, 2012), and ethnolichenological studies may assist in targeting and selecting species for future pharmacological research.

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Lichens used in the Traditional Medicine by the Pankararu Indigenous Community, Pernambuco-Brazil

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In Brazilian Northeast, in the west side, opposite to the Atlantic coast, it can be found the semi-arid region, that presents an exclusive biome – Caatinga, where endemic species of several taxa are reported (Leal et al., 2003), with an endemism level that varies from 4.3 % (birds) to 57 % (fishes)(Brasil, 2002).

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In this context, lichens are also found in this region, much of them are new report to Brazilian semi-arid northeast, or Country, and many species are new to the Science. Studies conducted by Cáceres (2007) refermainly crostose lichens, whereas Buril (2015) reports 22 new species and one new genus of foliose lichens, *Parmeliaceae* family, from semi-arid region of Pernambuco one of the States that makes part of Brazilian Northeast.

Even almost unknown the lichen biota of Brazilian semi-arid, the reported species have biologically-active substances in their chemical composition, that can be useful in the future in a sustainable way. By other hand, until this moment no report was found in traditional use of lichens in this region.

Among the traditional existent communities in Brazilian semi-arid, Lodoño-Castañeda (2010), selected Pankararu people for ethnobotanical studies, and observed that indigenous people use higher plants and also lichens for medicinal purposes.

This way, in this paper we show the use of foliose lichen species by indigenous Pankararu people in the semi-arid of Pernambuco State, Northeast of Brazil, and the biologically-active compounds found in these species.

II. Material and Methods

a) Site Description

The indigenous community Pankararu occupies an area of 8,100 ha with a population about 4,850inhabitants distributed in 13 villages (Socioambiental, 2009). The territory, inserted onto the Caatinga ecosystem, was homologated by the Brazil government, and named as “Pankararu land”. It is located in the “Sertão Pernambucano”, between the hills Serra Grande and Serra da Borborema, near the banks of São Francisco river, in the municipalities of Petrolândia, Tacaratu and Jatobá and the border of the states of Alagoas and Bahia – Brazil, whose Geographic coordinates are 09°07`16``S and 38°15`25``WGr North and 09°11`56``S and 38°13`52``WGr South (Fig. 1).

According to Köppen's classification, the climate is BSHs' (semi-arid of low latitudes), with mean annual temperature of 25°C, and mean annual pluviosity around 600 mm. The vegetation is dry tropical forest type, characterized by a predominance of xerophytic and deciduous species, endowed with a high floristic and physiognomic variation. Amongst the typical woody species there are found *Ziziphus joazeiro* Mart. (*Rhamnaceae*), *Schinopsis brasiliensis* Engler (*Anacardiaceae*), *Caesalpinia pyramidalis* Tul. (*Fabaceae*), *Bauhinia cheilanta*(Bong.) Steud. (*Fabaceae*), *Maprounea guianensis* Aubl. (*Euphorbiaceae*) (Araújo *et al*., 1995); succulent plants of *Cactaceae* and *Bromeliaceae* families are also typical, while lianas are scarce (Araújo and Martins, 1999).

In this study nine villages were considered, mainly that one known as "Brejo dos Padres", located in a valley between the Serra Grande and Serra de Tacaratu, near to the left margin of São Francisco, one of the main river of Brazilian Northeast.

The villages are inserted in areas with several stages of ecological succession, where often can be found fruit trees as "murici" (*Byrsonima crassifolia* (L.) H.B.K., *Bignoniaceae*) and "umbu" (*Spondias tuberosa* Arr. Câm., *Anacardiaceae*), as well as woody and medicinal species.

Pankararu people perform subsistence agriculture practices, spanning in some cases informal marketing of food stuffs in local markets, as "macaxeira" (*Manihotesculenta* Crantz.), maize (*Zea mays* L.), and bean (*Phaseolus vulgaris* L.).

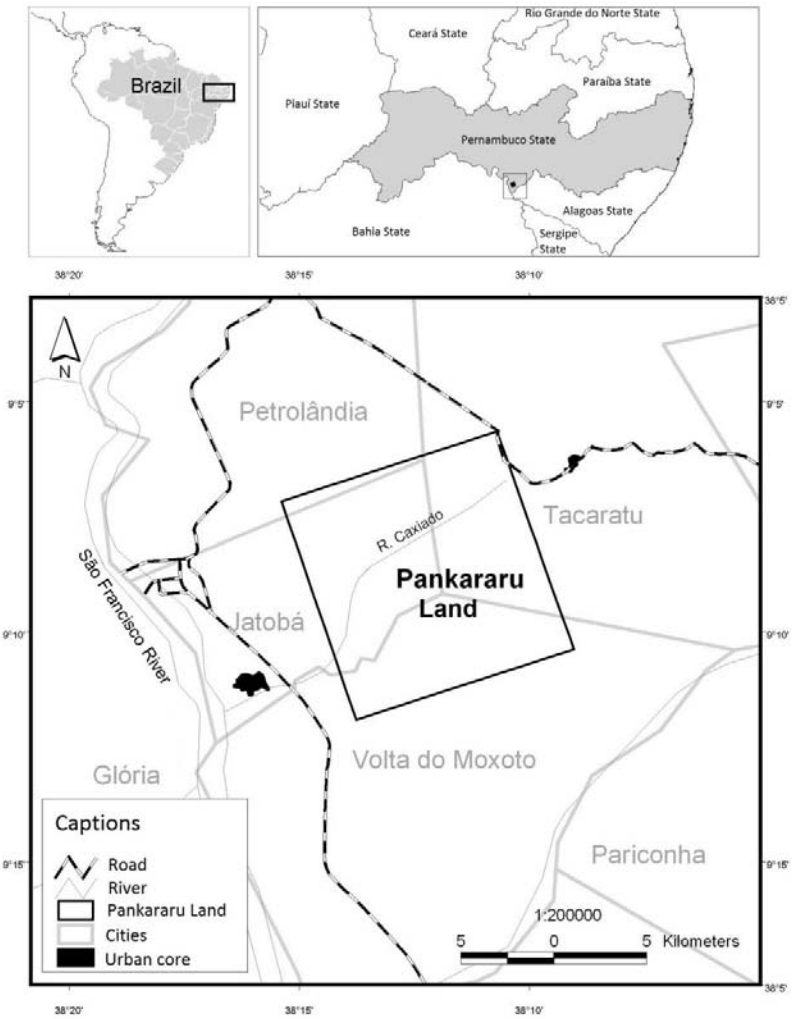

Fig. 1: Localization of Pankararu Land in Pernambuco State, Brazilian Northeast

Map designed by A.K.O. Silva (2014), adapted from FUNAI (National Indian Foundation) and IBGE (Brazilian Institute for Geography and Statistics) (2001).

b) Data Collection

The ethnobotanical information in the traditional medicine Pankararu were obtained within 60 days of field activities through semi-structured surveys, in which were employed standardized forms and recorded notes on the therapeutic indications of the local flora. The

survey was targeted to specialists of traditional medicine, using the "snowball" technique (Albuquerque et al., 2008).

