The Pharma Innovation Journal 2023; 12(3): 5259-5266

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(3): 5259-5266

www.thepharmajournal.com
Received: 14-01-2023
Accepted: 04-03-2023

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Serology and infectivity Assay based Survey for detection of *Peanut bud necrosis virus* (PBNV) and *tobacco streak virus* (TSV) causing necrosis in Black gram and Green gram in Andhra Pradesh and Telangana

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Abstract
Surveys for occurrence of necrosis disease caused by PBNV and/or TSV were undertaken during Rabi 2007-08, summer 2008 and kharif 2008 in major blackgram and greengram growing areas of Andhra Pradesh. In 2008 kharif season, the village wise mean incidence of necrosis disease ranged from 51.03-78.32 per cent incidence in Medak district followed by Guntur (1.68-3.63%) and Krishna (0-1.03%) in blackgram while it ranged from 2.85- 4.94% in Medak district followed by Guntur (1.19-2.77%) and Nalgonda (0-0.27%) in greengram. In 2007-08 Rabi the disease incidence ranged from 6.15 to 10.12% and 0.90-2.13% in Medak district in blackgram and greengram, respectively. Srikakulam district ranged from 28.96-42.55% disease incidence in summer 2008 in greengram. Krishna and Nalgonda districts showed negligible or no disease incidence. Necrosis/leaf curl disease in blackgram and greengram was manifested with similar symptoms such as veinal chlorosis associated leaf curl. These veins later turned to necrotic resulting in downward curling of leaves where necrotic veins were clearly observed on the under surface of the leaf, occasionally, chlorotic spots turning to necrotic, leaf necrosis leading to death of growing bud. Early infection caused death of the plant. PBNV appeared to be more prevalent than TSV in blackgram and greengram in DAC-ELISA tests where PBNV was 95.12% and 89.47% (NBPGR-RS, Hyderabad fields) and TSV was 7.31% (NBPGR-RS, Hyderabad fields) and 7.89% (Jogipet mandal of Medak district) in blackgram and greengram, respectively while mixed infections also were recorded.

Keywords: Peanut Bud Necrosis Virus (PBNV), *Tobacco Streak Virus* (TSV), Pulses

Introduction
Pulses are very important crops in India, with annual production of about 12-15 million tones from 22-24 million hectares (Moe *et al.*, 2008) [11]. Among pulses, blackgram (urdbean - *Vigna mungo* L. Hepper) and greengram (mungbean - *Vigna radiata* L. Wilczek) are major grain legumes and are cultivated in both upland and rice fallows in India. These two crops together contribute 80 per cent to the total pulse production. Major Blackgram states are Madhya Pradesh, Maharashtra, Andhra Pradesh, Tamil Nadu and Uttar Pradesh while Orissa, Maharashtra, Andhra Pradesh, Rajasthan, Madhya Pradesh, Bihar, Karnataka and Uttar Pradesh are major greengram growing states. In Andhra Pradesh, major blackgram growing districts are Krishna, Guntur, East Godavari, Srikakulam, Medak, Adilabad, Nizamabad, Prakasam and Nellore while greengram is being grown majorly in Nalgonda, Medak, East Godavari, Guntur, Krishna, Mehaboobnagar, Khammam, Karimnagar, Warangal, Prakasam, Srikakulam and Vizianagaram districts. In Andhra Pradesh, blackgram crop occupies an area of about 373.15 thousand ha with an annual production of 225.04 thousand tons where as greengram crop occupies an area of about 96.82 thousand ha with an annual production of 32.16 thousand tons.

Blackgram and greengram have been subjected to the attack of several biotic stresses such as fungi, bacteria and viruses, affecting the productivity and among them; viral diseases became great menace and are the great yield reducers. Of several viral diseases attacking greengram and blackgram necrosis disease caused by *Peanut bud necrosis virus* (PBNV) (= *Groundnut bud necrosis virus* – GBNV) (Amin *et al*., 1985) [1] transmitted by *Thrips palmi* (Karny) in a propagative manner (Sreekanth *et al.*, 2002) [18] was considered to be a major threat, causing 40% yield loss (Nene, 1972) [12].

