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Influence of weather parameters on *Aphis craccivora* and Bean common mosaic virus disease incidence in *Vigna unguiculata* L. Walp

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Abstract

Aim : Many crop modelling and climate simulation studies have reported variability in pest and pathogen distribution as well as their interaction with host. These prediction encourages to revisit the pest and pathogen epidemiological and distribution studies in light of changing climate. In line with above assumption, the present field study was carried out to study the population dynamics of cowpea aphid (*Aphis craccivora*) and its influence on BCMV disease incidence.

Methodology : In this field experiment, *A. craccivora* population and BCMV disease incidence data were collected in different standard meteorological weeks of 2013-2014 and 2014-2015. Impact of weather parameters on *A. craccivora* and BCMV disease incidence was statistically analyzed.

Results : The significant change in *A. craccivora* population and BCMV disease incidence was documented during the study. The maximum aphid population was observed during *rabi*

season and less was observed in *kharif* and summer season. Whereas, incidence of BCMV was recorded maximum in *rabi* season. Correlation analysis affirmed, minimum temperature (T_{min}) and rainfall had negative impact on *A. craccivora* whereas weather parameters had non-significant impact on BCMV disease incidence. The regression models developed from the present study explained 41.4 % to 78.8 % and 18.5 % to 46.1% variability (R^2) of aphid population and BCMV incidence, respectively.

Interpretation : The weather parameters had more impact on *A. craccivora* population than BCMV disease incidence and merely presence of maximum number of *A. craccivora* was not a deciding factor for outbreak of virus disease incidence.

Key words: *A. craccivora*, Bean common mosaic virus, Cowpea, Disease incidence, Weather parameters

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Introduction

Cowpea (*Vigna unguiculata* L.Walp.) is one of the most important but fairly neglected among legume crops. The crop has a global distribution with major intensity in the semiarid regions of all major continents like Africa, Asia, Southern Europe, Southern United States, and Central and South America (Timko *et al.*, 2008; Diouf, 2011) and estimated average annual worldwide production of about 6.5 metric ton on about 14.5 m ha (Boukar *et al.*, 2016). The crop is an excellent source of good quality protein, rich in essential amino-acids lysine and tryptophan (Badiane *et al.*, 2014), and is also valued for its richness in different vitamins and minerals (Boukar *et al.*, 2015). However, the beauty lies in the fact that the crop provides hungry and resource poor farmers of Asian and African continent a means of food and feed in diverse form (Khan *et al.*, 2015) and that too in less resource hungry manner along with a sustainable farming system via crop-rotation, mixed and inter-cropping, through nitrogen fixation and release of soil bound phosphorous (Gowda *et al.*, 2013; Huynh *et al.*, 2016). In Indian scenario, where resides the world's one of the largest human and livestock population, along with major population of under nourished children, the crop can provide a cheaper mean of nutrition. In the present scenario, India produces 2.24 mt of cowpea annually with an average productivity of 0.570 tons per hectare (Jukanti *et al.*, 2012; Manjunatha *et al.*, 2015) which is quite below to global production. Other than poor farming technology, biotic and abiotic stresses are major constraints for cowpea production. Biotic stress caused by plant pathogens such as fungi, bacteria, viruses and nematodes are known to affect the cowpea productivity.

Among these, viruses are the major group of pathogens infecting cowpea (Badiane *et al.*, 2014; Yadav *et al.*, 2015). Globally, about 140 viruses are known to infect cowpea (Hughes and Soyinka, 2003). Among them, *Bean common mosaic virus* (BCMV), a type of potyvirus is a serious threat to bean cultivation worldwide because it is easily transmitted through seeds, pollens and aphid insect vector (Kapil *et al.*, 2011; Manjunatha *et al.*, 2015). It is believed to originate from East Asia and now it has spread worldwide wherever legumes are grown (El-kady *et al.*, 2014). Infection in field may reach 100% (Li *et al.*, 2014) leading to 35%-98% yield loss. (Manjunatha *et al.*, 2015). Population dynamics of aphid and its influence on different potyviruses have been established for different crops, including legumes (Casteel *et al.*, 2014; Mondal and Gill, 2012; Perotto *et al.*, 2014). However, to the best of our knowledge no such study has so far been available for BCMV infecting cowpea. Hence, in the present field experiment, the effect of weather parameters on aphid population in relation to BCMV disease incidence was investigated.

