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Full Length Research Paper

Establishing inoculum threshold levels for *Bean common mosaic virus* strain blackeye cowpea mosaic infection in cowpea seed

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Bean common mosaic virus **strain blackeye cowpea mosaic (BCMV-BICM) is an important seed-borne virus infecting cowpea and is transmitted both by seeds and aphids. Infected cowpea seeds can act as primary source of inoculum for disease epidemics. Four field experiments were conducted during 2003 - 2006 to assess the role of different amounts of seed-borne inoculum in the dissemination of BCMV-BICM virus in cowpea under field conditions. The identity of BCMV-BICM was confirmed by ELISA and IC-RT-PCR. Plants infected at an early growth stage appeared to serve as the primary source for subsequent virus spread by aphids. The mean disease incidence during four field experiments reached 88-93% in plots sown with 10% infected seed. The disease incidence in plots sown with 5% infected seed recorded 46-63% while for plants raised from 3 and 2% BCMV-BICM seed infection, disease incidence reached 32-49% and 17-23%, respectively. Mean yield losses in terms of seed yield per plant from four field experiments were 74 and 54% for initial seed infection of 10 and 5%, respectively. Seed infection of 2% BCMV-BICM incidence resulted in an average of 24% mean seed yield loss/plant^{-1}. The infection appeared to decrease the seed yield in terms of number and size. The BCMV incidence in harvested seed ranged from 0.3 - 19% for the different levels of initial seed infection. The field experiments demonstrated that sowing > 1% BCMV-BICM infected seed can lead to significant losses in grain yield, while the spread of BCMV-BICM infection resulting from sowing 1% infected seed did not significantly decrease seed yield. The role of establishing damage or inoculum thresholds from BCMV-BICM seed-borne infections is discussed in the present study.**

Key words: Cowpea, potyvirus, seed-borne virus, thresholds, yield loss.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is an important and widely adapted nutritive legume grown for grain and fodder worldwide. The majority of world production takes place in Africa where its importance derives from its relative tolerance to poor, dry soil conditions and extensive application. It is estimated that in Africa, more than 200 million people consume cowpea on a daily basis and rely on it as a cheap protein source. Cowpea, besides providing a variety of foods, is a source of income and livestock feed (Popelka et al., 2004).

Viral diseases are significantly contributing to the reduced yield of cowpea in Asia, Africa and Latin America. Worldwide, more than 20 viruses have been identified as naturally infecting cowpea (Mali and Thottappilly, 1986) and many are transmitted through seed (Hampton, 1983). Among the seed-borne potyviruses infecting cowpea, *Bean common mosaic virus* strain blackeye cowpea mosaic (BCMV-BICM) is economically important as it causes crop losses as high as 40% under field conditions (Zettler and Evans, 1972). The seed transmission of particular importance since germplasms are primarily conserved and exchanged through seeds both nationally and internationally.

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The seed-borne inoculum acts as serious contributing factor to disease epidemics. Since viral diseases cannot be controlled by any chemicals, use of healthy seed lots is an alternative and feasible strategy for controlling seed-borne viral diseases necessitating an effective seed health certification scheme, establishing tolerance levels, as well simple and reliable seed testing procedures for routine seed health testing.

Establishing inoculum threshold levels for seed-borne diseases is difficult. It is influenced by many factors, such as macro- and micro-environment, cultural practices, agricultural systems, locations, certification requirements and class of seed, i.e., breeder, foundation, or certified seed. For example, Lettuce mosaic virus can be controlled by using seed certified to have 0 infected seeds in 30,000 in California and 0 in 2,000 in the Netherlands (Grogan 1980).

Attempts have been made in the past to establish tolerance limits to some of the seed-borne viruses; Lettuce mosaic virus (LMV) in lettuce (Grogan 1983), Cucumber mosaic virus (CMV) in narrow leafed lupin (Bywe et al. 1995), subterranean clover *(Lupinus subterraneus)* (Jones 1998 and 2000) and *Alfalfa mosaic virus* (AMV) in Burr medic (*Medicago polymorpha*) (Jones and Nicholas 1998). Limited information is available for other economically important seed-borne viruses viz., *Bean yellow mosaic virus* (BYMV; Kaiser 1973), *Broad bean stain virus or Broad bean true mosaic virus* in faba bean (Jones 1980), BYMV in yellow lupin (Corbett and Edwardson 1957), *Peanut mottle virus* in groundnut (Adams and Kuhn 1977), *Cucumber mosaic virus* in lentil (Fletcher et al. 1999) and *Pea seed borne mosaic virus* in pea (Masmoudi et al., 1994) with regard to contribution of seed-borne inoculum to the disease spread and its impact on yield.

Seed-borne viruses that are also transmitted by aphids are of particular concern when infected seed serves as the primary source of inoculum. Some of the most economically important plant virus diseases are fall within this category. This appears to be the case for many poty-viruses (Stace-Smith and Hamilton, 1988).

Information on germination of infected seeds and survival of resulting plants, virus disease progress during the growing season, magnitude of yield loss and amount of infection in harvested seed in replicated field experiments is required to establish acceptable threshold levels of seed-borne infection (Thackray et al. 1998). Infection of one-week-old cowpea plants resulted in a higher proportion of seed infection than in those infected later (Sumana and Murthy, 1992; Nain et al., 1994). Puttaraju et al. (2004a) observed that early infection to cowpea plants leading to severe yield losses and many of the seed-lings showed stunted growth. Yield in plants, infected after 50 days did not differ much from those of healthy plants. BCMV-BICM infection was higher in seeds of plant infected within 10 days than in those infected at a later stage. The seed yield per plant was lower in early-infected plants than late infected ones.

This paper presents the results on the contributionof different levels of BCMV-BICM seed infection in disease development and yield in cowpea, based on which threshold values could be established in seed certification.