Recently, *Tobacco streak virus* (TSV) has also been reported to be a cause of necrosis and leaf curl symptoms on blackgram (Prasada Rao *et al.*, 2003c; Ladhalakshmi *et al.*, 2005) [14, 10] and greengram (Bhat *et al.*, 2002; Prasada Rao *et al.*, 2003c) [3, 14] paving confusion in field diagnosis to assess the disease incidence. Although both the viruses cause necrotic symptoms and are transmitted by thrips, the method of transmission and the virus vector relationship vary and hence need different approaches of management practices. It is necessary to identify and differentiate necrosis-causing viruses and their incidence on blackgram and greengram to follow the appropriate management practices. To determine the natural distribution and prevalence of necrosis and also to confirm the virus in blackgram and greengram in Andhra Pradesh, field surveys based on ELISA and infectivity assays were conducted.

Materials and Methods

Survey

Surveys were undertaken during 2007-08 *Rabi* and *kharif* seasons on blackgram and greengram in major growing districts of Andhra Pradesh. The districts for survey were chosen based on statistics of preceding year where crop concentration was more. Krishna, Guntur and Medak districts were chosen for blackgram and Nalgonda, Medak and Guntur districts for greengram. Based on the information in each district, three mandals and in each mandal, three villages, in each village, five fields were surveyed at random. In each field 1 m^2 area from each of the four corners, leaving the border rows and another 1 m^2 area at the centre were fixed to record the necrosis disease incidence. Leaves from infected plants showing leaf curl, necrosis and chlorosis were collected in polythene bags for Direct Antigen coating Enzyme Linked Immunosorbent Assay (DAC-ELISA) test and infectivity assays.

Direct Antigen Coating Enzyme Linked Immunosorbent Assay (DAC-ELISA)

Leaves showing necrotic and chlorotic symptoms collected from blackgram and greengram fields during the survey as well as from the experimental fields of NBPGR-Regional Station, Hyderabad were tested for the presence of PBNV and TSV by DAC-ELISA (Hobbs *et al.*, 1987) [7] using polyclonal antisera. ELISA was performed in 96 well polysterene microtitre plates (Coaster, Sigma, USA).

Infectivity assay

Some of the leaf samples collected from infected plants showing necrosis, leaf curl and chlorosis that were positive in DAC-ELISA, were used for infectivity assays. Young trifoliate leaves of blackgram and greengram showing clear necrotic and chlorotic symptoms from the infected plants were collected and washed thoroughly in running tap water to remove dirt and blotted dry. Necrotic portion of the leaves along with healthy portion was taken for inoculation. The inocula were prepared in pre-chilled 0.05M potassium phosphate buffer (pH 7.0) containing 2-mercaptoethanol at the rate of one ml per gram of infected leaf tissue by macerating in pre-chilled sterile mortar and pestle. The resulting pulp after maceration was squeezed between two folds of sterile muslin cloth and used for inoculation. The sap inoculations were made by conventional leaf rubbing method using carborundum powder (600 mesh) as an abrasive to provide injury on the leaf. Leaves were dusted with carborundum prior to inoculation. Seedlings were inoculated at cotyledonary stage and washed with water 5-10 min post-inoculation to remove the excess of inoculum and abrasive on the leaves, with a jet of sterile water from a squeeze bottle. Each sample was inoculated on five plants each of cowpea cv. C-152, groundnut, cv. JL-24, blackgram cv. LBG-20, greengram cv. K-851 and kept in insect-proof glasshouse and carefully observed for symptom appearance.