Materials and Methods

Plant material and experimental site : The seeds of cowpea variety (C-152) were collected from All India Co-ordinated Research Project (AICRP) on Arid legumes, Zonal Agricultural Research Station (ZARS), GKVK, Bengaluru and it was

considered to be highly susceptible for BCMV infection in our previous study (Manjunatha *et al.*, 2015). To conduct field experiment, crop was sown at Main Research Station, University of Agricultural Sciences (UAS), Hebbal, Bengaluru, in three plots of area, 10 m² (5 m X 2 m) during different standard meteorological weeks [37th week (September), 46th week (November) and 8th week (February)] of 2013-2014 and 2014-2015. Recommended agronomical practices were followed during the field experiments, and all the plots were kept free off insecticidal spray throughout the experimental period in order to record the number of aphids.

RNA extraction and cDNA synthesis : Total RNA was extracted from BCMV infected plant samples using RNA Extraction kit (Spectrum™, Sigma Aldrich). The obtained total RNA was further used for synthesis of cDNA. A total of 20 µl RT mixture was prepared by adding 2 µl of 10X RT buffer, 1.0 µl of 25 µM MgCl₂, 2.0 µl of 10 mM dNTP mixture, 2.0 µl of 10 µM Reverse Primer (5'AGGCATGTACGGCTTCTCG3'), 1.0 µl of Reverse transcriptase (100 units µl⁻¹), 5.0 µl of template RNA and sterile distilled water added to make up final volume. Reaction mixture template RNA (5.0 µl) + reverse primer (2.0 µl) was incubated at 65 °C for 15 min. Finally, the RT-PCR mixture was reverse transcribed at 42 °C for 60 min and then at 94 °C for 5 min to synthesize cDNA.

Reverse transcriptase PCR (RT-PCR) : The synthesized cDNA was subjected to PCR amplification using BCMV coat protein based primer (5'CGCAGGCTCCAAAGGAAAAG 3'). A total of 25 µl reaction mixture (2.0 µl of cDNA, 12.5 µl of master mix (Takara), 2.0 µl of primer and 8.5 µl of sterile water) was amplified in Eppendorf thermocycler. Amplification was carried out with the following reaction conditions: initial denaturation at 94 °C for 3 min followed by 35 cycle reaction profile involving denaturation at 94 °C for 1 min, annealing at 54 °C for 1 min and extension at 72 °C for 2 min followed by a final extension at 72 °C for 10 min, finally reaction was hold at 4 °C. The amplified product (25 µl) was separated on 1% agarose gel along with 1 kb DNA ladder (Fermentas, USA) and visualized by a gel documentation unit.

Aphis craccivora population and BCMV disease incidence : Occurrence of different aphids was identified with the help insect taxonomist. But only the presence of *Aphis craccivora* was recorded for each sowing date at weekly interval for 60 days. The number of *A. craccivora* per plant up to 60 days was recorded by observing the top 20 cm of plant each sampling unit. Similarly, the BCMV disease incidence was documented at weekly interval in field upto 60 days from first appearance of virus disease symptoms. Virus disease incidence (%) was estimated by counting number of BCMV infected plants to total number of plants observed in an individual plot.

Weather parameters : Data on weather parameters viz., maximum temperature (T_{max}), minimum temperature (T_{min}), maximum relative humidity (RH_{max}), minimum relative humidity (RH_{min}), rainfall and sunshine hours were collected from Agro-meteorological observatory located in UAS, Bengaluru, India.

Statistical analysis: The data obtained (aphid population and virus disease incidence) from the present study were statistically analyzed. The data were further subjected to correlation and regression analysis to find out relationship between weather parameters, *A. craccivora* population and BCMV disease incidence. Statistical analyses were performed using *INDOSTAT* statistical software. Step-wise regression analysis was performed to find outbreak of virus disease in cowpea.