MATERIALS AND METHODS

Detection and identification of *Bean common mosaic virus* strain blackeye cowpea mosaic

Seed samples of cowpea cultivar C-152 from different seed lots were subjected to growing-on tests to determine the levels of seed-borne infection. The identity of the virus was serologically confirmed by DAC-ELISA according to Hobbs et al. (1987) employing polyclonal antibodies raised in rabbits against Bean common *mosaic virus* strain blackeye cowpea mosaic (Puttaraju et al., 2004b). BCMV-BICM infected leaf samples dried over CaCl2 from the Danish Seed Health Centre, University of Copenhagen, Denmark was used as positive control.

For molecular confirmation the samples were subjected to immunocapture reverse transcription polymerase chain reaction (IC-RT-PCR). Immunocapture was performed by PAb raised against BCMV-BICM (Puttaraju et al., 2004b). The primers specific to the sequence located at the 3' end part of the genome of Potyviruses,'oligodTBamHI'5'gCgGgATCCTTTTTTTTTTTTTTTTVN-3' and 'WCIENGTS'5'-TgAggATCCTggT gYATHgARAAYgg-3' (Langeveld et al., 1991) were employed for reverse transcription and polymerase chain reaction.

Reverse transcription of the captured RNA was performed in a 20 µl reaction mixture containing 1 µl downstream primer BAM HI (100 µM), 4 µl 5X reaction buffer, 2 µl DTT (0.1 M), 2 µl dNTP (10 mM each), 0.25 µl RNase inhibitor (10 u/µl), 0.5 µl MMLV reverse transcriptase (20 u/µl), and 10 µl sterile, distilled water. Each reaction vial with 20 µl reaction mixture was placed in a gradient thermocycler (Eppendorf, Germany) at 37 oC for 10 min followed by 42 oC for 30-40 minutes. Amplification of viral cDNA was accomplished by adding 80 µl of a reaction mixture containing 10 µl 10X reaction buffer, 4 µl MgCl2 (50 mM), 1µl upstream primer "WCIENGTS" (100 µM), 0.5 µl Taq DNA polymerase (5 u/ml), and 64.5 µl of sterile, distilled water. The cycling parameters were an initial denaturation at 95 oC for 3 min followed by 35 cycles of denaturation at 94 oC for 60 s, annealing at 53 oC for 90 s, extension at 72 oC for 90 s and final extension at 72 oC for 10 min.

The resulting PCR products were randomly selected and cloned in the pGEM vector using a TA cloning kit (GeNei, India) according to manufacturer's instructions. The ~700 bp band was amplified, eluted from the gel and ligated onto pGEM easy vector and transformed into *E. coli* JM 109 cells by heat shock. DNA sequencing of the cloned PCR product was done for confirmation of the identity of BCMV-BICM.

Seed and inoculation

For each experiment, a seed stock of cowpea cv. C-152 free from BCMV-BICM infection was used throughout the experiment. Based on growing-on test results, cowpea seed samples with different levels of BCMV-BICM infection were obtained by mixing the infected seed lots with healthy seeds in order to obtain seed lots with different levels of infection.

Experimental design and layout

A series of field experiments were conducted during monsoon (kharif) season in Mysore, Karnataka, India over a period of four

years (2003-2006). Seeds were sown during mid-June for all four years/-experiments as the majority of significant disease problems in cowpea production are reported in kharif crops. The zone receives 1064 mm of average rainfall per annum and has an average day temperature ranging from 29-34 oC and 87% humidity during April-September. All the above trials were conducted in red loamy soil (pH 6.7). During the crop growing period for all four years, the crop received rain in 20-70 days, with a maximum and minimum relative humidity in the range 50-95%. The field was maintained according to the cowpea growing conditions, conducting weeding at regular intervals and as practiced in cowpea production. The details of the experiments are presented in figure 1.

For each experiment, cowpea seeds were sown in plots of 5 X 5 m in four replicates, except for the 0.05% seed infection plot, for which one replicate of 10 X 10 m was used, in order to obtain accommodate the number of seedlings required for such low infection levels (1 infected seedling out of 2000 seedlings). Control plots were kept away from infected plots (Figure 1). Plots were sown with seed samples having 10, 5, 3, 2, 1, 0.75, 0.5, 0.05 and 0% BCMV-BlCM infection. Field layout was designed with the highest level of infection at the end of wind direction, beginning with control plots. Each plot was separated by maize crop (non host for BCMV-BlCM) buffers to minimise plot-to-plot virus spread. Normal seeding rate was followed with a row-to-row distance of 50 cm and plant-to-plant distance of 10 cm, except for the 0.05% infected plot. This was also done in order to avoid high plant density and early canopy development that can cause seed-infected plants to be shaded out before aphids arrive.

Records and assessment of BCMV-BICM infection

The BCMV-BlCM infected plants were recorded at 10-days interval based on symptoms to study disease progress and percentage infection was tabulated. The cumulative disease incidence was computed. Representative cowpea leaf samples (10 plants row^{-1}) for confirmation of BCMV-BlCM infection were collected from replicate plots of different levels of BCMV-BlCM infection. A small (1 - 2 mm^2) piece of leaf tissue was employed as antigen in DAC-ELISA. Representative leaf samples collected from plants at different levels of seed-borne inoculum were macerated immediately in liquid nitrogen and used as antigen in IC-RT-PCR.

Trapping of aphids

Aphids were trapped using cylindrical traps consisting of plastic jars (20 cm height x 12.5 cm diameter) covered with yellow sticky paper mounted above the crop on wooden stakes. Five traps were placed per plot, at each of the four corners and one at the centre. Traps were also placed at a distance of 10 meters from the test plot to check the movement of aphids into the test plots.

Grain yield

The plants were tagged when the first sign of BCMV-BlCM infection was evident. The pods from plants were harvested plant-wise at maturity. The effect of BCMV-BlCM infection on yield was determined in terms of average number of pods $plant^{-1}$, average number of seeds pod^{-1}, average plant height, pod length and 100 seed weight (Figure 6).

BCMV-BICM infection in harvested seed

On maturity, the seeds were pooled replicate-wise and subjected to growing-on tests to determine the infection levels. From each treatment, 400 seeds (100 seeds x 4 replicates) were planted in growing -on tests to determine percentage of virus transmission to seedlings. The seedlings were monitored for the appearance of symptoms caused by seed-borne BCMV-BlCM and their presence was confirmed by DAC-ELISA and IC-RT-PCR as described earlier.