Results and Discussion

Field symptoms

It is difficult to distinguish symptoms of PBNV and TSV on blackgram as well as greengram under field conditions unless observed in the early stages of infection. The first disease symptoms usually appear within first fortnight after sowing on the leaf as veinal chlorosis either on the secondary or tertiary veins, which later spread to the primary vein. These veins later become necrotic resulting in downward curling of the leaves. The necrotic veins are clearly observed on the under surface of the leaf. Sometimes show chlorotic spots, which later become necrotic finally leading to leaf necrosis and ultimately killing the growing bud. Early infections cause death of the plants. Leaf curl symptoms were prominent in late infected plants. Sometimes plants were severely stunted with reduced internodal length and many axillary shoots. Most of the TSV positive plants were completely necrotic leading to death revealing the rapid necrosis than PBNV. Abnormal reduction in the size of the leaves was observed on stunted blackgram plants. Mixed infections of the both the viruses were also observed in both the crops where plants were about to die. A range of chlorotic to necrotic symptoms recorded under field conditions in blackgram (Fig.1) and greengram (Fig.2) are presented here.

Survey

Surveys for occurrence of necrosis disease caused by PBNV and/or TSV were undertaken during *Rabi* 2007-08, summer 2008 and *kharif* 2008 in major blackgram and greengram growing areas of Andhra Pradesh when the crop was 30-45 days age. A total of 270 blackgram fields (135 each in *Rabi* and *kharif*) and 315 greengram fields (135 each in *Rabi* and *Kharif* and 45 in summer), surveyed spread over 36 villages in Andhra Pradesh, revealed varied occurrence of necrosis in these crops. The mandal wise mean necrosis disease incidence ranged from 0.37-72.52% in blackgram and 0.08-34.17% in greengram among the three districts surveyed. The average of mandal wise mean disease incidence of blackgram in *kharif* season was highest in Medak (72.52%) followed by Guntur (2.56%) and Krishna (0.37%) districts (Table 1) while highest incidence in greengram was recorded in Srikakulam (34.17%) (Table 2) followed by Medak (4.18%), Guntur (2.15%) and Nalgonda (0.08%) districts (Table 3). No necrosis disease incidence on blackgram was found in Guntur and Krishna districts in *Rabi* 2007-08 while 8.84% was recorded in Medak. In greengram, 1.53% disease incidence was recorded in Medak while no disease incidence was observed in Guntur and Nalgonda districts.

The village wise mean incidence in Medak district during *Rabi* 2007-08 on blackgram ranged from 6.15 to 10.12% while it was 51.03-78.32% *kharif* 2008-09. Highest incidence was recorded in S. Itikyal village and lowest in Pulkal village of Pulkal mandal in *Rabi* while highest recorded in

Ismailkhanpet village and lowest in Gajwel village of Sangareddi mandal in *kharif*. Necrosis disease was not found in *rabi* 2007-08 in Guntur district where crop was severely affected by MYMV. The village wise mean incidence on blackgram during *kharif* 2008 showed a range of 1.68-3.63% where Karlapalem recorded highest disease incidence while lowest was in Appikatla of Bapatla mandal. The disease incidence in *rabi* was negligible due to MYMV disease occurrence as in case of Guntur district. Village wise mean incidence on blackgram in Krishna district during *kharif* 2008 showed a range of 0-1.03% where highest necrosis incidence was recorded in Rolupadi village of Thiruvuru mandal and the lowest/zero per cent disease incidence was recorded in Vavilala village of Thiruvuru mandal, Arlapadu village of Gampalagudem mandal and Kammampadu village of A.K onduru mandal (Table 1).