Results and Discussion

Predicting epidemics of plant virus disease constitutes a challenge due to complexity of three-cornered factors (virus, insect vector and host) involved and their interactions with the environment (Jones *et al.*, 2010). Weather parameters exert a great effect on aphid population dynamics and disease development as well. Variation in physical and biological factors has direct influence on aphid population density and spread of aphid borne viruses (Ingwell *et al.*, 2012). Many researchers have reported the spread of aphid borne non-persistent viruses follows seasonality of vectors (Jones, 2014; Manjunatha *et al.*, 2015). Four different species of aphids including *A. craccivora* were

recorded during study (Fig.1). Identification of these species were confirmed by taxonomist at ICAR-National Bureau of Agriculturally Important Insects (NBAIL), Bangalore. The presence of BCMV in cowpea plant (Fig. 2a) was confirmed by coat protein (CP) specific primer pair. A fragment size of approximately 750 bp (Fig. 2b) was amplified for the virus infected cowpea plant.

Fluctuation in *A. craccivora* population and BCMV disease incidence was observed in different weeks as shown in Fig.3, 4. During 2013-2014, the aphids occurred in 40th week (50 aphids/plant) whereas in 46th week aphids count continued to be high showing two peaks. First peak was observed at 48th week (234 aphids/plant), second peak at 51st week (470 aphids per plant). Subsequently, the number of aphids was reduced. Similar trend of aphid population and BCMV disease incidence was recorded in different standard meteorological weeks of 2014-2015. Between 38th and 41st weeks, aphid population was less. whereas, absence of aphids was observed between 42nd and 46th week. Afterwards aphid population was drastically increased at 50th (385 aphids per plant) and 52nd (510 aphids per plant) week. Weather parameters are frequently suggested as a cause of population fluctuations. The results clearly displayed that aphid

Fig.1: Different aphid species were observed in cowpea experimental field: (a) *Aphis craccivora*; (b) *Aphis gossypii*; (c) *Aphis nerii* and (d) *Myzus persicae*.

Fig. 2: Molecular confirmation of BCMV from infected cowpea plant sample using CP specific primers. M: 1kb DNA ladder; Lane 1: Virus infected leaf sample; Lane 2: healthy leaf sample; Lane 3: BCMV infected French bean leaf sample; (Positive control); Lane 4: French bean healthy leaf sample.

Fig. 3 : Relationship between weather parameters, *A. craccivora* and BCMV disease incidence observed during the year 2013-2014.

Fig. 4 : Relationship between weather parameters, *A. craccivora* and BCMV disease incidence observed during the year 2014-2015.

population was widely fluctuated among the standard meteorological weeks. The present findings is in confirmation with the reports of Purnima Das and Dutta (2002) who observed infestation of aphid and peak population at 2nd week of November and December. Similarly, Shahzad *et al.* (2003) observed occurrence and an increased trend of aphid numbers during winter months. *Bean common mosaic virus* (BCMV) disease incidence ranged from 16.5 % to 35.5 % and 5 % to 23.51 % in 2013-2014 and 2014-2015 respectively. Cowpea crop sown before 38th week recorded highest virus disease incidence (35.5 % and 23.51 %) whereas relatively low incidence was recorded in crop sown before 45th week during both the years. More disease incidence (23.51 %) was recorded in cowpea crop sown before 9th week during 2013-2014. The observations made in this study is in accordance with the findings of Permey *et al.* (1997) and Prasad

et al. (2007) where date of sowing was reported to have greater impact on incidence of BCMV disease in French bean.

To estimate the additive effects of different weather parameters on *A. craccivora* population and BCMV disease incidence were statistically analyzed. As, aphid dynamics were largely dependent on temperature and relative humidity, however, *A. craccivora* population was not significantly correlated with rainfall (Shakeel *et al.*, 2014). T_{max} and T_{min} had significant negative association with aphid population (Nisha, 2014). The coefficient of correlation showed negative relation with T_{min} , i.e., aphid population started building up when T_{min} showed downward trend. Civitanes *et al.* (2012) observed that *B. brassicae* and *M. persicae* were more when the days with T_{min} was >15 °C and days with relative humidity 70 %. It was concluded that the less

Table 1: Correlation and multiple regression between *A. craccivora* population and weather parameters