Statistical analysis

The data were subjected to regression analysis using the SSPS package. The disease incidence of BCMV-BlCM in the field, mean number of seeds/pod, size of pods, height of plants and seed weight of 100 seeds, were calculated. Data for symptoms were recorded in the maps, with one square cell representing 100 plants; The number of infected plants from the primary infection foci of was calculated; the total number of plants with symptoms in the plots with the different levels of infection was presented in percentage with the mean value from the four experiments.

RESULTS

Detection and identification of *Bean common mosaic virus* strain blackeye cowpea mosaic

A high incidence of BCMV-BlCM ranging from 0.3-12% was detected in the cowpea seed lots of the cv. C-152 from growing-on tests. Identification of the presence of the potyvirus was initially based on disease symptoms which became visible after 10 days. The infected seedlings displayed mosaic symptoms on primary leaves followed by mosaic, vein clearing, mottling and vein banding symptoms caused by BCMV-BlCM infection on trifoliate leaves (Figure 2a & b). The polyclonal antibodies employed in DAC-ELISA confirmed the virus present in the infected cowpea leaf samples as BCMV-BlCM. High ELISA values were recorded in comparison to the negative control and on par values with positive control (data not shown). As potyviruses share high serological homology, the identity of BCMV was further confirmed by IC-RT-PCR analysis.

The primer pair 'oligo dTBamHI' and 'WCIENGTS' amplified the coat protein coding region of ~700 bp product from all the leaf samples subjected to IC-RT-PCR (Figure 3). The ~700 DNA fragments, amplified by IC-RT-PCR from samples were cloned and sequenced. In the BLAST analysis, the nucleotide sequence of the part of the coat protein of the BCMV-BlCM matched with *Bean common mosaic potyvirus* strain blackeye cowpea mosaic (BCMV-BlCM). Comparisons with other sequenc-ed potyviruses recorded highest similarity with *Bean common mosaic potyvirus* strain *blackeye cowpea mosaic* (BCMV-BlCM) with accession No. AF395678.

Record and assessment of BCMV-BICM infection

The extent of spread was related to the amount of infection in the seed. The BCMV-BlCM disease spread faster in field plots sown with 10, 5 and 3% infected cowpea seeds, than in plots sown with 2, 1, 0.75, 0.5, and 0.05% infected seeds (Figure 4). The spread of BCMV-BlCM across the cereal buffers into the control plots was evident only during the first field trial, merely at the end of the growing season. as noticed during harvesting of seeds. In the four replicate

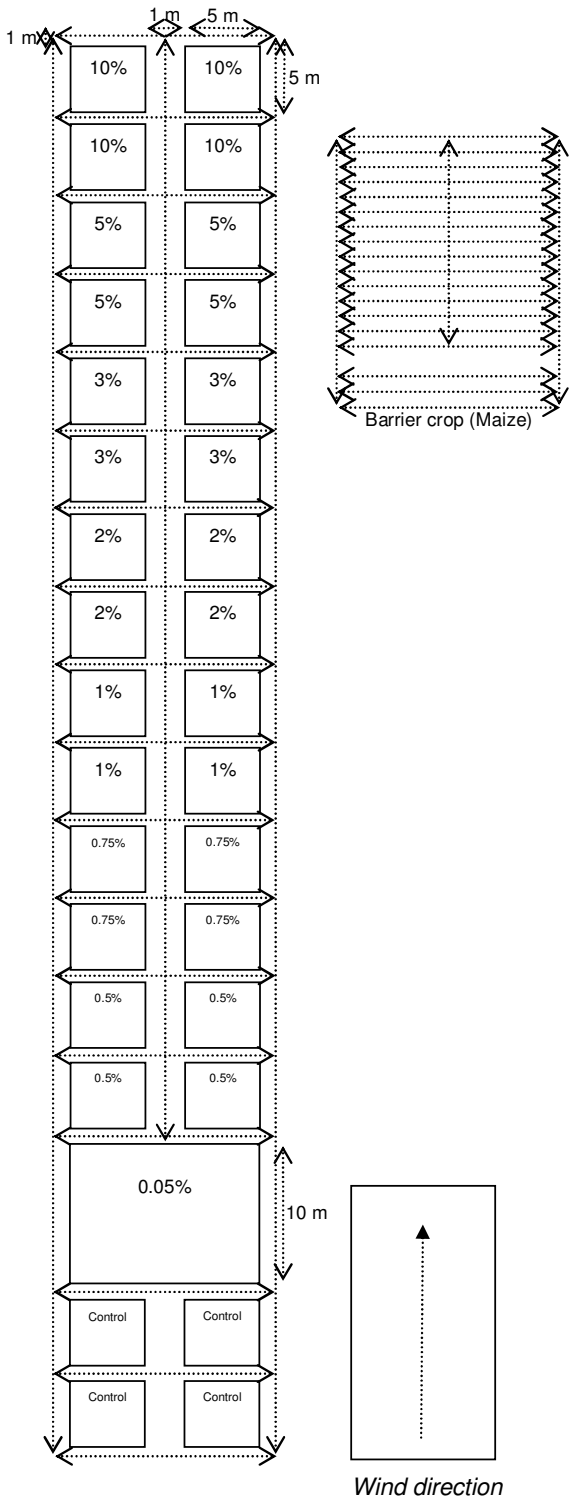

Figure 1. Details of a series of experiments conducted during 2003-2006 examining the spread of BCMV-B1CM in plots. Test rows were sown with seed infection of 10, 5, 3, 2, 1, 0.75, 0.5 and 0.05%, respectively. Control plots were sown first initiating with wind direction. The plot sizes were 5 x 5 m, except for 0.05% seed infection, plot size was 10 x 10 m. The distance between each plot was 1 m along with barrier crop (maize)