The village wise mean incidence on summer greengram crop in Srikakulam district during 2008 showed a range of 28.96-42.55% where highest necrosis incidence was recorded in Narasapuram village of Kotabommali mandal and the lowest was in Nandagiri village of Amudalavalasa mandal. Kancharam village of Ponduru mandal was recorded with 40.56% disease incidence (Table 2). Perusal of village wise mean incidence in Medak district, during *rabi* 2007-08 on greengram recorded with an overall average village wise ranged from 0.90-2.13%, while it was 2.85- 4.94% in *kharif* 2008. Highest per cent incidence was recorded in Talemla village of Jogipet mandal and lowest in Laxminagar village in *Rabi* 2007-08 while highest was in Laxminagar village of Pulkal mandal and lowest was in Pulkal village of Pulkal mandal in *kharif*. Necrosis disease was not found in *rabi* 2007-08 in Guntur district where crop was severely affected by MYMV. The village wise mean incidence on greengram during *kharif* 2008 showed a range of 1.19-2.77% where Chintalapudi village of Ponnur mandal was recorded with highest per cent disease incidence while lowest was in Karlapalem village of Bapatla mandal (Table 3). The village wise mean incidence on greengram in Nalgonda distrct during *kharif* 2008 showed a range of 0-0.27% where highest necrosis incidence was recorded in Chivvemla village of Chivvemla mandal and zero per cent incidence was recorded in all villages of Suryapet mandal, Bebigudem village of Chivvemla mandal and Appajipet and Rayanigudem villages of Nalgonda madal. No disease was found in Suryapet mandal, while mean incidence of 0.08% was found in Nalgonda (Table 20). Necrosis disease was not found in *rabi* 2007-08 in Nalgonda district where crop was severely affected by MYMV.

During the survey across the locations it was found that yellow mosaic disease caused by *Mungbean yellow mosaic virus* (MYMV) was more prevalent in Guntur, Krishna and Nalgonda districts where complete crop was ruined by MYMV in *Rabi* 2007-08 where the farmer had to plough the field with the standing crop and the same trends were followed in *Kharif* 2008-09 with a little lesser severity to that of *Rabi*. Whereas in Medak district, 30-40% incidence of MYMV was observed in blackgram along with higher necrosis disease incidence, where the crop was looking green with apical necrosis and ended up with no flowering there by any podding, indicating late infection. Negligible or no MYMV incidence was found in summer greengram crop of Srikakulam district whereas 40-100% MYMV incidence was found in the other districts (Nalgonda, Medak and Guntur) surveyed for greengram.

DAC-ELISA

The blackgram and greengram samples showing symptoms ranging from chlorosis to necrosis, collected from different districts during the survey as well as NBPGR-RS experimental fields subjected to DAC-ELISA showed varied serological affinities (Table 4). Out of 374 blackgram samples tested, 287 samples were positive to PBNV while 17 samples were positive to TSV. Whereas in greengram, 395 samples were positive PBNV while seven samples were positive to TSV out of 480 samples. Out of 244 blackgram samples collected from Medak district 183 samples were positive to PBNV and 11 samples were positive to TSV while 73 greengram samples were positive to PBNV and three were positive to TSV from a total of 96 greengram samples collected from Medak. Among 184 greengram samples collected from Srikakulam district, 144 samples were positive to PBNV while no sample was positive to TSV. Of 82 blackgram and 76 greengram samples collected from experimental fields of NBPGR-RS, Hyderabad 78 and 68 samples were positive to PBNV while six and four samples were positive to TSV, respectively. Mixed infections of PBNV and TSV also were recorded in both, blackgram and greengram.

Infectivity assay

Three samples of blackgram from each district *viz.*, Medak, Guntur, Krishna and experimental fields of NBPGR-RS, Hyderabad and three samples of greengram from Srikakulam, Medak, Guntur and experimental fields of NBPGR-RS, Hyderabad, tested positive to PBNV in DAC-ELISA and three samples of blackgram and greengram positive to TSV from Medak and experimental fields of NBPGR-RS, Hyderabad, were chosen for infectivity assays. Infectivity assay of all the blackgram and greengram samples tested positive to PBNV and TSV in DAC-ELISA test expressed symptoms, typical of PBNV and TSV, respectively, on indicator hosts *viz.* cowpea cv. C-152 (Fig. 3) and groundnut cv. JL-24 (Fig. 4) as well as blackgram cv. LBG-20 (Fig.5) and greengram cv. K-851 (Fig. 6).

