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Relationship of Epidemiological Factors with Urdbean Leaf Crinkle Virus Disease and its Management using Plant Extracts

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ABSTRACT

Urdbean (*Vigna mungo* (L.) Hepper) is an important pulse crop grown worldwide. ULCV is an important disease of urdbean in Pakistan, because it causes huge losses in production of urdbean. The susceptible germplasm and favorable environmental conditions also contribute towards the wide spread outbreak of viral diseases. Forty urdbean lines were screened against ULCV under field conditions. Correlation studies between environmental factors (temperatures & relative humidity) and ULCV infection in selected lines revealed positive interaction for maximum and minimum temperatures but a negative one for relative humidity. The comparative efficacy of 2% concentration of different plant extracts (neem, akk, garlic) against ULCV was determined. Plants sprayed with neem extract have least whitefly population followed by garlic and results showed that minimum disease severity was observed on plants that were sprayed with neem followed by akk. The results are helpful in the understanding of epidemiological factors that play vital role in disease spread. Furthermore, the plant extracts can be used to manage the vector populations, which ultimately reduce the disease incidence.

Key Words: Urdbean; ULCV; Germplasm; Plant extracts

INTRODUCTION

Urdbean or mash, also called black gram is native to India, from where it has spread to American, African, European and Asian countries. It belongs to the family Leguminosae and it is often included in rice or corn-based crop rotation to replenish nitrogen, improve soil fertility and control pests and diseases. As a leguminous plant, it could be nodulated by rhizobia, causing the formation of a new organ (i.e., nodule) and establishing a nitrogen-fixing symbiosis (Loh & Stacey, 2003). Urdbean seed contain protein (24%), phosphoric acid and lycine but is a poor source of sulphur containing amino acids. However, in combination with cereal it fulfills the requirement of protein in diets (Duffus & Slaughter, 1980). In Pakistan it covers an area of 31.5 thousand hectares, yielding an annual production of 13.7 thousand tones of grains with 20.8% decrease in production as compared to last year (Anonymous, 2009).

Low yield of urdbean is mainly attributed to the prevalence of viral diseases. Among the viral diseases, Urdbean leaf crinkle virus (ULCV) is considered to be the most serious depending on the season and variety cultivated (Reddy *et al.*, 2005). ULCV is known to spread through seeds and insects (Nene, 1972; Kadian, 1980). According to Ahmad *et al.* (1997), ULCV is transmitted through seed at the rate of 2.7 to 46%. Leaves feeding beetle (*Henoewpilachna dodecastigma* Wied), whitefly and two aphid species have been reported as vector of ULCV (Narayanasamy & Jaganathan, 1973; Dhingra, 1976; Beniwal & Bharathan, 1980).

Weather is one of the important parameters that influence plant disease epidemics. Hence, understanding of weather data and climatic conditions is required to provide baseline information for developing simple and reliable disease prediction system. Adequate work on the influence of environmental conditions conducive for urdbean leaf crinkle disease development is not available. The impact of environmental conditions and their fluctuations in relation to build up of inoculums and potential spread of disease has not been studied quantitatively. Prediction of environmental factors that have vital role in disease spread are regarded as an economical method for controlling plant diseases, especially those caused by viruses. A good deal of research work has been directed towards screening urdbean germplasm against ULCV and to identify adverse environmental conditions under which the virus causes severe crop losses, The main objective of the study was to quantify the environmental factors that play important role in the disease spread and to identify the plant extracts that can reduces the disease incidence as these extracts are economical and environment friendly.

MATERIALS AND METHODS

Urdbean disease screening nursery was established in

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Research Area of Department of Plant Pathology, University of Agriculture Faisalabad Pakistan. Each test entry was planted in a row of 3 m. One row of the most susceptible check (Kabali mash) was repeated after every two entries in the experiment. Spread of ULCV in the experimental plot was recorded at seven day intervals till maximum infection was achieved. Plants showing clear symptoms such as crinkling, curling, puckering, rugosity and enlargement of the leaves were counted and percent infection was calculated based on 0-5 arbitrary scale as suggested by Ashfaq *et al.* (2007). The number of genotypes infected per week was calculated. The results were expressed in percentages. The data of different environmental factors such as maximum and minimum temperature, relative humidity during the growth period of the crop was acquired from the website www.uaf.edu.pk.

For management of ULCV three plant extracts were used to control the vector (whitefly) and disease development. For this purpose 2% plant extracts of Neem, Akk and Garlic were used. For the use of plant extracts four susceptible varieties/lines were selected and grown in pots with CRD, where disease inoculums and virus spreading vector population were maximum. Progression of ULCV in the experimental pots was recorded at weekly interval; percent disease incidence was calculated by counting the total healthy and diseased plants and taken their percent. Whitefly population was calculated before and after 48 h when plant extracts was applied. The spray was conducted after seven days interval.

The environmental and disease incidence data were statistically analyzed using Pearson's correlation coefficient for disease incidence and environmental factor. It was subjected to correlation and regression analysis to find out their relationship with development of ULCV and in case of plant extracts and whitefly population the data were subjected to analysis of variance using MStatC statistical software and treatment means were compared with the help of Least Significance Difference (LSD) Test at 5% level of probability (Steel *et al*., 1997).