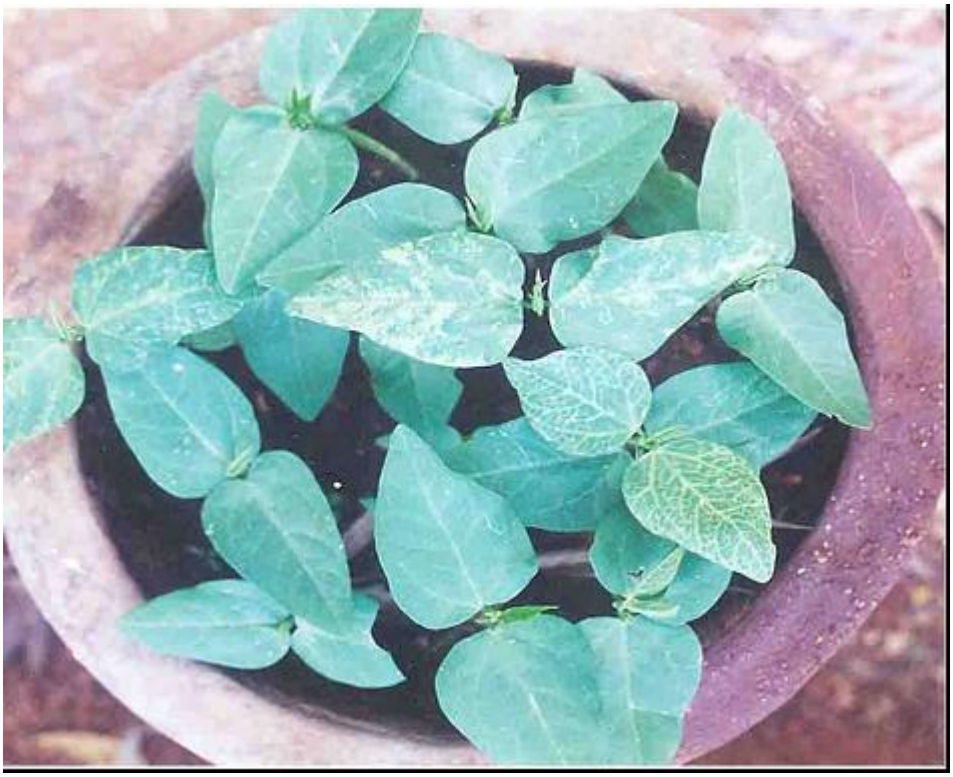

Figure 2a. Detection of BCMV-BlCM by growing-on test. Two-leaf stage seedlings showing mosaic and banding symptoms.

Figure 2b. Cowpea seedlings displaying mosaic, mottling, vein-clearing, vein banding symptoms due to BCMV-BlCM infection in growing-on test.

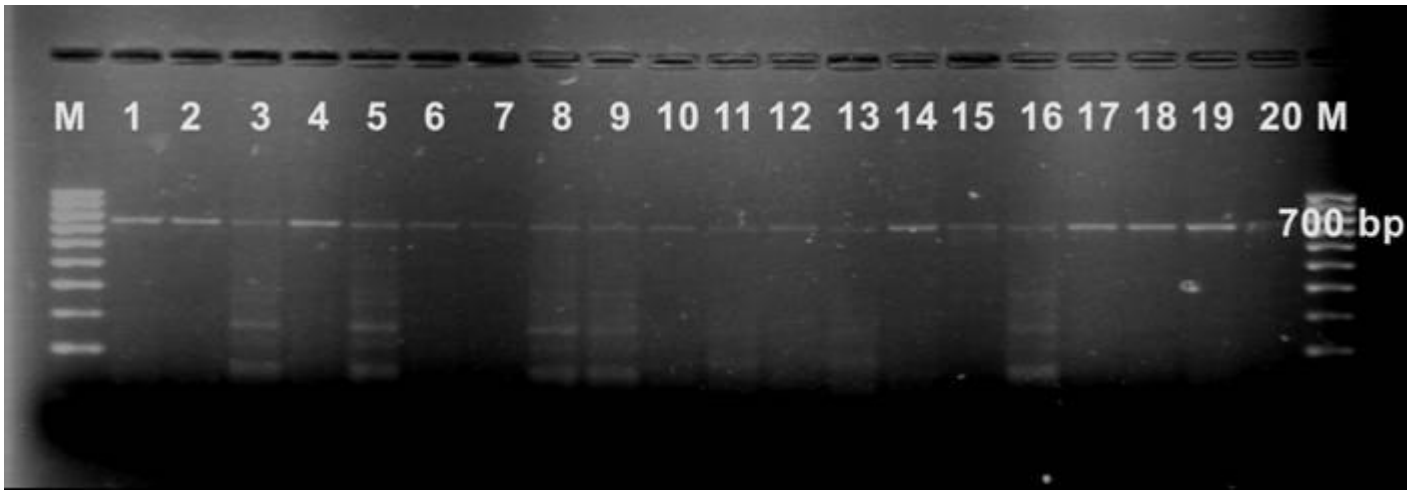

Figure 3. Detection of BCMV-BICM in cowpea by IC-RT-PCR. Lanes 1 - 20 test samples; M-Molecular weight marker 100 bp DNA ladder.

plots sown with 10% infected seed, the BCMV-BICM disease incidence reached up to 88 -93%. The final score of BCMV-BICM disease incidence for plots sown with 5% infected seed was 46-63%. In the case of 3, 2 and 1% infected seed plots, cumulative BCMV-BICM disease incidence reached 32-49%, 17-23% and 8-12%, respectively in the four field trials (Table 1). Maximum spread was evident between 20-40 days after sowing. The disease incidence at crop maturity was 7- 9%, 5 - 7% and 0.5-1.3%, for 0.75, 0.5 and 0.05% seed infection respectively. The spread was slow in these plots (Table 1). The initial spread of virus (from 10-20 days after sowing) was restricted just about the time of the primary infection foci (Figure 4).