Further, both the blackgram and greengram crops were recorded with negligible to high levels of leaf curl/necrosis incidence in different districts during the survey. Occurrence of PBNV on groundnut from negligible to high levels was reported in different districts of Andhra Pradesh (Amin, 1985) [1]. Higher incidence of necrosis in Medak and Srikakulam districts may be due to early and increased activity of vectors due to favorable weather conditions and more number of plants was infected as it is an established fact that susceptibility of the plants will be high at early stages of the crop growth. The rapid spread of the disease could be attributed to the movement of viruliferous thrips both by natural and artificial means as opined by Cho *et al*. (1989) [4]. Lower/no disease incidence in Guntur, Krishna and Nalgonda districts was perhaps due to absence of thrip vector for transmission and/or incidence of whitefly transmitted *Mungbean yellow mosaic virus* (MYMV) in the most severe form which could have ruined the entire crop. The outbreak of whitefly population may be due to indiscriminate use of pesticides either alone and/or as mixtures which is a common practice in Guntur, Krishna and parts of Nalgonda adjacent to Krishna district, might have resulted in the development of resistance against and there by spreading the disease under field conditions. The rapid multiplication of whitefly population might have suppressed the multiplication of thrips

thereby reducing PBNV incidence.

Necrosis caused by viruses is posing a serious threat to *Vigna* species in Andhra Pradesh. TSV was identified on several crops and weeds in the peninsular India (Prasada Rao *et al*., 2000, 2003a, 2003b, 2003c; Ravi *et al*., 2001; Jain *et al*., 2005; Arunkumar, 2006) [14-16, 17, 8, 2]. In the present study TSV has been identified as one of the viruses in necrosis affected plants of blackgram and greengram. TSV was detected in mungbean from Andhra Pradesh (Prasada Rao, 2003c) [14] and Tamil Nadu (Bhat *et al*., 2002) [3] and urdbean from Andhra Pradesh (Prasada Rao, 2003c) [14] and from Tamil Nadu (Ladhalakshmi *et al*., 2005) by ELISA. Serological tests conducted on the necrosis samples collected from several fields, indicated the presence of PBNV in more samples than TSV. Mixed infections of PBNV and TSV also were recorded in both, blackgram and greengram. Under field conditions it was observed that TSV infected blackgram or greengram have succumbed due to rapid necrosis compared to PBNV. Reduction in the size of the leaves with mosaic mottling was often observed on stunted blackgram plants when compared to greengram. No significant differences were observed in the symptoms observed under field conditions produced by PBNV and TSV. The appearance of TSV in blackgram and greengram under natural conditions is a warning to g*et al*ert as the virus can result in no yield with infection at both early and late stages of the crops. Host range of TSV is wide, infecting more than 200 plant species belonging to 30 dicotyledonous and monocotyledonous plant families (Fulton, 1985; EPPO, 2005) [6, 5]. Appropriate management practices can be recommended based on the occurrence of necrosis caused by PBNV and/ or TSV for controlling the corresponding vectors along with the cultural practices like destroying the virus sources, installing the barrier crops, maintaining optimum plant population (Kumar *et al*., 2008) [8] with an integrated management strategy with a focus on virus-vector relationships.

Table 1: Incidence of necrosis disease of blackgram in different mandals of Medak, Guntur and Krishna districts during *rabi*, 2007-08 and *kharif*, 2008.