Among the reports, it was quoted the use of plants, as well as lichens, and the cited species were collected to identification. The lichen material was collected and kept in paper bags until laboratory tests. Vouchers were deposited in UFP Herbarium of Botany Department of Universidade Federal de Pernambuco (*Parmelinella salacinifera* (Hale) Marcelli & Benattin° 61069, *Heterodermia galactophylla* (Tuck.) W.L. Culb. n° 75448 and *Parmotrema wrightii* Ferraro & Elixn°61212).

To develop the field activities in indigenous PankararuLandit was required the approval of the Research Ethics Committee – CEP, the National Committee of Ethics in Research – CONEP, and the Board of the Genetic Heritage Management - CGEN, with subsequent approval of FUNAI (National Indian Foundation) (Proc. n° 1253/08).

c) Identification of lichen species

i. *Morphotaxonomic Analysis*

To identify the species of lichenized fungi, anatomical and morphological characters were studied. Structures as cilia, rizines, maculae, type, size and form of thallus, cortex and medulla, apothecia, ascospores and others were analyzed under stereo microscope (10-50X) and optical microscope (40-1000X).

d) Chemical Analysis

i. *Obtainment of extracts from the thallus in natura*

A chemical study of the species was performedto confirm the secondary metabolites.

The phenolic composition was analyzed from organic extracts obtained from each lichen species. Samples of lichen thalli (50 mg) were successively extracted by maceration with diethyl ether (5 mL), chloroform (5 mL) and acetone (5 mL), with infusion time of 15 minutes in each solvent and then filtered, reunited into one single extract for each lichen sample and stored until evaporation at room temperature (28 ± 3 °C).

ii. *Thin layer chromatography (TLC)*

For a general characterization of lichen phenols contained in the species, the organic extracts obtained from the thallus *in natura* were subjected to thin layer chromatography (TLC). The samples were applied on silica gel chromatoplates $F_{254\ +366}$, along with the standards of norstictic acid, salazinic acid, atranorin, and the ether extract of *Heterodermia leucomela*, containing as main compounds atranorin and zeorin. The samples and standards were previously dissolved in a concentration of 0.01 mg.$\mu L^{-1}$and then applied5 μL of each extract. It allows a more careful and accurate chromatographic analysis. TLC was developed in a solvent system A (toluene/dioxane/acetic acid 90:25:4, v/v/v),according to Culberson (1972), and spots formed were visualized under UV light and subsequently revealed by spraying 10% sulphuric acid (H_2SO_4) over the plates and subjecting them to heat.

For a more detailed evaluation additional TLC assays were performed with acetone extracts of the species using the following solvent systems: toluene: ethyl acetate: formic acid (139:83:8, v/v/v); toluene: ethyl acetate: acetic acid (6:4:1, v/v/v), using salazinic and norstictic acids, as well as atranorin.

In all tests, value of Rf spots were calculated and compared to the Rf of standard substances.

III. Results

Although the records of lichen species which therapeutic value are rare in ethnobotanical studies in Brazil and non-existent for the Northeast regionso far, three lichen species were recorded being used by Pankararu as medicine (Fig. 2):

Parmelinella salacinifera(Hale) Marcelli & Benatti (*Parmeliaceae*)
Heterodermia galactophylla (Tuck.) W.L. Culb. (*Physciaceae*)
Parmotrema wrightii L. I. Ferraro & Elix(*Parmeliaceae*)

These species are commonly called stone flower (flor-de-pedra in Portuguese) by the community and are used to treat digestive system problems such as diarrhoea and vomiting. The mix of three species is employed in an aqueous extract. They are also used for the treatment of epilepsy and cultural diseases through the smoker.

The species are the first report for Pernambuco state, being *H. galactophylla* the first report for Brazilian Northeast.

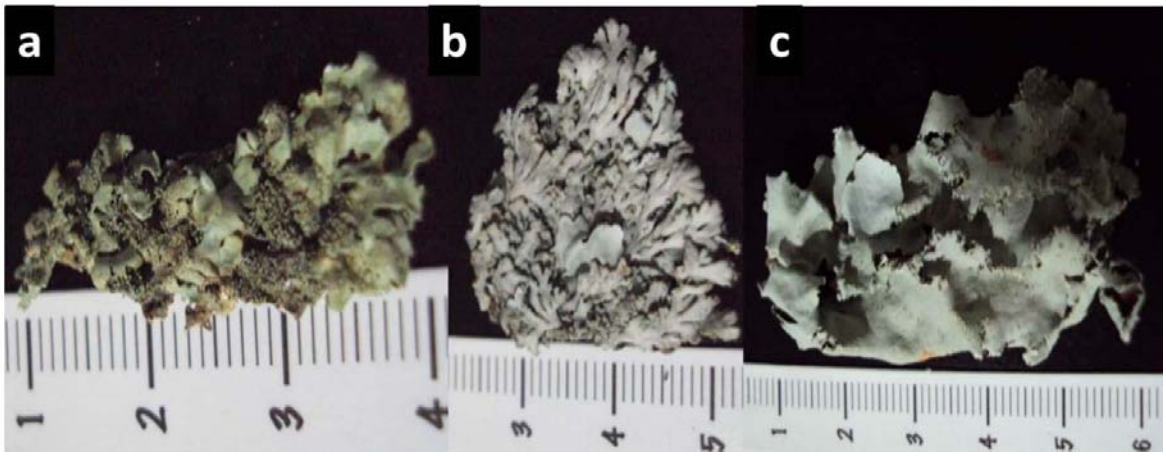

Fig. 2: Lichen species used as medicine by Pankararu people: a. *Parmelinella salacinifera* (Hale) Marcelli & Benatti, b. *Heterodermia galactophylla* (Tuck.) W. Culb., c. *Parmotrema wrightii* L. I. Ferraro & Elix

According to traditional knowledge, the different types of stone flower have contraindications of use: *P. wrightii* does not present any restrictions, while *H. galactophylla* is contraindicated for children and pregnant women, and *P. salacinifera* also presents restrictions of its use by pregnant women. The species are differentiated by the community by the colour and shape of the thallus. By other side, in the revised papers no mention about contraindications was found.