Virus infection was successfully monitored and assays on field collected leaf samples confirmed the diagnosis utility of DAC-ELISA and IC-RT-PCR tests. The affected leaves were ELISA-positive, confirming the presence of BCMV-BICM, while non-affected were ELISA-negative. The results from ELISA tests with mean absorbance values that ranging from 0.258-0.5268 are presented in Table 2. The leaf samples collected randomly from replicated plots with different levels of initial seed-borne BCMV-BICM infection were positive in IC-RT-PCR tests with the expected final amplified product of ~700 bp of the coat protein gene from all leaf samples showing virus symptoms.

Trapping of aphids

Aphids namely, *Aphis craccivora* and *Myzus persicae* were observed in and around field sown cowpea plots. Aphids were observed on weed hosts and were trapped on aphid traps in air. Aphids were observed in cowpea plots from the first week after sowing. Aphids colonised cowpea plants and *A. craccivora* and *M. persicae* were the most abundant species.

Grain yield

In plots sown with 10 and 5% infected seed, the grain yield decreased significantly by 74% and 54% respectively in comparison to control plants. The replicate plots sown with 3 and 2% BCMV-BICM seed infection resulted in an average yield loss of 36 and 24% respectively in the four field experiments in comparison to the control plots. The average seed yield/plant-1 in plots sown with low levels of seed infection (1, 0.75, 0.5 and 0.05%) was not significantly different when compared to control plot plants (Figure 7f).At the end of the growing season, the average plant height was considerably reduced to 15.42 cm in plots sown with 10% seed infection and to 18.08 cm in plots sown with 5% seed-borne BCMV-BICM in comparison to 32.80 cm in control plants. The average plant height was not significantly reduced in plots sown with 0.05, 0.5, 0.75 and 1% seed-borne inoculum in comparison to control plants (Figure 5a).

The average number of pods per plant was reduced from 22.30 in control plants to 6.78 and 11.36 in replicate plots sown with 10 and 5% BCMV-BICM seed infection respectively. In plots sown with 3 and 2% BCMV-BICM seed infection, the average number of pods per plant was 14.03 and 15.22 respectively (Figure 5b).

BCMV-BICM seed infections

The seeds harvested from plots sown with 0.5% infection showed the lowest BCMV infection. Maximum infection was found in seeds harvested from plots sown with a 10% BCMV infection (Table 3).

DISCUSSION

Most potyviruses infecting large-seeded legumes are

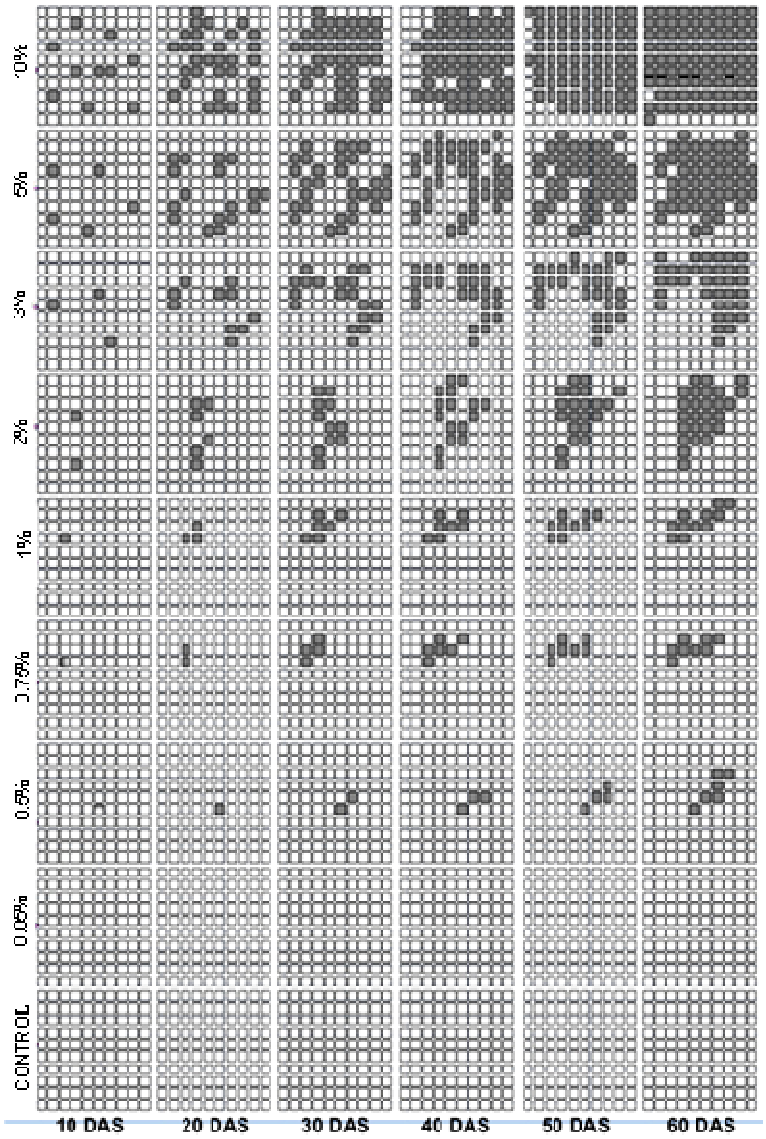

Figure 4. Final maps for experiments showing the distribution of BCMV-BICM (shaded) in blocks representing plants with symptoms from initial seed infection of 0.05 - 10% levels for 10 - 60 DAS. Each square box represents one plant with average of 100 plants considered for analysis. The figure indicates average movement of virus in four individual experiments at the end of growing season. DAS: Days after sowing.

economically important because they are transmitted through seed and spread naturally by aphids (Edwardson and Christie, 1991). *Bean common mosaic virus* strain blackeye cowpea mosaic has a restricted host range. Since BCMV-BICM survives in seeds of cowpea, seed transmission plays a key role in the epidemiology of this disease. The presented data clearly demonstrate the spread of the BCMV-BICM organism and disease symptoms in cowpea plants raised from infected seed with different levels of infection under field conditions in the Karnataka state. The levels of disease infection were higher in plant plots sown with the highest levels of infection. Virus-infected seeds provide random primary infection foci within the crop field. The prevalent rain and temperatures during the four growing seasons appear to favour the amount of aphids in the fields.