Name of District	Name of Mandal	Name of Village	Rabi			Kharif		
			No. of fields	PDI*		No. of fields	PDI*	
				Range	Average		Range	Average
Medak	Pulkal	Pulkal	5	6.42-11.01	6.15	5	68.50-77.96	72.44
		S.Itikyal	5	8.21-11.15	10.12	5	66.35-87.50	75.27
		Laxminagar	5	6.79-9.54	7.67	5	61.53-91.48	73.39
	Sangareddi	Sadasivapet	5	8.46-10.82	9.81	5	70.17-82.24	76.57
		Ismailkhanpet	5	8.44-11.14	9.88	5	69.49-84.31	78.32
		Gajwel	5	6.10-9.58	8.40	5	61.90-72.30	51.03
	Jogipet	Dakuru	5	7.19-10.94	9.48	5	63.26-86.27	73.81
		Rollapad	5	7.88-11.24	9.27	5	61.61-82.97	75.70
		Talelma	5	6.94-10.68	8.84	5	70.90-80.99	76.19
Guntur	Bapatla	Appikatla	5	Nil	Nil	5	0-2.04	1.68
		Etheru	5	Nil	Nil	5	0-4.65	2.04
		Karlapalem	5	Nil	Nil	5	1.61-5.88	3.63
	Ponnur	Chintalapudi	5	Nil	Nil	5	0-3.96	2.03
		Munipalle	5	Nil	Nil	5	1.63-4.39	2.95
		Mamillapallli	5	Nil	Nil	5	0-4.54	2.56
	Chebrolu	Budampadu	5	Nil	Nil	5	0-5.55	2.16
		Narakoduru	5	Nil	Nil	5	0-5.97	3.29
		Patareddypalem	5	Nil	Nil	5	0-4.93	2.72
Krishna	Thiruvuru	Vavilala	5	Nil	Nil	5	0	0
		Rolupadi	5	Nil	Nil	5	0-2.56	1.03
		Mallela	5	Nil	Nil	5	0-1.61	0.63
	Gampalagudem	Penugolanu	5	Nil	Nil	5	0-1.51	0.30
		Ootukuru	5	Nil	Nil	5	0-1.38	0.53
		Arlapadu	5	Nil	Nil	5	0	0
	A. Konduru	Kammampadu	5	Nil	Nil	5	0	0
		Cheemalapadu	5	Nil	Nil	5	0-1.16	0.44
		Madhavavaram	5	Nil	Nil	5	0-1.19	0.45

*PDI – Per cent disease index

Table 2. Incidence of necrosis disease on greengram in different mandals of Srikakulam district during *summer*, 2008-09

Name of Mandal	Name of Village	*Rabi*		
		No. of fields surveyed	PDI*	
			Range	Average
Amudalavalasa	Nandagiripeta	5	24.48-32.67	28.96
	Cheemalavalasa	5	29.16-35.41	31.52
	Chintada	5	21.97-45.45	32.17
Ponduru	Kancharam	5	35.48-47.50	40.56
	Nakkapeta	5	30.48-35.13	33.55
	Lolugu	5	29.16-35.13	31.61
Kotabommali	Raagulapadu	5	28.57-37.17	33.81
	Gottapadu	5	28.73-36.11	32.33
	Narasapuram	5	41.17-43.75	42.55

*PDI – Per cent disease index

Table 3: Incidence of necrosis disease of greengram in different mandals of Medak, Guntur and Krishna districts during *Rabi*, 2007-08 and *kharif*, 2008.