Through general TLC assays (Fig. 3) the presence of atranorin and zeorinin *H.galactophylla*, of atranorin and salazinic acid in *P. Salacinifera* and atranorin and norstictic acid in *P. Wrightii* was detected. It is quite likely that these substances are related to the therapeutic potential of these species as well as to their restrictive nature.

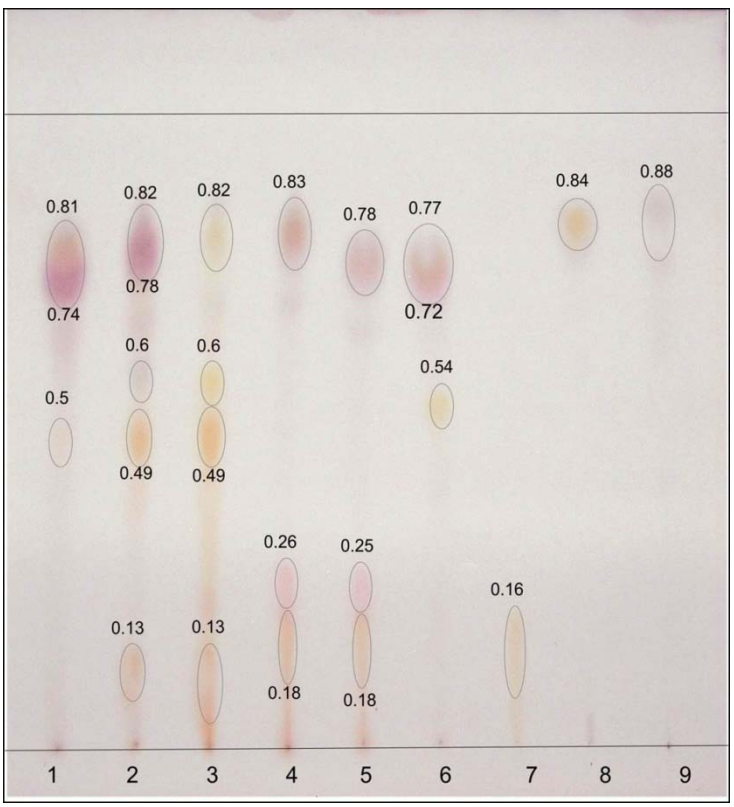

Fig. 3: Thin Layer Chromatography of 1, 2 – *Heterodemia galactophylla* extracts; 3 – *Parmotrema wrightii* extract; 4, 5 – *Parmelinella salacinifera* extracts; 6 –Norstictic acid (Rf0.54); 7 –Salazinic acid (Rf0.16); 8 - Atranorin (Rf0.84); 9: Ether extract of *Heterodermia leucomela* containing zeorin (Rf0.88)

IV. Discussion

Yavus (2012) investigating the pharmacopoeias written by Dioscorides, a physician who acted in the army of Roman legion in Italy, France, Greece and Turkey, had observed that in great part of species used as medicinal, *Parmelia* species were used among other species.

Most of *Parmeliaceae*, grows on different substrate as branches, barks, rocks etc. forming a total or partial rosette (Yavus, 2012), due to its radial growth form, resembling a flower, probably reason of the name "stone flower" given by Pankararu people.

Despite of the knowledge of lichen use in traditional medicine comes from ancient times (Agelet and Vallés, 2003), the relationship between their use with vascular plants is unbalanced. Soukand and Kalle (2013) studying plants used for tea with medicinal and/or recreational purposes in several places of Estonia, reported the use of 69 vascular plants and only one lichen species, *Cetraria islandica.* From 180 interviewed persons, 22 used this lichen as tea for medicinal purposes, as cough, cold, bronchitis, lung diseases, respiratory problems and fever. From these persons, only one used *C. islandica* tea for recreational purpose, and the predominance of its use as medicinal was justified due to be considered culturally unpleasant.

Singh et al. (2014) studied medicinal plants in sacred groves of Kumaon region of central Himalaya, and found 89 species, two of them were the lichens *Everniastrum cirrhatum* and *Parmotrema reticulatum* (*Parmeliaceae*), both used for cold.In the same region, in Nepal mountains Devokta et al. (2017) documented the use of lichens in nine different communities. The authors found ethnic and different value uses for lichens, since medicinal (most part) to spiritual and aesthetic. In addition, three species had been mentioned their use for cooking. Probably due the high availability of lichens in mountain regions, all kind of thallus were reported by using (fruticose, foliose and crustose).

Agelet and Vallès (2003) worked in Iberian Peninsula and mention 272 medicinal plants used by traditional communities, being five of them lichen species. They refer *Alectoria sarmentosa, Cetraria cucullata, C. islandica, Pseudevernia furfuracea* and *Ramalina capitata* as antiasthmatic, as well as an anticatarrhal and hypotensive activity for *P. Furfuracea* and hypotensive and antituberculosis action to *C. islandica.*

Crawford (2015) summarizes studies made throughout the world, describing the use of 52 lichen genera as medicinal. The author consider *Usnea* the most common used genus, except in Australia, and so many others in Europe, USA, Canada, China, etc. For South America the data are scarce and many of them few informative. As example in Ecuador there are reported *Usnea* spp and *Dictyonema huaorani*, while in Argentina four species of *Usnea*are mentioned; the same genus is reported as useful in Uruguay, Venezuela and Chile. Marcelli (personal communication, 2015) mentions a saxicolous *Usnea* sp occurrant at Santa Catarina and Rio Grande do Sul coast (states of Brazilian South), used by local people for genitourinary diseases. The lichen thallus is mixed to "chimarrão" (typical drink of Brazilian South, made from infusion of "erva mate" – *Camelia sinensis*), and the users recognize the efficiency of the lichen thallus from its coloring; the more yellow it is, more effective its action. In Peru one *Roccella*sp is used by traditional communities. To Brazil the use of *Cladonia miniata*is reported, besides an inaccurate information about *Usnea*barbata with a local nomination with a Tupi Guarani term "membyrakú í ja", that means "hot daughter" (Ms Priscela Navarro, personal communication, 2015), and used for woman fertility. However, this species is more common in Brazilian South and neighbours Countries. The information to occur in Brazil is very much imprecise, due to size of this Country. In this context, the same author mention crostose white lichen in Peru used mixed to resins, as hallucinogen; a mix of five species (Chácobo) for several problems in Bolivia, and an unidentified species used for constipation in babies.