Aphid vectors spread the disease from primary infection foci leading to steep gradient of disease progress in the field. In the present study, cowpea seed samples with 0.05 - 10% infections con-tributed to the spread of BCMV-BICM inoculum, with a negative effect on yield and in the perpetuation of the virus in the harvested seed. The initial spread of BCMV-BICM was concentrated around primary foci by infected seeds.The infected patches increased in

Table 1. BCMV-BICM disease incidence in cowpea field plants grown from seed with different levels of infection.

Per cent seed infection	Per cent disease incidence **				
	Experiment 1	Experiment 2	Experiment 3	Experiment 4	Average
Control	0.4	0.0	0.0	0.0	0.1 ± 0.1^{f}
0.05	1.05	0.93	0.5	1.29	0.94 ± 0.16^{f}
0.5	3.53	5.52	5.0	5.12	4.79 ± 0.43^{ef}
0.75	4.95	5.36	7.0	6.75	6.01 ± 0.50^{ef}
1	8.56	9.46	8.0	11.8	9.45 ± 0.83^{e}
2	16.54	18.66	17.0	22.94	18.78 ± 1.45^{d}
3	32.15	34.68	32.0	48.88	36.92 ± 4.03^{c}
5	51.36	52.85	46.0	62.75	53.24 ± 3.49^{b}
10	90.56	92.56	88.0	89.7	90.20 ± 0.94^{a}

Regression constant = 3.11; Regression coefficient = 9.156; F_X = 406.87*; * = Significant at P < 0.001 level; ** = Per cent cumulative disease incidence at the end of growing season, the virus identity was confirmed by ELISA and PCR tests.

Table 2. Mean DAC-ELISA values for tests conducted with representative leaf samples collected from cowpea plots sown with different levels of seed infection.

Seed infection (%)	DAC-ELISA Absorbance values*
Control	0.080^{a}
0.05	0.264^{b}
0.5	0.300^{b}
1.0	0.464^{d}
2.0	0.500^{e}
3.0	0.258^{b}
4.0	0.350^{c}
5.0	0.466^{d}
10.0	0.356^{c}
ELISA Positive control	0.564^{e}
ELISA Negative control	0.088^{a}

*Mean A405 values of infected seedlings and corresponding negative and positive controls, respectively. Readings taken after 60-90 min.
Values were means from four repeated experiments with four replications.
Means fallowed by the same letter in a column do not differ significantly to Duncan's multiple range test at P = 0.05.

size and number eventually tending to coalesce. Similar observa-tions were made with narrow leafed lupin and CMV combination by Jones and Proudlove (1991). Close grouping of infected plants around primary infection foci resulting in obvious 'pools' of spreading infection is particularly characteristic of diseases caused by viruses. In the work reported here, BCMV-BICM appeared to spread rapidly outwards from primary infection foci to form patches of infection, which met and coalesced resulting in more widespread infection.

Sowing seeds with 10, 5, 3, 2, 1, 0.75, 0.5, 0.05% infection resulted in an average cumulative disease incidence of 90, 53, 37, 26, 12, 8, 6 and 1% respectively in four field experiments. Puttaraju et al. (2004a) reported that sowing cowpea seeds with 4 - 10% infection resulted in a 65-100% BCMV-BICM infection. Jones (2000) reported that sowing 5 and 1% CMV infected lupin seed resulted in 50 and 10% disease incidence, respectively. Sowing 0.5 and 5% CMV infected narrow leafed lupin resulted in a final disease incidence of 13 and 58% infection respectively (Jones and Proudlove, 1991). The seed yield reduction was directly correlated to levels of seed infection Seed infection of 10, 5 and 1% resulted in 74, 54 and 18% seed yield loss per plant. Infection of 0.75 and 0.5% reduced the seed yield per plant by less than 1%. Reports from Jones and Proudlove (1991) revealed that sowing 0.5% CMV-infection lupin.

The seed yield reduction was directly correlated to levels of seed infection Seed infection of 10, 5 and 1% resulted in 74, 54 and 18% seed yield loss per plant. Infection of 0.75 and 0.5% reduced the seed yield per plant by less than 1%. Reports from Jones and Proudlove