Name of District	Name of Mandal	Name of Village	Rabi			Kharif		
			No. of fields	PDI*		No. of fields	PDI*	
				Range	Average		Range	Average
Medak	Pulkal	Pulkal	5	0-2.12	1.03	5	2.85-5.31	2.85
		S.Itikyal	5	0-2.51	1.41	5	2.04-6.52	4.00
		Laxminagar	5	0-1.54	0.90	5	3.03-7.44	4.94
	Sangareddi	Sadasivapet	5	0-3.14	1.82	5	4.25-5.94	4.88
		Ismailkhanpet	5	0-2.64	1.79	5	3.92-5.76	4.82
		Gajwel	5	0-342	1.91	5	2.70-5.21	3.67
	Jogipet	Dakuru	5	0-2.64	1.19	5	3.12-5.88	4.29
		Rollapad	5	0-2.58	1.65	5	2.88-5.10	4.03
		Talelma	5	0-2.68	2.13	5	3.06-5.20	4.24
Guntur	Bapatla	Appikatla	5	Nil	Nil	5	0-5.26	2.60
		Etheru	5	Nil	Nil	5	0-4.16	2.11
		Karlapalem	5	Nil	Nil	5	0-2.62	1.19
	Ponnur	Chintalapudi	5	Nil	Nil	5	0-6.89	2.77
		Munipalle	5	Nil	Nil	5	0-6.45	2.47
		Mamillapallli	5	Nil	Nil	5	0-5.55	1.92
	Chebrolu	Budampadu	5	Nil	Nil	5	0-5.63	2.21
		Narakoduru	5	Nil	Nil	5	0-4.34	2.38
		Patareddypalem	5	Nil	Nil	5	0-4.22	1.79
Nalgonda	Suryapet	Balemla	5	Nil	Nil	5	0	0
		Dasaiguda	5	Nil	Nil	5	0	0
		Kesaram	5	Nil	Nil	5	0	0
	Chivvemla	Chivvemla	5	Nil	Nil	5	0-1.38	0.27
		Bebigudem	5	Nil	Nil	5	0	0
		B. Chanupatla	5	Nil	Nil	5	0-1.28	0.26
	Nalgonda	Appajipet	5	Nil	Nil	5	0	0
		Pillalamarri	5	Nil	Nil	5	0-1.21	0.24
		Rayanigudem	5	Nil	Nil	5	0	0
*PDI – Per cent disease index								

Table 4: Serological affinities of blackgram and greengram samples collected in the survey carried out in different districts and experimental fields of NBPGR-RS, Hyderabad.

Crop	District	Mandal	DAC-ELISA			
			PBNV		TSV	
			No. infected/ tested	Per cent incidence	No. infected/tested	Per cent incidence
Blackgram	Medak	Pulkal	62/84	73.80	5/84	5.95
		Sangareddi	53/78	67.94	3/78	3.84
		Jogipet	68/82	82.92	3/82	3.65
	Guntur	Bapatla	10/20	50	0/20	0
		Ponnur	5/12	41.66	0/12	0
		Chebrolu	8/14	57.14	0/14	0
	Krishna	Thiruvur	1/2	50.00	0/2	0
		Gampalagudem	1/1	100	0/1	0
		A. Konduru	1/1	100	0/1	0
	Hyderabad	NBPGR-RS	*78/82	95.12	*6/82	7.31
Greengram	Srikakulam	Amudalavalasa	45/64	70.31	0/64	0
		Ponduru	51/64	79.68	0/64	0
		Kotabommali	48/56	85.71	0/56	0
	Medak	Pulkal	27/32	84.37	0/32	0
		Sangareddi	22/26	84.61	0/26	0
		Jogipet	24/38	63.15	3/38	7.89
	Guntur	Bapatla	12/15	80.00	0/15	0
		Ponnur	10/14	71.42	0/14	0
		Chebrolu	9/12	75.00	0/12	0
	Nalgonda	-	1/1	100	0/1	0
	Hyderabad	NBPGR-RS	68/76	89.47	4/76	5.26

*Mixed infection of PBNV and TSV was recorded.