The mentioned papers did not mention if any species are used together, or due to their morphological similarity can be ethnosynonymous for these communities. This is the case of our study. Although *H. galactophylla*, *P. salacinifera* and *P. Wrightii* being ethnosynonyms for the Pankararu people being employed for the same purpose, they are differentiated by the degree of concentration of the therapeutic effect.

Considering the use and contraindications by popular and/or traditional use of lichens, neither Sõunkand and Kalle (2013), nor Agelet and Vallès (2003), Singh et al. (2014) and Crawford (2015) mentioned the active principles of the species, as well as reports of literature about effectiveness or toxicity of compounds contained in the studied species. By other hand, Agelet and Vallès (2003) reported studies performed by other colleagues about biological activities of related species or genus, nevertheless without refer any lichen compound.

It is known that substances like atranorin, zeorin, stictic and salazinic acids (Fig. 4) have antimicrobial and antibacterial activity (Tay et al., 2004; Yilmaz et al., 2004; Vicente et al., 2006; Marijana et al., 2010; Molnar and Farkas, 2010). To establish these relationships, more studies referring to their pharmacological uses are needed, due to the scarce information about toxicity at acute and both chronic and subchronic levels.

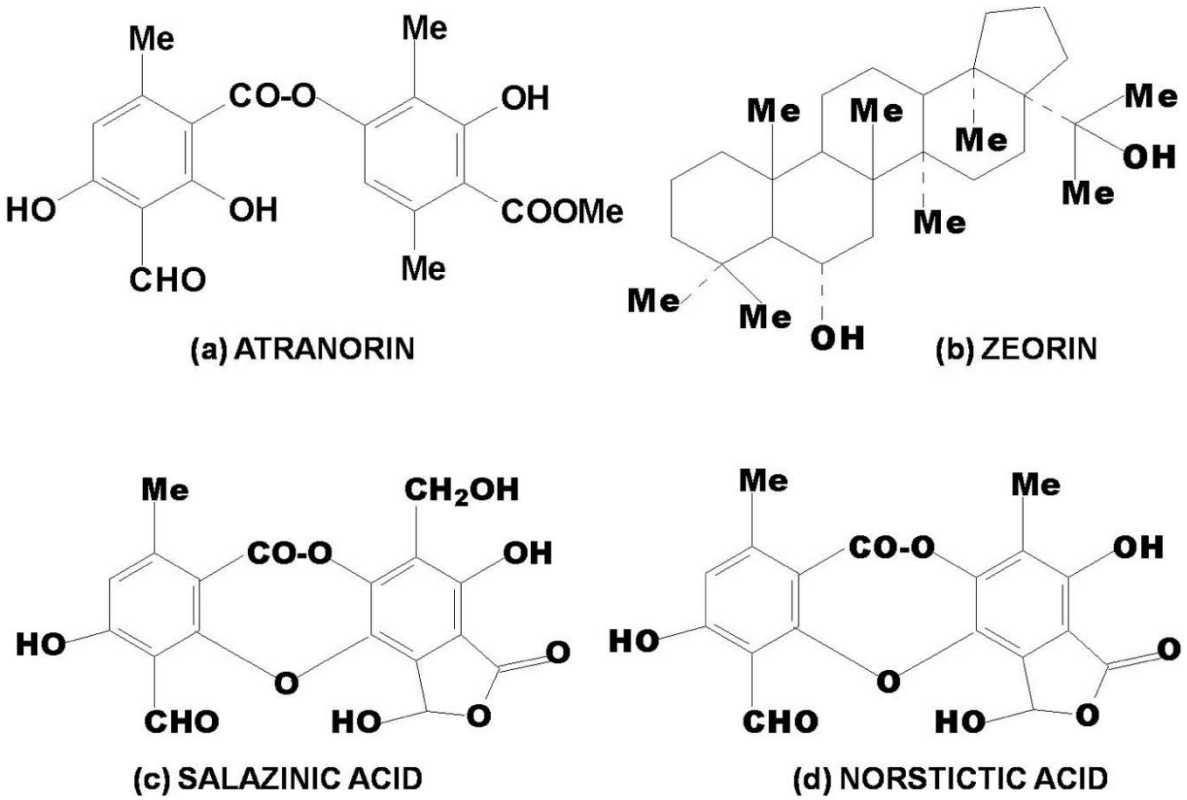

Fig. 4: Structural formulae of atranorin (a), zeorin (b), salazinic (c) and norstictic (d) acids

Graphical Abstract

Regarding to toxicity of substances found in studied species, it is known the low toxicity of atranorin. Melo et al. (2011) in anti-inflammatory assays with this substance (100 mg/kg and 200 mg/kg) obtained from *Cladina kalbii* a meaningful activity, but no significant toxicity at acute and subchronic levels was detected, as well as cytotoxity. These data are coincident to ones described to Maia et al. (2002), when tested the antinociceptive action of atranorin and crude extracts from *Cladina dendroides.*

Asakawa et al. (2013) describes cytotoxic activity of α-zeorin, isolated from several liverworts, against P-388 cells, whose IC50 was 1.1 μg mL^{-1}. Data is almost nonexistent for such compounds, and no information was found about salazinic acid.

By other hand, it is possible to attribute a more remarkable action, depending on the chemical group the lichen compound is placed. In this context, Correche et al. (2002) mention that the depsidones, in general, exhibit a stronger cytoxity than the depsides, attributing this bioactivity to the structural characteristics of their chemical group, where the aldehyde function is always linked to a C3, with an OH to the adjacent C4. This way, both salazinic and norstictic acids have these characteristics, while atranorin, being a depside, exhibit a lower toxicity. Regarding to zeorin, this compound is a terpenoid. Harrewijn et al. (2001) mention that several terpenoids have minimal toxicity to vertebrates, besides their usefulness in the cosmetics and pharmaceutics, due to be biologically actives. By other hand, some of them have evidenced their toxicity, suggesting a more accurate discussion about toxic effects of this chemical group, mainly concerning the terpenes obtained from lichens and lower plants.

V. Conclusions

Our results show that Pankararu people use lichens with active principles for several treatments, andknow the right dose, side effects and restrictions of each species.