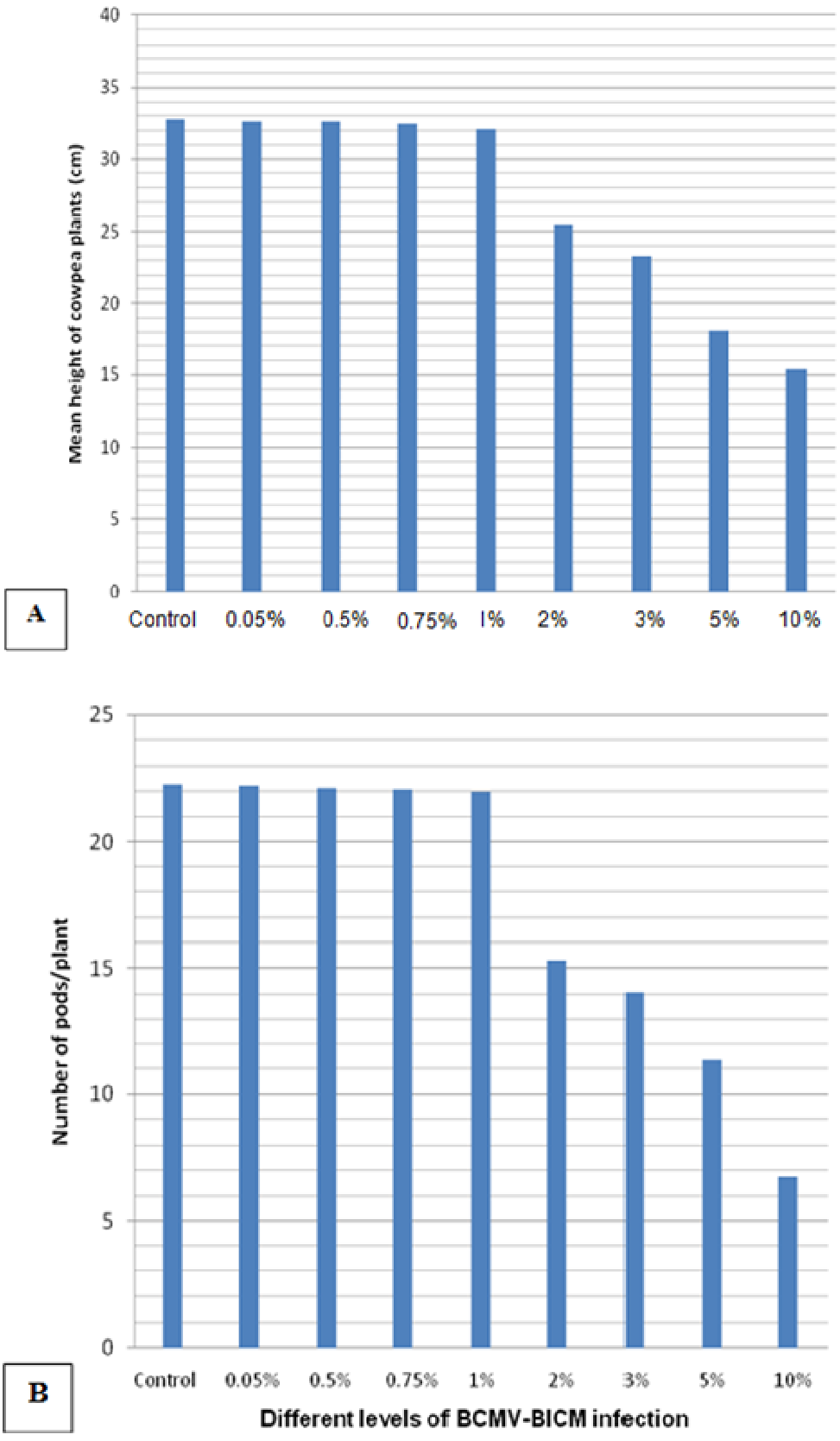

Figure 5. Effect of sowing cowpea seed with different levels of BCMV-BICM infection on (A) plant height and (B) number of pods/plant.

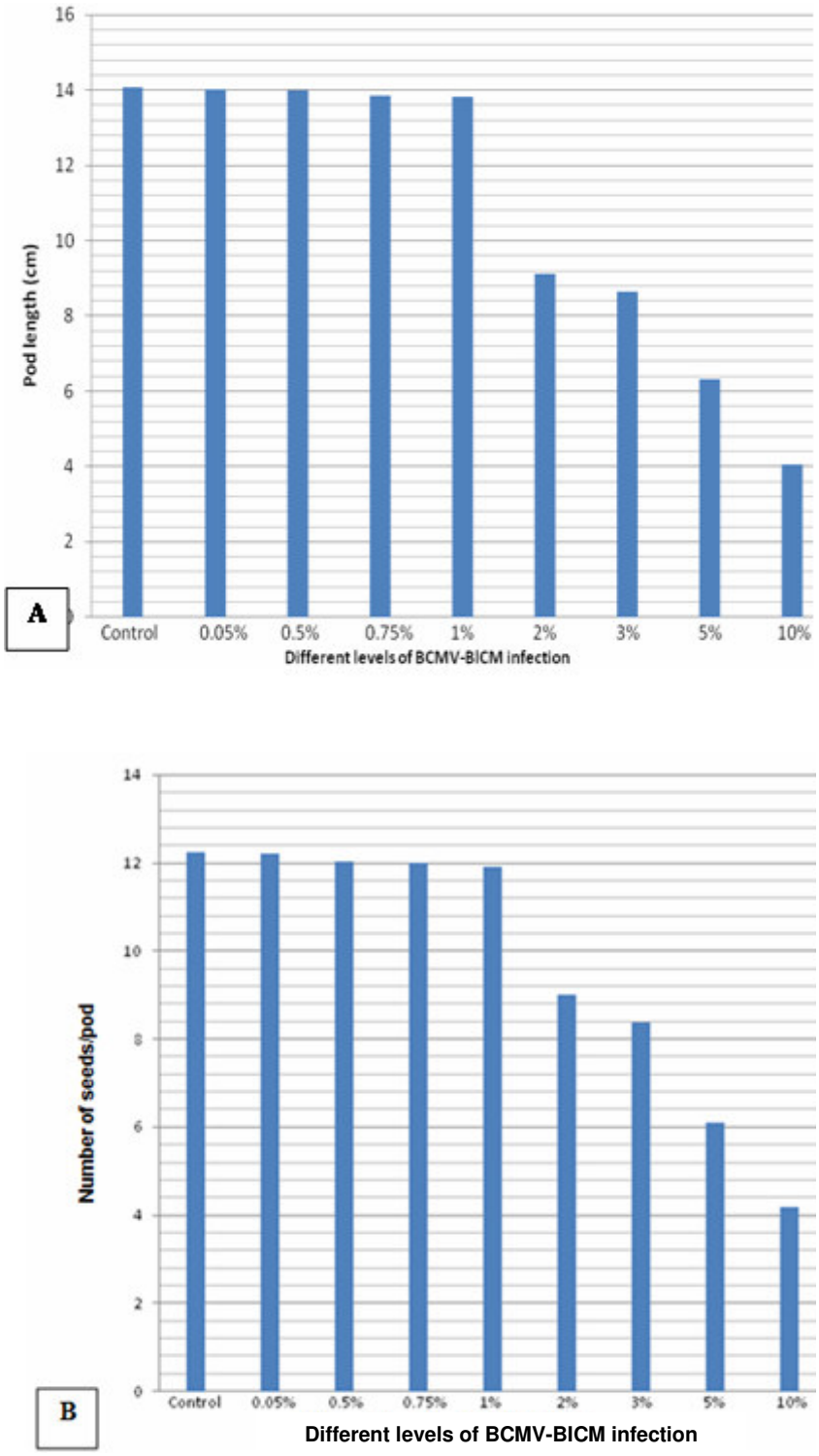

Figure 6. Effect of sowing cowpea seed with different levels of BCMV-BICM infection on (A) pod length and (B) number of seeds/pod.