RESULTS AND DISCUSSION

The spread of ULCV disease in all urdbean lines was recorded at one week interval for seven week after the germination from 25th April to 6th June 2009 based on symptom expression. Infected plants were counted and percent disease increment was calculated on each occasion.

The correlation of maximum temperature with % ULCV infection was found to be positive in all six lines. Temperature was highly significant within the lines 62027, ES-1 and significant on other four remaining lines (Table I). With one degree increase in maximum temperature, infection was found to be increased by 5.87 and 5.66% in 62027 and ES-1 and in AARIM-32, Mash-189, 6036-14 and IAM 382-1, infection increased by 2.82, 2.26, 2.12 and 1.82%, respectively. The correlation between minimum temperature and disease incidence was also positive in all six lines. One degree increase in minimum temperature increased the rate of ULCV infection by 5.60, 5.42, 2.69, 2.13, 2.08 and 1.70% in ES-1, 6036-14, AARIM-32, Mash-189, 6036-14 and IAM 382-1, respectively and these results are the confirmation of Ashfaq (2007), who reported that maximum disease incidence was developed at maximum temperature of 35-42Ċ. There was negative correlation between relative humidity and infections of ULCV in six urdbean lines. All the lines showed highly significant correlation (Table I). One percent decreased in relative humidity increased the infection rate by 2.82, 2.49, 1.30, 1.08, 1.03 and 0.87%, in 62027, ES-1, AARIM-32 , Mash-189, 6036-14 and IAM 382-1, respectively and these results are in disagreement with Kadian (1980), who reported that RH above 70% is favorable for disease development.

Analysis of variance for the effect of plant extracts on ULCV showed that there was a significant variation within the varieties and also significant variation within the plant extracts. The disease incidence of ULCV was calculated on the basis of disease rating scale on ES-1, 6065-3, 62027, M-17. It indicated that in case of neem extracts at 2% concentration the disease incidence was 2.17, 1.67, 2.00 and 2.17, on ES-1, 6065-3, 62027, M-17, respectively. Similarly by akk plant extract at 2% concentration disease incidence was 2.58, 2.17, 2.67 and 2.67 on ES-1, 6065-3, 62027, M-17. But with the application of garlic at 2% concentration incidence was 2.83, 2.50, 2.92 and 3.171 on ES-1, 6065-3, 62027, M-17. In case of control, maximum disease incidence was 4.25 (Table II). Similar results were reported by various other scientists like Thirumalaisamy *et al.* (2003), Reddy *et al.* (2006) and Karthikeyan *et al*. (2008). They reported the anti viral properties of various plant extracts, which showed significant inhibition in disease incidence of ULCV.

On ES-1 the minimum whitefly population was found to be 5.0 adults/plant before the application of 1st spray on ES-1. All of the plant extracts i.e., neem, akk and garlic were used at 2% concentrations. After the application of plant extracts the whitefly population was reduced. Minimum population was observed in neem extract i.e., 2 whitefly/plant after the 3rd spray. All of these plant extracts at 2% concentration were effective as compared to control 7 whitefly/plant (Table III).

On 6065-3 mean whitefly population before 1st spray in all treatments varied between 5 to 7 per plant (Table III). Among the treatments neem and garlic at 2% concentrations were effective showing 1.3 to 3.3 whitefly per plant after 3rd spray, respectively. On the other hand akk extract at 2% concentration, the whitefly population was 3.6 whitefly per plant. These results also showed that the plant extracts reduced the whitefly population and in case of neem extract minimum vector population was seen followed by the garlic and on the other hand in case of control whitefly population was maximum, where only water was sprayed.

Table I: Correlation matrix among different environmental variables

Varieties/lines	Temperatures (°C)		Relative Humidity (%)
	Maximum	Minimum	
IAM 382-1(y1)	r= 0.781* y1=1.82x-52.43	r=0.856* y1=1.70x-21.46	r= -0.761* y1= -0.87x+46.26
AARIM-32(y2)	r= 0.808* y2=2.82x-88.28	r=0.905** y2=2.69x-39.64	r= -0.763* y2= -1.30x+63.38
Mash-189 (y3)	r= 0.796* y3=2.26x-63.62	r=0.880** y3=2.13x-25.50	r= -0.779* y3= -1.08x+59.26
ES-1 (y4)	r= 0.844* y4=5.66x-78.65	r=0.949** y4=5.42x-75.51	r= 0.759* y4= -2.49x+78.89
6036-14 (y5)	r= 0.768* y5=2.12x-57.85	r=0.887** y5=2.08x-24.20	r= -0.763* y5= -1.03x+57.90
62027 (y6)	r=0.771* y6=5.87x-88.58	r=0.921** y6=5.60x-79.60	r= -0.804* y6= -2.82x+79.40

* = Significant (P<0.05); ** = Highly significant (P<0.01)

Table II: Evaluation of plant extracts against ULCV recorded on various varieties of urdbean