Acknowledgements

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Highlights

- Traditional indigenous community in semi-arid Brazil use lichens as medicine.
- Lichens used have their properties and contraindications recognized by this people.
- This is the first report of use of lichens as medicament in Brazilian semi-arid.
- Active compounds were found in lichens used by Pankararu people.

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Characterization of Environmental Conditions Conducive for Spread of Whitefly Population and Epidemic Development of ChiLCV

By Maryam Iftikhar, M. Aslam Khan & Sajjad Haider

Abstract- Chilli (*C.annum* L.) is one of the main solaneaous crop in Pakistan and treated by many viral diseases. This experiment was performed to check the effect of environmental conditions on whitefly population and disease incidence development as well as correlation and regression analysis of environmental factors like maximum, minimum temperature, relative humidity, rainfall and wind speed with whitefly population on Chilli plants. For the determination of effect of environmental factors on the incidence of virus and whitefly Population, five environmental factors were kept in consideration which were maximum temperature, minimum temperature, relative humidity, rainfall and wind pace. The data recorded on disease incidence and whitefly population was subjected to correlation and regression analysis for determining the relationship between environmental variables and incidence of disease and whitefly population. All environmental parameters including (maximum temperature, minimum temperature, and relative humidity) showed positively significant correlation and wind speed and rainfall showed negatively significant correlation.

Keywords: *chiLCV, regression, correlation, epidemiology, conducive.*

GJSFR-C Classification: *FOR Code: 060704*

Characterization of Environmental Conditions Conducive for Spread of Whitefly Population and Epidemic Development of ChiLCV

Maryam Iftikhar [α], M. Aslam Khan [σ] & Sajjad Haider [ρ]

Abstract- Chilli (*C.annum* L.) is one of the main solaneaous crop in Pakistan and treated by many viral diseases. This experiment was performed to check the effect of environmental conditions on whitefly population and disease incidence development as well as correlation and regression analysis of environmental factors like maximum, minimum temperature, relative humidity, rainfall and wind speed with whitefly population on Chilli plants. For the determination of effect of environmental factors on the incidence of virus and whitefly Population, five environmental factors were kept in consideration which were maximum temperature, minimum temperature, relative humidity, rainfall and wind pace. The data recorded on disease incidence and whitefly population was subjected to correlation and regression analysis for determining the relationship between environmental variables and incidence of disease and whitefly population. All environmental parameters including (maximum temperature, minimum temperature, and relative humidity) showed positively significant correlation and wind speed and rainfall showed negatively significant correlation.

Keywords: chiLCV, regression, correlation, epidemiology, conducive.

I. Introduction

Chilli (*Capsicum annum* L.) originate from south and central America and are members of Solaneaous family. Viral diseases annually reduce the quality and yield of all kind of pepper. Symptoms of virus infection widely vary in expression and severity including mild mottle, mosaic, vein banding, ring spots, necrosis, leaf discoloration, deformation and blistering and severe stunting of the whole plant. Viruses could not just identified based on symptoms, because symptoms could vary with respect to the strain of the virus, the host cultivar, the age of the host, environmental conditions and co-infection with other viruses.

Different viruses may cause similar symptoms, as well as insect damage, particularly by thrips and mites, may mimic virus symptoms. Chilli leaf curl virus (ChiLCV), Chilli vein mottle virus (ChiVMV) and cucumber mosaic virus (CMV) are the main viruses in all Chilli growing areas of Pakistan and also in some other parts of the world. ChiLCV is the most important pathogen related to Chilli crops (Shah *et al*., 2001).

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ChiLCV is more susceptible to all Chilli varieties. Begomoviruses are the main cause of this disease. It is one of the largest group of Gemini viruses with more than 50 members described so far by different workers (Markham *et al.,* 1996). In worldwide review more than 65 viruses have reported to infect different crops. Viruses are most disturbing agents of chili crop, causing serious losses in reduction of both fruit quality and quantity (Green and Kim., 1999). Approximately 40 to 60 % losses in Pakistan and some other parts of world has been recorded due to ChiLCV because it is major virus most common in Chilli producing areas and decreasing yield badly (Shah and Khalid, 1999).

II. Historical Background of Chilcv Disease

Chilli leaf curl virus for first time was reported by Verma (1962). The virus has been found to be transmitted though vector whitefly *Bemisia tabaci* Gen. (Moghe, 1977). Later on it was reported by venkatesh *et al*., (1998) that Chilli leaf curl complex caused by Chilli leaf curl geminivirus (ChiLCV) is transmitted by *Bemisia tabaci* and also by thrips (*S.dorsalis* and *polyphagotarsonemus latus*). In Pakistan Hussian *et al.,* (1992) reported chilli leaf curl complex in 1992. Severe yield losses in Chilli crop along with other Chilli varieties has been found. Tomato mosaic virus, Potato virus Y, Chilli leaf curl virus, Pepper veinal mottle virus, Tomato yellow leaf curl virus and Tomato spotted wilt virus has considered as economically most important viral diseases in Africa and Asia among the economically important vegetables (Nono-Womdim, 2001).

III. Symptomology Of Chilcv Disease

Chilli pepper (*C.annum* L.) use as a spice and it is an important vegetable. For the cultivation of Chillies in Pakistan Diverse ecological, environmental and soil conditions are very suitable (Briddon *et al.*, 2003; Shih *et al*., 2003). Leaf curling, wrinkling, vein clearing and vein swelling and yellowing are major symptoms of ChiLCV. In severely affected plants the size of leaves and branches reduced resulting in a bushy appearance of plant. Such plants have very few flowers and very few fruits (Peiris, 1953; Joshi and Dubey, 1976). Begomoviruses are the major cause of Chilli leaf curl

disease (CLC), which is most important viral disease of chilies. Typical symptoms of ChiLCV include stunting, a reduction in leaf size, leaf curling as well as a reduction in fruit size and number (Hussian, 2009).