(1991) revealed that sowing 0.5% CMV-infection lupin seed did not cause significant loss of yield. In lupin seed with a CMV infection of 0.5 - 5% Bwye et al. (1994) reported yield losses of 16 - 42%. In the present study, even in a crop with 90% disease incidence, the level of seed infection did not exceed 19%. Puttaraju et al. (2004a) reported 12% infection in seeds harvested from plots with 100% disease incidence.

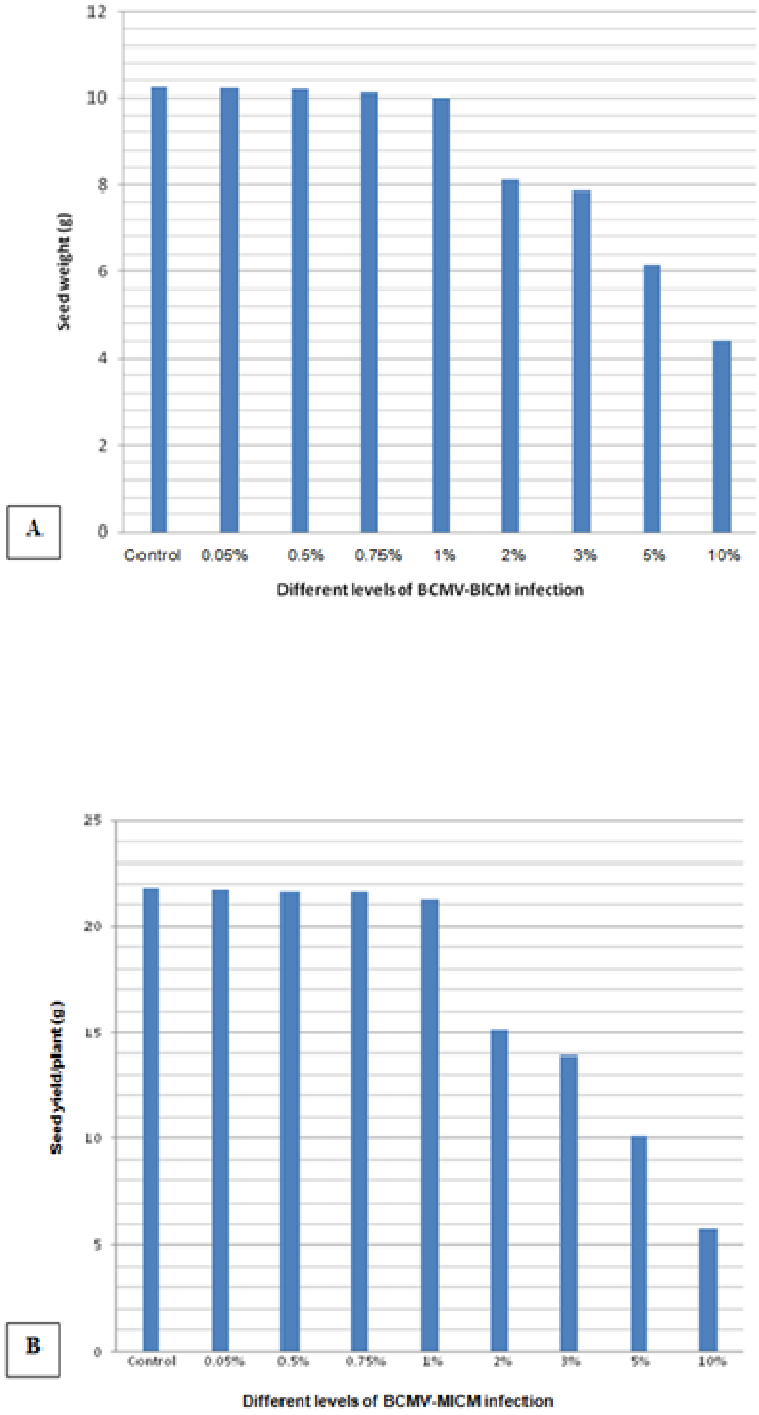

Figure 7. Effect of sowing cowpea seed with different levels of BCMV-BlCM infection on (A) seed weight and (B) seed yield/plant.

In Northeast China, where aphid intensity is high, a 0.01% tolerance has been considered with regards to *Soybean mosaic virus*. Besides, near the seashore where aphid intensity is low, the tolerance can reach 0.5% (Guo, 1992). An inoculum threshold of 0.1% has been found suitable in France for *Lettuce mosaic virus* /lettuce seed (Marrou and Messiaen, 1967). Masmoudi et al. *(*1994) observed that an inoculum threshold in the range of 0.1 to 0.5% was realistic in France for PSbMV. Jones and Proudlove (1991) advised growers to sow seed with a CMV infection of 0.5% or less to avoid yield loss in grain crops in South Australia. In the present study, sowing

seeds with 0.75% BCMV-BICM infection did not result in significant yield losses. Pio-Ribeiro et al. (1978) and Frison et al. (1990) reported the synergistic effect of co-infection of cowpea with BCMV-BICM / CMV resulting in high yield loss combined with seed transmission of up to 30%. Seed infection is epidemiologically important because it ensures that the virus will be associated with the plant crop, because infected seeds are randomly dispersed in the field, and because infected seedlings serve as primary sources of inoculum from which secondary spread can be initiated. Therefore, establishment of an inoculum threshold of seed-borne BCMV-BICM is of utmost importance (Stace-Smith and Hamilton, 1992). In the preset study, it was possible to show that sowing seed even with a low incidence of BCMV-BICM (> 1%) would result in substantial virus spread with a major impact on grain yield of cowpea. Thus a 'threshold level' below 2% infection for cowpea seeds was judged to be adequate to avoid the risk of economic losses due to the spread of BCMV-BICM in cowpea.

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