Weather parameters	Correlation coefficients	Regression coefficients	Standard Error	't' value	R ²
2013-2014					
Intercept		-101.36			
X ₁ = T _{max} (°C)	-0.332	26.770	31.880	0.840	0.790**
X ₂ = T _{min} (°C)	-0.822**	-68.034	18.772	3.624	
X ₃ = RH _{max} (%)	0.110	8.560	5.504	1.610	
X ₄ = RH _{min}	-0.012	-1.981	5.880	0.337	
X ₅ = Rainfall (mm)	-0.315	-0.591	2.954	0.200	
X ₆ = Sunshine (hr)	0.300	0.412	13.320	0.031	
2014-2015					
Intercept		941.73			
X ₁ = T _{max} (°C)	-0.325	-14.927	24.431	0.611	0.414
X ₂ = T _{min} (°C)	-0.461*	-26.937	22.757	1.184	
X ₃ = RH _{max} (%)	0.134	9.480	9.043	1.050	
X ₄ = RH _{min}	-0.061	-11.996	7.772	1.540	
X ₅ = Rainfall (mm)	-0.400	-7.350	6.552	1.122	
X ₆ = Sunshine (hr)	0.037	-21.856	19.640	1.113	

or no rainfall and T_{min} with high RH had profound positive effect on *A. craccivora* population. The results of the present study are in accordance with the findings of Patel *et al.* (2010) who stated that T_{max} had negative impact on *A. craccivora* in cowpea ecosystem. The higher cowpea aphid infestation during January, February and lower in April was also documented by Aheer *et al.* (2007). These results indicated that disease incidence had positive association with RH_{min} and sunshine hours. Remaining weather parameters showed inappreciable relationship. This type of association indicated that there may be several other factors associated with occurrence of BCMV disease in cowpea (Table 1).

The predictive model for *A. craccivora* population and BCMV disease incidence on cowpea were developed based on two year data (2013-14 and 2014-15). The models showed that weather parameters together were responsible for variation in *A. craccivora* population and occurrence of disease incidence. The multiple regression model equations for *A. craccivora* population and BCMV disease incidence is given below :

$$\begin{aligned}
 \text{2013-14} \quad Y_{DI} &= 34.790 - 3.208X_1 + 4.679X_2 + 0.161X_3 + 0.153X_4 - 0.257X_5 + 1.289X_6 + 0.028X_7 \\
 \text{2014-15} \quad Y_{DI} &= 35.229 + 0.025X_2 - 1.361X_2 - 0.057X_3 + 0.126X_4 + 0.501X_5 - 0.66X_6 - 0.001X_7
 \end{aligned}$$

* Significant @ P < 0.05, ** Significant @ P < 0.01

where, Y_{AP} is aphid population (number); Y_{DI} is disease incidence (%); X₁ is T_{max} (°C); X₂ is T_{min} (°C); X₃ is RH_{max} (%); X₄ is RH_{min} (%); X₅ is rainfall (mm); X₆ is sunshine (hr) and X₇ is the number of aphids. The regression model for 2013-2014 had a R² value of 0.790, which indicates that variability of *A. craccivora* population was 79% in the model. The coefficient for each of the variables indicate the amount of change one could expect in aphid population given a one-unit change in the value of variables, given all the variables in the model are constant. For example, if we consider variable X₁, i.e., T_{max}, we could expect a decrease of 101.36 in the aphid population for every unit increase in X₁, assuming that all variables in the model held constant. The regression equation (R² = 0.790) further indicated that among weather parameters, T_{min} (-68.034X₂) had negative impact on aphid population, which showed that one unit increase in T_{min} there was 68.034 unit decrease in aphid population whereas, other factors viz., T_{max} (26.77X₁), RH_{max} (8.560X₃), RH_{min} (-1.981X₄), rainfall (-0.591X₅) and sunshine (0.412X₆) did not exhibit profound effect on aphid population. It was evident from the model that T_{min} influenced variation on aphid numbers, whereas regression model equation fitted for 2014-2015 data revealed that weather parameters did not show any significant impact on aphid population. These observations are in accordance with Khan *et*