IV. Influence Of Environmental Factors On Whitefly Population And Disease Incidence

An experiment was performed to check the effect of environmental conditions on whitefly population and correlation of environmental conditions like maximum, minimum temperature, relative humidity, rainfall and wind speed with whitefly population on tomato plants by (Zeeshan *et al.,* 2015). Maximum temperature has positive correlation with whitefly population. Whitefly population increase with increase in temperature and decrease with decrease in relative humidity

V. Materials And Methods

a) Establishment of disease screening nursery against ChiLCV disease incidence

A disease screening nursery of eight varieties/Lines i.e.V1 (Maha), V2 (Hot Queen), V3 (7-Ph), V4 (9-Patayla), V5 (Tatapuri), V6 (Biaddy), V7 (Hot Shot), V8 (5-Glory) were established against ChiLCV disease.

b) Epidemiological studies of ChiLCV disease

Five varieties viz. 9-patayla, Hot-Shot, Five-Glory, 7-ph, Tatapuri were used in the experiment. The experiment was conducted in a randomized complete block design (RCBD) with three replications. Each variety was planted in a sub-plot with row length 3m, row to row spacing 60cm and plant to plant spacing of 30cm. The disease on every variety was assessed by coefficient of infection according to available disease rating scale.

c) Collection of environmental and whitefly population data

The data of different environmental factors (maximum, minimum temperature, relative humidity and rainfall) during the growth period of chili crop (April-July) was obtained from the Department of Crop Physiology, University of Agriculture Faisalabad. The data regarding whitefly population was recorded on weekly basis for each variety. Ten plants from each plot were selected at random and population of whitefly was recorded from upper middle and lower leaves/plant and averaged for 5 leaves.

d) Correlation of environmental factors with ChiLCV incidence

Correlation of ChiLCV incidence with maximum temperature, minimum temperature, relative humidity, rainfall and wind speed were determined on weekly basis at variety level. The variety used for this purpose were 9-Patyala, Hot Shot, 5-Glory, Maha and Hot Queen. A significant correlation was observed between maximum temperature and disease incidence. Similarly, minimum temperature showed significant correlation with disease incidence on all the varieties.

Relative humidity had a significant relationship with disease incidence on all the varieties. Rainfall showed a significant but negative correlation with disease incidence as increase in rainfall suppresses the rate of increase of disease on all varieties while wind velocity also showed the significant positive correlation on all varieties used.

Table 1: Correlation of environmental factors with ChiLCV

Varieties	Max Temp	Min Temp	RH	Rainfall	Wind Speed
9-Patyala	0.5419* 0.0267	0.8960* 0.0157	0.7092* 0.0146	-0.3593* 0.0483	-0.6037* 0.0244
Hot Shot	0.6528* 0.0159	0.9044* 0.0133	0.6258* 0.0183	-0.3187* 0.0351	-0.3478* 0.0493
5-Glory	0.5169* 0.0239	0.8935* 0.0164	0.7576* 0.0180	-0.3218* 0.0354	-0.5452* 0.0263
Maha	0.5607* 0.0247	0.9151* 0.0105	0.7374* 0.0494	-0.3434* 0.0155	-0.5313* 0.0287
Hot Queen	0.5812* 0.0264	0.9289* 0.0074	0.7272* 0.0114	-0.3766* 0.0146	-0.5532* 0.0254

Upper values indicate Pearson's correlation coefficient while lower values indicate significance at 5% level of probability.

e) Correlation of environmental factors with whitefly population

Correlation of whitefly population with maximum temperature, minimum temperature, relative humidity, rainfall and wind speed were also determined on weekly basis at variety level. Same varieties were used for this purpose i.e. 9-Patyala, Hot Shot, 5-Glory, Maha and Hot Queen. A significant correlation was observed between maximum temperature and whitefly population. Similarly, minimum temperature showed significant correlation with whitefly population on all the varieties.

Relative humidity had a significant relationship with whitefly population on all the varieties. Rainfall showed a significant but negative correlation with whitefly population as increase in rainfall suppresses the rate of increase of disease on all varieties while wind speed also showed significant but negative correlation with all varieties used.

Table 2: Correlation of environmental factors with whitefly population

Varieties	Max Temp	Min Temp	RH	Rainfall	Wind Speed
9-Patyala	0.6204* 0.0188	0.9098* 0.0118	0.6762* 0.0143	-0.2720* 0.0126	-0.4227* 0.0437
Hot Shot	0.5988* 0.0291	0.8809* 0.0204	0.6850* 0.0132	-0.1964* 0.0792	-0.3129* 0.0456
5-Glory	0.5020* 0.0132	0.8480* 0.0329	0.7767* 0.0296	-0.1083* 0.0382	-0.2783* 0.0359
Maha	0.4557* 0.0367	0.8006* 0.0557	0.7600* 0.0579	-0.9576* 0.0283	-0.2554* 0.0252
Hot Queen	0.4900* 0.0328	0.8732* 0.0231	0.7969* 0.0577	-0.1836* 0.0277	-0.4246* 0.0144

Upper values indicate Pearson's correlation coefficient while lower values indicate significance at 5% level of probability

f) Correlation of ChiLCV disease incidence with whitefly population

Correlation of ChiLCV disease incidence with its vector population was also determined at variety level. The results indicated that a significant correlation was observed between disease incidence and whitefly population on all varieties.

Table 4.6: Correlation of ChiLCV disease incidence with whitefly population on all chili varieties

Varieties	Disease Incidence and Whitefly Population
9-Patyala	0.9152* 0.0105
Hot Shot	0.9025* 0.0138
5-Glory	0.8923* 0.0168
Maha	0.9083* 0.0122
Hot Queen	0.9741* 0.0010

Upper values indicate Pearson's correlation coefficient while lower values indicate significance at 5% level of probability.