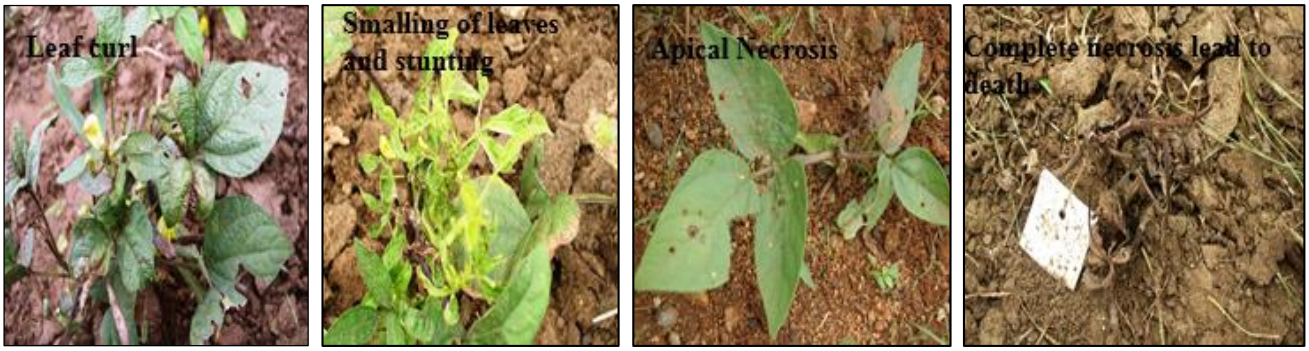

Fig 1: Symptoms produced by blackgram due to necrosis/leaf curl disease under field conditions

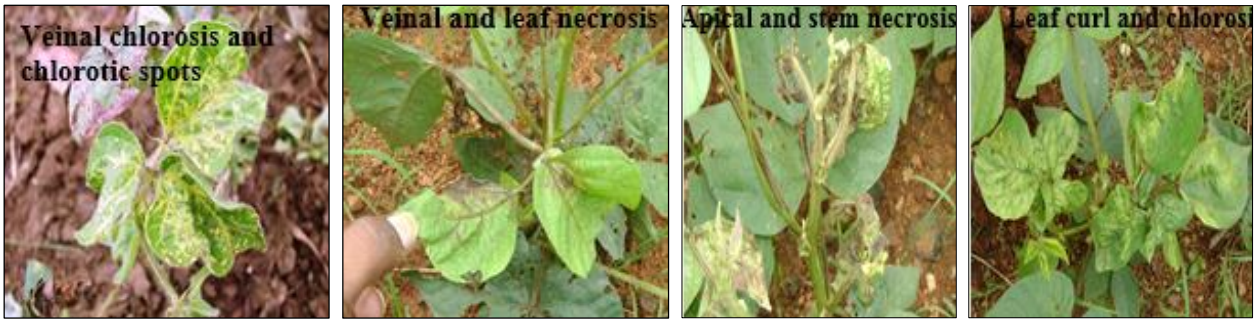

Fig 2: Symptoms produced by greengram due to necrosis/leaf curl disease under field conditions

Fig 3: Symptoms produced by symptomatic blackgram and greengram samples tested positive in DAC ELISA, collected during survey, on *Vigna unguiculata* cv. C-152

Fig 4: Symptoms produced by symptomatic blackgram and greengram samples tested positive in DAC ELISA, collected during survey, on *Arachis hypogaea* cv. JL-24

Fig 5: Symptoms produced by symptomatic blackgram and greengram samples tested positive in DAC ELISA, collected during survey, on *Vigna mungo* cv. LBG-20

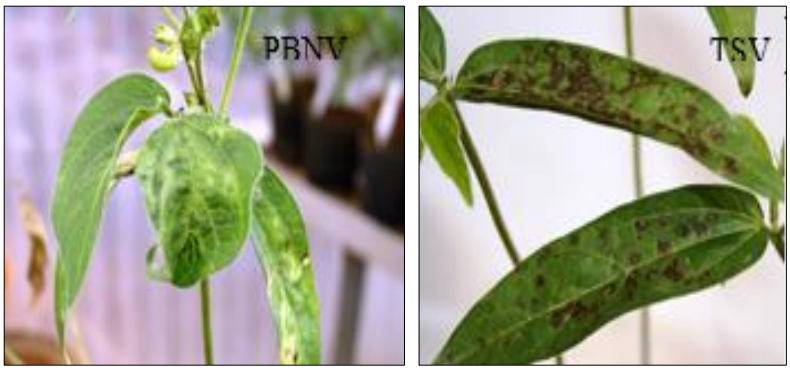

Fig 6: Symptoms produced by symptomatic blackgram and greengram samples tested positive in DAC ELISA, collected during survey, on *Vigna radiata* cv. K-851

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