Year	Regression equation	R ²
<i>The multiple regression equations for aphid population</i>		
2013-14	$Y_{AP} = -101.36 + 26.77X_1 - 68.03X_2 + 8.56X_3 - 1.98X_4 - 0.59X_5 + 0.41X_6$	0.790**
2014-15	$Y_{AP} = 941.73 - 14.92X_1 - 26.93X_2 + 9.48X_3 - 11.99X_4 - 7.34X_5 - 21.85X_6$	
<i>The multiple regression equations for BCMV disease incidence</i>		
		0.414

Table 2 : Correlation and multiple regression between BCMV disease incidence, *A. craccivora* and weather parameters

Weather parameters	Correlation coefficients	Regression coefficients	Standard Error	't' value	R ²
2013-2014					
Intercept		34.800			
X ₁ = T _{max} (°C)	0.055	-3.208	4.718	0.680	0.185
X ₂ = T _{min} (°C)	0.232	4.680	3.624	1.291	
X ₃ = RH _{max} (%)	0.090	0.161	0.857	0.291	
X ₄ = RH _{min}	-0.002	0.153	0.855	0.190	
X ₅ = Rainfall (mm)	-0.105	-0.260	0.430	0.180	
X ₆ = Sunshine (h)	0.056	1.290	1.931	0.601	
X ₇ = Number of aphids	-0.120	0.028	0.035	0.670	
2014-2015					
Intercept		35.230			
X ₁ = T _{max} (°C)	-0.380	0.025	1.090	0.023	0.461
X ₂ = T _{min} (°C)	-0.253	-1.361	1.045	1.302	
X ₃ = RH _{max} (%)	0.420*	-0.057	0.412	0.140	
X ₄ = RH _{min}	0.554**	0.126	0.366	0.346	
X ₅ = Rainfall (mm)	0.477*	0.501	0.300	1.671	
X ₆ = Sunshine (h)	0.460**	-0.662	0.900	0.300	
X ₇ = Number of aphids	-0.054	-0.001	0.010	0.130	

al. (2011) who studied two season data, stepwise regression indicated significant impact of weekly T_{max}, RH and wind speed on aphid population. Similarly, multiple regression models for aphid population were developed under different weather conditions in Sawat, Pakistan (Hayat Zada et al., 2004). The present investigations are in partial agreement with Selvaraj et al. (2010) who reported that among various weather factors, evening relative humidity, sunshine hours and wind velocity were found to be the most influencing factors, which contributed 80% variation in aphid population and also reported significant negative impact of evening relative humidity, sunshine hours and wind velocity on aphid population, i.e., one unit increase in relative humidity, sunshine hours and wind velocity there was 0.99, 0.29 and 1.53 unit decrease in aphid population. Even the experiments of Panwar et al. (2014) also indicated significant negative impact of T_{max}, T_{min} and RH_{min} on aphid population. A multiple regression model containing weather parameters and *A. craccivora* population explained 18.5 % and 46.1 % variability in BCMV disease incidence. The regression equations further indicated that weather parameters and aphid population didn't have significant effect on disease development, either positively or negatively. The model equations obtained for disease incidence were not reliable to use as predictive models, because the R² values were very small. Multiple regression models were poorly fitted to the data. This situation was due to the complexities in aphid population dynamics. No a regular patterns of aphid population, was observed, i.e., there were ups and down in occurrence of aphids. The data included only number of aphids, it did not account for viruliferous aphids.

The spatial and temporal distribution of *A. craccivora* population was also not included. Moreover different factors such as landing of aphids and their movement with wind direction are some epidemiological factors which play crucial role in spread of insect vectors. There are many problems in developing statistical models of insect vector-virus pathosystems and the source of inoculum (Radcliffe and Ragsdale, 2002). Seasonal changes in environmental factors may cause serious inconsistencies. In the present study, weather parameters had greater impact on *A. craccivora* population than BCMV disease incidence. Though, aphid is a vector of BCMV disease but aphid population did not show significant relationship with BCMV incidence which may be due to lack of source of virus inoculum or viruliferous aphids during the course of experiment. A number of research reports have revealed that transmission efficiency of any virus is influenced by insect vector species. Hence, there is a necessity to understand the aphid vector dynamics and role of weather parameters on virus disease epidemiology. Presently, BCMV is spreading in different parts of Karnataka and causing substantial grain yield loss of pulse and legumes.

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