Extracts	Varieties/lines				Mean
	ES-1	6065-3	62027	Mash-17	
Control	4.08	3.17	4.08	4.25	3.90A
Neem	2.17	1.67	2.00	2.17	2.00D
Akk	2.58	2.17	2.67	2.67	2.52C
Garlic	2.83	2.50	2.92	3.17	2.85B
Mean	2.92A	2.38B	2.92A	3.06A	

Mean sharing similar letters in a row or in a column are statically non-significant (P>0.05). LSD for varieties (5%) = 0.259, LSD for extracts (5%) = 0.259

Table III: Effect of plant extracts on whitefly population

Varieties	Extracts	Ist Spray		2nd Spray		3rd Spray	
		Before	After	Before	After	Before	After
ES-1	Neem	5.0a	3.3c	2.0c	1.0d	3.0c	2.0c
	Akk	6.6a	5.6ab	3.3bc	2.6bcd	7.0b	3.3b
	Garlic	6.0a	4.0bc	3.0bc	1.6cd	4.0c	3.0bc
	Control	6.3a	6.67a	7.3a	7.0a	9.0a	7.3a
	LSD (*P*=0.05)	2.04	1.87	1.98	1.78	1.51	1.81
6065-3	Neem	5.6a	2.6d	4.3b	2.3d	4.3b	1.3d
	Akk	7.0a	6.0b	5.0ab	4.0b	5.0ab	3.6c
	Garlic	6.0a	3.0cd	4.3b	3.3cd	4.3b	3.3c
	Control	7.6a	8.3a	5.6a	6.0a	5.6a	6.0a
	LSD (*P*=0.05)	2.92	1.68	1.28	1.10	1.28	1.61
62027	Neem	5.0c	3.0c	4.3b	2.3c	2.3b	1.3c
	Akk	7.3b	7.0b	5.0ab	3.6b	4.6ab	3.3b
	Garlic	5.6bc	4.0c	4.3b	3.6b	3.0b	2.3bc
	Control	9.0a	8.6a	5.6a	5.6a	5.6a	6.0a
	LSD (*P*=0.05)	1.81	1.57	1.28	0.89	1.41	1.69
Mash-17	Neem	3.6b	2.3c	2.3c	1.3c	1.6c	1.0c
	Akk	4.6a	4.0b	4.6b	3.3b	2.3bc	1.6bc
	Garlic	3.6b	3.3bc	2.6c	2.0bc	2.3bc	1.3bc
	Control	5.3a	6.0a	5.0a	5.6a	7.0a	7.3a
	LSD (*P*=0.05)	0.78	1.29	1.10	1.73	1.20	0.93

In case of variety 62027 statistical analyses showed significant effects of extracts (Table III). The lowest population of whitefly before the application of plant extracts at 2% concentration was 5 per plant. The application of 2% concentration of neem, garlic and akk of all mean whitefly population was 1.3 to 4.0 whitefly per plant. Among the treatments neem 2% concentration showed remarkable reduction of whitefly population per leaf i.e., 1 as compared to control 6 whitefly/leaf. Similarly garlic and akk at 2% concentration reduced whitefly population per leaf i.e., 2.3 and 3.3 whitefly per plant.

The lowest population of whitefly before the application of plant extracts at 2% concentration was 3.6 per plant Mash-17 (Table III). By the application of 2% concentrations of neem, garlic and akk of all mean whitefly population was 1.0 to 2.0 whitefly per plant as compared to control 7.3 whitefly per plant. Among the treatments neem 2% concentration showed remarkable reduction of whitefly population per leaf i.e., 1 as compared to control 7.3 whitefly per plant. Similarly garlic and akk at 2% concentration reduced whitefly population per leaf i.e., 1.3, and 1.6 whitefly per plant.

The results clearly indicated that the initial period of 4 weeks for urdbean crop is highly critical for the development and spread of ULCV. The initiation of primary infection was considered to be originated through seed borne inoculums of the virus (Negi & Vishunavat, 2004). Field environment was highly conducive for natural spread of the disease due to high vector population and the buildup of inoculums potential of virus from the very beginning. It also indicates that there are minimum chances that any disease escape mechanism could become operative except the changes in environmental factors.

The plant extracts effect has been attributed to inhibitory principles present in the leaf extracts. Consequently the number of virus particle entering the plants might have been reduced. According to Chowdhury and Shah (1985) the ULCV can be inhibited to 30% over control but Reddy *et al.* (2006) reported 40% reduction over control. In our study it was observed that neem extract reduced 50% of disease incidence over control. As the disease incidence and whitefly population both reduced due to plant extracts spray so the mechanism of disease reduction was quite clear, because the whitefly was the major vector of ULCV. The results obtained in the present studies have indicated a great potential of plant extracts for the control of plant disease. Thus there is a need to identify and purify the active ingredients and their formulation will lead to a practical method for viral disease management.

CONCLUSION

Environmental factors showed positive interaction in case of maximum and minimum temperature but in case of relative humidity the interaction was negative. With an increase in temperature the infection rate increased and with the increase in relative humidity the infection rate decreased. The neem extract gave better results for reducing vector population as well as disease incidence.

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