GENERAL DISCUSSION
Cucurbitaceous crops expressed disease symptoms due to RNA and DNA viral pathogen in Maharashtra region. These viral diseases were major constraints in cucurbits, particularly in the last five years. The RNA viral pathogens affecting cucurbitaceous crops belonged to the genera Cucumovirus, Potyvirus, Tobamovirus and Tospovirus, in cucumber, melon, bottlegourd, and bittergourd across plants grown at study regions such as Aurangabad, Nashik, Pune and Sangamner. The additional symptoms expressed, besides chlorotic leaves, leaf distortion, and necrotic spots in addition to mosaic symptoms, these symptoms were prominent in watermelon and melon. Certain crop plants expressed overlapping or complex symptoms including necrotic ring and mild green mosaic, which could be due to infection by more than one viral pathogen, necessitating proper disease diagnosis.

The typical symptoms caused by the two Tospovirus pathogens were mottling, yellowing of leaves, stunting of plants and death of shoots, which were first recorded in Southern India in 1992 (Singh and Krishnareddy 1996). During 2002, symptoms such as mosaic, puckering, blistering and stunted vine and fruit damage were associated with Papaya ring spot virus (PRSV-W), Watermelon mosaic virus (WMV-2) and Zucchini
yellow mosaic virus (ZYMV) in Pune district Maharashtra (Verma, 2004). These three RNA viral pathogens belonged to potyviridae.

The disease incidence in 45-65 days-old-crops was high (100%) in Aurangabad in 2008-2009 and Sangamner in 2009-2010, and it was low (20%) in Sangamner and Aurangabad regions respectively. The occurrence of virus disease incidence varied in cucurbit crops grown in Sangamner, Nashik, Aurangabad, Jalna, Paithan and Sillod locations. This could be due to the difference in susceptibility in various cucurbitaceous hosts, availability of viral inoculum for transmission or environmental conditions. Watermelon cv crimson sweet was frequently cultivated genotype in Sangamner, and Pune districts and was seriously affected. Cucumber, muskmelon, bottlegourd and watermelon probably served as alternative hosts to the viral pathogens occurring in these regions. The study also indicated that the disease incidence due to CMV, ZYMV, WMV and WBNV was high in most cucurbitaceous crops, and particularly in cucumber, and muskmelon in Sangamner, Nashik, Aurangabad, Sillod, Jalna and Paithan. The incidence of WBNV was noticed in almost all watermelon and muskmelon growing locations in Maharashtra.

Cucumber mosaic virus was successfully mechanically transmitted from field infected watermelon to cucumber and tobacco; the latter one is a diagnostic host for Cucumber mosaic virus infection. The systematic infection caused in solanum and cucurbit species confirmed the occurrence of Cucumber mosaic virus in cucumber. Verma et al., (2004), also noted that cucumber plants in commercial fields in Pune, western Maharashtra, suffered the yield loss of 29-38%, due to CMV. The study indicated that the successful mechanical transmission of ZYMV could be achieved from
field infected cucumber to cucumber varieties during greenhouse. The ZYMV transmission occurred in few hosts, since some level of resistance prevailed in certain host species. Experiments, conducted in the study clearly noted the predominance of CMV, ZYMV and WBNV infections in cucurbitaceous crops grown in Maharashtra. Protocols used for the bio and immunoassays could be useful in screening cucumber for resistance and breeding programs, and also in disease management in cucurbitaceous crops in Maharashtra.