g) *Relationship between environmental factors with ChiLCV disease incidence*

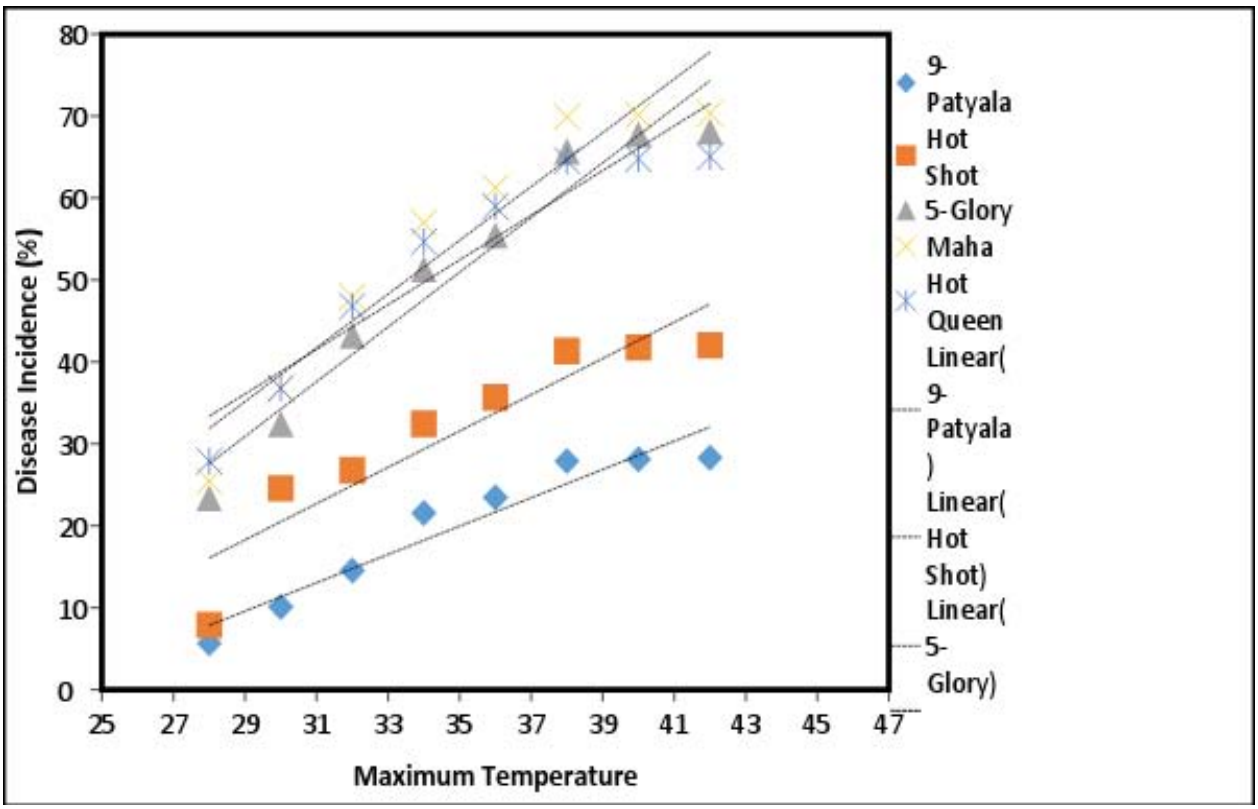

Figure 1: Effect of Maximum temperature on ChiLCV incidence

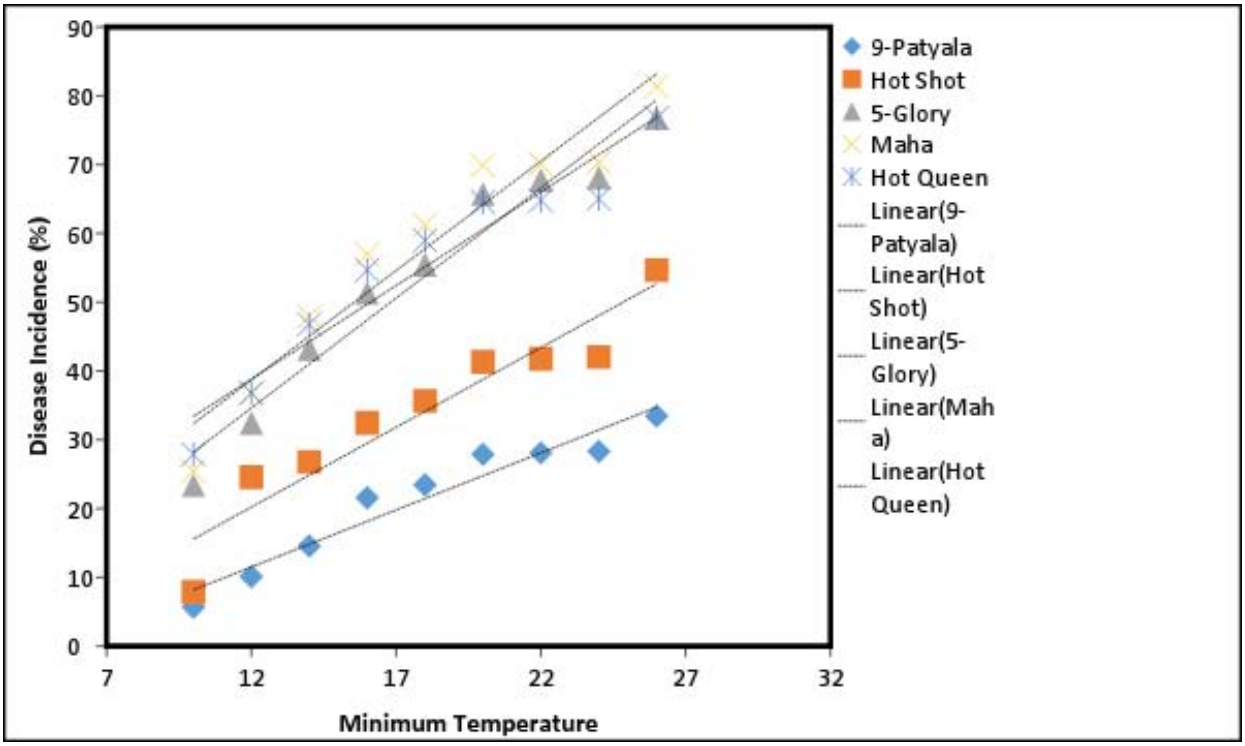

Figure 2: Effect of Minimum temperature on ChiLCV incidence

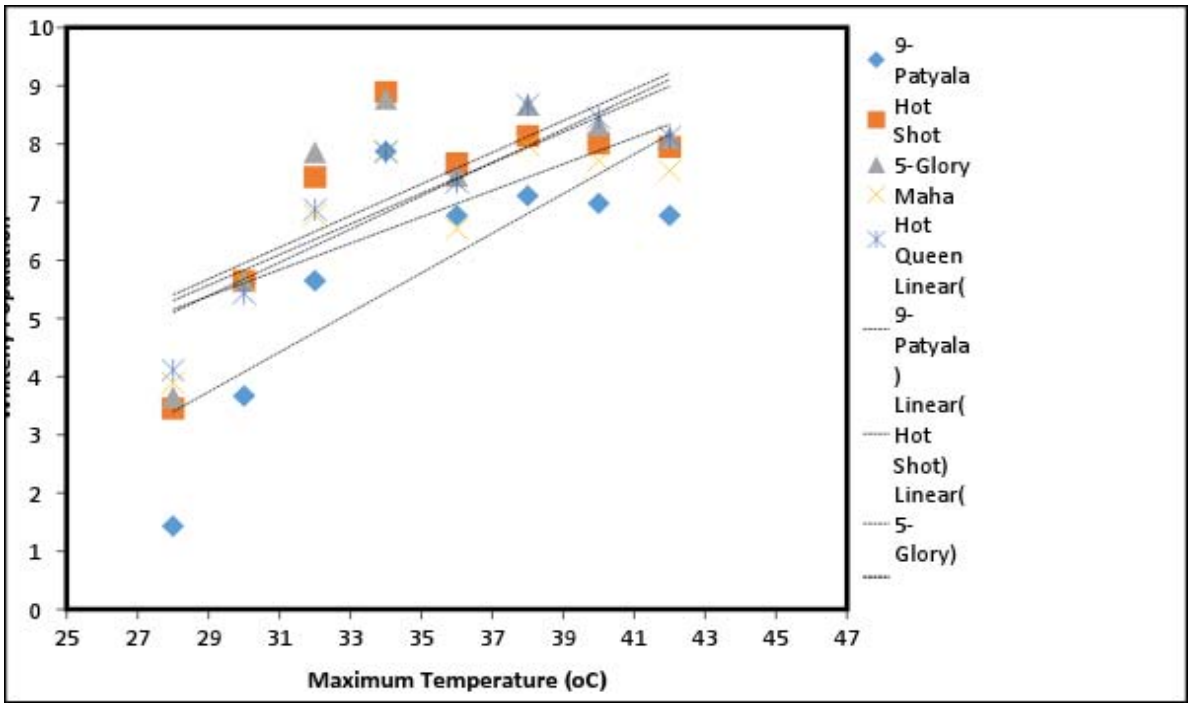

Figure 3: Effect of Maximum temperature on Whitefly Population

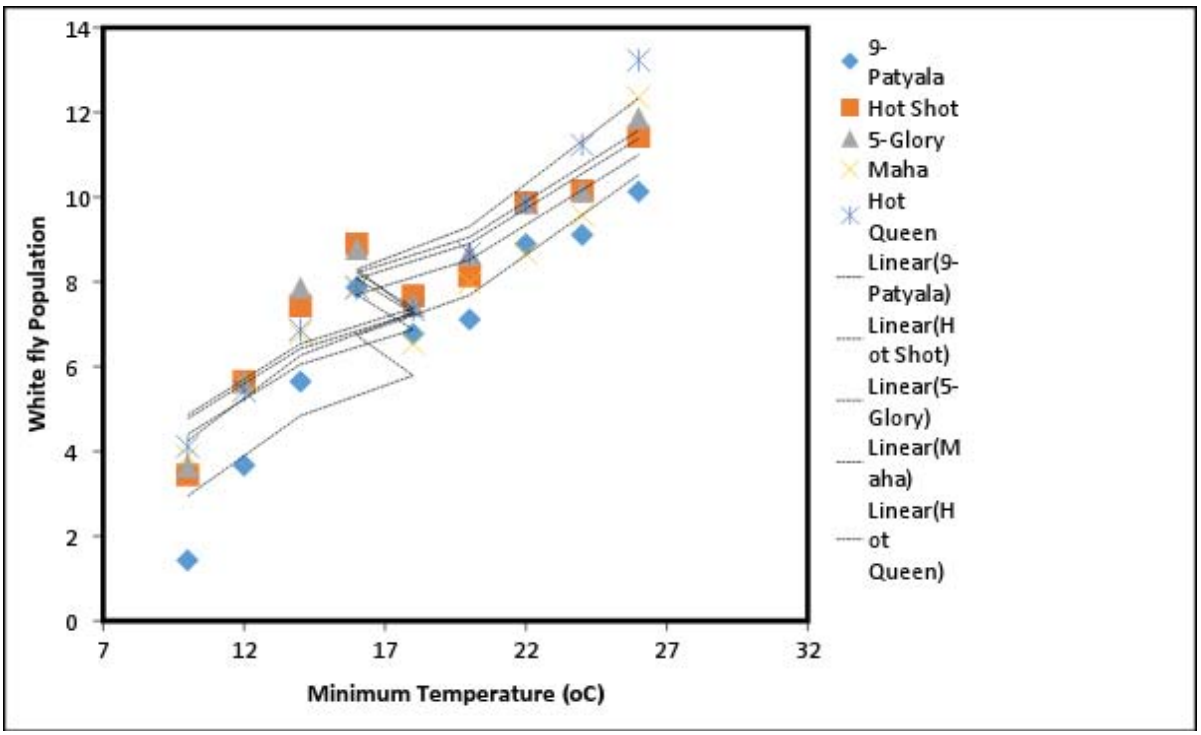

Figure 4: Effect of Minimum temperature on Whitefly Population

VI. Result and Discussion

Correlation of weekly maximum and minimum air temperature, relative humidity, rainfall, wind speed and aphid population with ChiLCV disease incidence was determined at variety level. There was significant correlation between environmental factors, time and ChiLCV disease on chili varieties. A significant correlation was found between maximum temperature and disease incidence on five varieties/lines and non-significant on the three varieties/lines. Minimum

temperature had significant correlation with disease incidence on five varieties while three varieties and non-significant on the three varieties. There was a significant correlation of relative humidity with disease incidence on five varieties/lines while a non-significant correlation on three varieties. There was a significant correlation of rainfall on four lines/varieties and remaining lines/varieties showed non-significant correlation. Wind speed had significant correlation with disease incidence on five varieties while others showed non-significant correlation. A significant correlation was found between maximum temperature and disease incidence on five varieties and non-significant on three varieties/lines. Minimum temperature had also significant correlation with disease incidence. The relative humidity and rainfall had negative correlation with whitefly population.

The results indicated that there was negative impact of rainfall on whitefly population more rainfall resulted in decrease in whitefly population but it had positive effect on disease intensity. Similarly Singh (1990) reported that cooler weather with high relative humidity and rainfall negative impact on whitefly population and spread. Morales and jones (2004) also reported that the disease caused by various Gemini viruses were more intense in wet and humid climatic conditions than in dry conditions. Khan *et al.,* (1998) explained the relationship of weekly air temperature, relative humidity, wind velocity and wind speed to ChiLCV disease development by linear regression in eight varieties. Plant infection by ChiLCV increased on all varieties at maximum and minimum air temperature of 33-45 celercious and 25-30 degree celicus respectively.

However negative correlation between the minimum temperature sunshine hours and pest abundance and a positive correlation between maximum temperature and pest abundance of *B.tabaci* was found by Men *et al.,* (1997).

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- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
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- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
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Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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