The survey was also conducted during 2008-2010 in Aurangabad, Nashik and Pune districts of Maharashtra state on DNA viral diseases occurring in cucumber crop. The symptoms caused were yellow mottling of leaves, leaf distortion, and crumpling. These symptoms typically resembled symptoms of viral disease caused whitefly transmission. Systemically affected matured plants were stunted and started withering, with low fruit set; if fruit set occurred, they were malformed. Symptoms of DNA viral pathogens varied from mild mottling to severe crumpling. In the study, the conspicuous symptoms of yellowing of leaves and severe crumpling were discussed and hence viral disease was named as ‘yellow leaf crumple disease’. Infected cucurbitaceous crops such as bittergourd, cucumber, muskmelon, winter squash, ridgegourd, bottlegourd, spongegourd, ivygourd, pumpkin and watermelon produced typical symptoms of yellow vein mosaic, leaf distortion, curling, and stunting (Verma and Giri, 1998; Singh et al., 2001; Muniyappa et al., 2006; Singh et al., 2007). In cucumber, yellow mosaic disease was caused due to whitefly transmission (Raja and Singh, 1996). The disease symptoms documented in this study were not recorded in the previous studies, and hence this is the first documentation of this disease.
The disease incidence ranged from 55 to 70% in Daund, Shikrapur and Rajgurunagar villages of Pune districts. The low disease incidence of 2% to high disease incidence of 90%, the mean disease incidence of 50% was recorded. The high disease incidence correlated with high whitefly population, with the exception in Saykheda where the disease incidence was low, inspite of the high whitefly population (98). This situation could be due to the prevalence of low inoculum load. The whitefly transmission study indicated that a single whitefly is capable of transmitting the viral disease to 40% of plants, and to obtain 100% infection 7 to 10 viruliferous whiteflies were required. The yellow leaf crumple disease was caused by Begomovirus. However, similar symptoms could be produced by more than one viral pathogen. Therefore, the identification of the viral pathogen as associated with the disease could be done was by PCR amplicon and sequencing of the clones.

By dot-blot hybridization test, out of 400 samples, nearly 250 samples showed strong signal of positive hybridization with radio labeled coat protein gene of ToLCNDV, clearly indicating that the DNA viral pathogens associated with the disease samples were Begomoviruses. Further, all these 250 samples, were also subjected to PCR with Roja’s (for Rep) and Core protein genes (CP) flanked with two sets of primers (that were extremely conserved in majority of the Begomoviruses). 129 samples yielded PCR amplicons-850 bp length with Roja’s primer and 550 bp length with CP primers. The PCR amplicons were generated for nearly 125 clones. Since clones obtained for Rep primers were less, only 38 clones derived from 17 samples were sequenced. In the case of CP, 91 clones from 35 samples were characterized. On the basis of CP gene sequence, the amplicons of 550 bp obtained using CP gene primers of Wyatt and Brown spanned from
approximately the nucleotide coordinate of 480-1000 which represented the core region of coat protein gene.

Twenty-nine samples of the 52 studied (23 for CP and 6 for Rep gene) showed >90% sequence identity with ToLCNDV-Luffa isolate (GeneBank accessions No EU 36616). In one sample, one each from Aurangabad and Nashik district, the nucleotide identity of three clones was only 86-88%, with Rep gene of ToLCNDV. Identity with other Begomovirus was less than 80%. Therefore, the above two samples could be infected by a new unidentified viral pathogen.

In addition to ToLCNDV, the sequence analysis inferred that Croton yellow vein mosaic virus (CYVMV) was detected in 20 samples (7 samples on the basis on CP gene, 13 samples on the basis of Rep gene). The nucleotide identity in the CP gene and with the Rep gene with CYVMV (Genebank accession no- JN8175816) is 97.8% and 91-98%, respectively. One sample each from Aurangabad and Nashik district, the nucleotide identity recorded for the Rep amplicon clone with CYVMV was 87 and 80%, respectively. Hence these samples could be infected by an unidentified viral pathogen.

Similarly, Tomato leaf curl Karnataka virus (ToLCKaV) was identified in these samples with nucleotide identity of 94-97% in CP gene. Other than ToLCNDV, CYVMV and ToLCKaV, and Begomovirus identified with CP and Rep initiation genes was Mesta yellow vein mosaic virus (GeneBank accession No FJI 59262) MeYVMV caused disease in mesta (Chatarjee et al., 2008; Das et al., 2008). This is the first report of its kind reported in a non-malvaceous host. The survey of three districts of Maharashtra indicated that all the above four viral pathogens recorded in this study were documented previously.
from India from other host systems, however they were for the first time recorded in cucumber.

In conclusion, the cucurbitaceous crops grown in Maharashtra during summer and rainy seasons were vulnerable to both RNA and DNA viral pathogens. RNA viral pathogens included CMV, ZYMV, WMV, CGMMV, and WBNV. The occurrence of DNA viral pathogens in cucumber not reported hitherto was recorded in Aurangabad, Nashik and Pune districts of Maharashtra for the first time. The DNA viral pathogens were ToLCNDV, CYVMV, ToLCKaV and MeYVMV. The above viral pathogens need further investigation for their host range and other epidemiological understanding.