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AN OVERVIEW ON THE CAUSAL AGENT, VECTOR AND MANAGEMENT OF LEAF CRINKLE DISEASE IN URDBEAN (*VIGNA MUNGO* L. HEPPER)

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ABSTRACT

Urdbean (*Vigna mungo* L. Hepper) is comparatively more vulnerable to leaf crinkle disease than other pulses. Urdbean leaf crinkle disease (ULCD) is a widespread and devastating disease of economic significance resulting in extreme crinkling, puckering, curling and roughness of leaves, malformation of floral organs and stunting of plants. The ULCD causes substantial yield losses annually in major urban-producing countries around the world. Aphids, insects, and whiteflies have been identified as disease vectors. The virus is also transmitted via inoculation, grafting, and seed sap. The seed yield loss in ULCD-affected Urdbean crops ranges from 35%-81%, which depends on the genotype, location type and time of infection. The diseased material and favorable climatic conditions lead to a widespread viral illness. In germplasm screening, genetic variations have been reported indicating continuous screening of available varieties and new germplasm to identify new traits (different genes) and new sources of disease resistance. Reports on breeding programs for the production and release of ULCD tolerant varieties are very limited. There are various RNA viruses, which evolved strategies to counter the silencing process by encoding suppressor proteins. However, in the case of ULCV, there is no report available indicating which protection pathway operates in the plants for its resistance and whether this virus causing leaf crinkle disease in Urdbean also follows the same silencing suppression strategy. This review provides an overview of different aspects of *Urdbean leaf crinkle virus*.

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INTRODUCTION

Urdbean (*Vigna mungo* L.) belongs to family Leguminosae, locally called as "Blackgram". It is native to India and grown in almost all the continents (Asia, Europe, Australia, America and Africa). Urdbean leaf crinkle virus (ULCV) is one of the most destructive virus

of Urdbean in Pakistan and all over the world (Ashfaq et al., 2007). Leaf crinkle is a major viral disease in all the urdbean growing countries across the globe as well as in Pakistan. Leaf crinkle virus infects mashbean and mungbean crops and causes severe disease losses. *Urdbean leaf crinkle virus* belonging to the family *Geminiviridae* genus *Begomovirus* is a single (+) standard RNA unipartite genome with the extent of 25-30 nm containing 17% nucleic acid. Replication does not depend on a helper virus (Zerbini et al., 2017). Urdbean leaf crinkle virus shows many types of symptoms which depend upon the viral strain. Significant symptoms are crinkling, curling and malformation, which decrease the yield of urdbean crop. Whitefly is the vector of Urdbean leaf crinkle virus (Sravika et al., 2018). Bashir and Zubair (1985) studied leaf crinkle virus on urdbean genotypes for the first time in Islamabad, Pakistan. Plant disease analysis in the ensuing years described that ULCV was broadly distributed in all over the region where mungbean and urdbean were cultivated. However, disease incidence was high in urdbean (Bashir and Malik, 1988). Bashir et al. (2006) collected infected plant samples from different research stations and farmers' fields and examined that the disease incidence of *Mungbean yellow mosaic virus* (MYMV) ranged up to 4-40% as a major disease while *Urdbean leaf crinkle virus* (ULCV) was the second viral disease with incidence up to 5-28%. However, ULCV was more serious than MYMV. Only 213 (39%) plant samples were detected as positive. Beniwal and Bharathan (1980) and Singh (1980) studied the effect of *Urdbean leaf crinkle virus* on the yield of mungbean and stated that due to ULCV yield was reduced up to 76-100%. According to Kadian (1982), age of plant has a significant effect on yield losses and intensity of ULCV at the time of infection. Direct relationship was examined between the early stage of plant when infection occurred and a decline in yield. Disease on the first stage decreased the number of pods. Heavy yield losses also occurred in term of 1000 grain weight in infected plants. Bashir et al. (1991) studied the heavy losses due to leaf curl virus in urdbean. They examined 90.8% reduction in pods, 18.5% in pod length and 81% in yield. Sahay and Varma (1999) reported that in India, the primary urdbean disease was *Urdbean leaf crinkle virus* and recommended to manage using tolerant varieties. Bashir et al. (2005) screened 132 breeding lines under field conditions against *Urdbean leaf crinkle virus and Mungbean yellow mosaic virus*. They observed that 26% of urdbean genotypes were highly resistant to *Urdbean leaf crinkle virus* and 53% to *Mungbean yellow mosaic virus*. Negi and Vishunavat (2006) determined the highest mortality, lowest germination and percentage of diseased plants from ULCV infected seeds. They also measured reduction in yield from 21.20 to 23.50% and the lowest values of thousand seeds weight 31.64-33.18. Seed transmission of ULCV was also confirmed from these results. Narayanasamy and Jaganathan (1975) described the impact of physical factors on ULCV inactivated at 48 hours at room temperature and even at 60 ^{o}C. This virus has dilution endpoint (DEP) 1:100000, thermal inactivation point (TIP) between 60-70 and longevity *in vitro* (LIV) up to 5 days at room temperature. Thermal inactivation of *Urdbean leaf crinkle virus* from urdbean was pH dependent with a maximum at 7.8. Bhaktavatsalam et al. (1983) examined physiological factors which were responsible for longevity and infectivity of *Urdbean leaf crinkle virus*. The infectivity depends on pH and maximum at 7.8. Rapid loss of virus infectivity occurred at pH 7.8 as compared to pH 7.0. Phenol treatment decreases the infectivity of the virus. Longevity and infectivity of ULCV can be increased by adding 1% mercapto ethanol and 5% sucrose to inoculums. Bhaktavatsalam et al. (1986) examined virus-like particle in urdbean cytoplasm, chloroplast, and in infected leaves but not in cells of healthy leaves. The size of the virus-like particle ranged between 25-30 nm in diameter with a spherical shape. They also examined the hypertrophy of contaminated cells. This literature will help researchers to understand about ULCV and develop the ecofriendly management strategies in future.

Symptomology and host range: Many scientists studied the *Urdbean leaf crinkle virus* and identified the disease based on symptoms. Chohan and Kalia (1967) reported for the first time a new condition in India under the name "curly top *Phaseolus mungo* L" but Williams et al. (1968) observed this disease on Mmungbean and urdbean in of Uttar Pradesh, India and then the same condition was observed in 1966 and 1967 in Delhi and Terai region of Uttar Pradesh. They followed the symptoms of ULCV puckering, rugosity, crinkling of leaves and stunting of leaves which is caused by *leaf crinkle virus* (Figure 1). During a survey of plant diseases, Bashir and Zubair (1985) reported that *leaf crinkle virus* was present in all area where urdbean and mungbean are grown, but disease severity

was more on urdbean as compared to mungbean. According to Brar and Rataul (1986) curly emergence on third trifoliate was the most characteristic symptom followed by reduction of petioles and crinkling of lamina resulting in crowding of leaves. The flower sepals became greenish and thicker than normal. The area of the leaf of healthy trifoliate was smaller than the diseased ones. In infected plants, distribution of disease, symptoms were not uniform; some branches were infected by crinkling while other branches of the same plant remained healthy. Reddy et al. (2005) studied the effect of *Urdbean leaf crinkle virus* on plant age and stated that 72-80% infection was caused due to inoculation at early leaf stage.

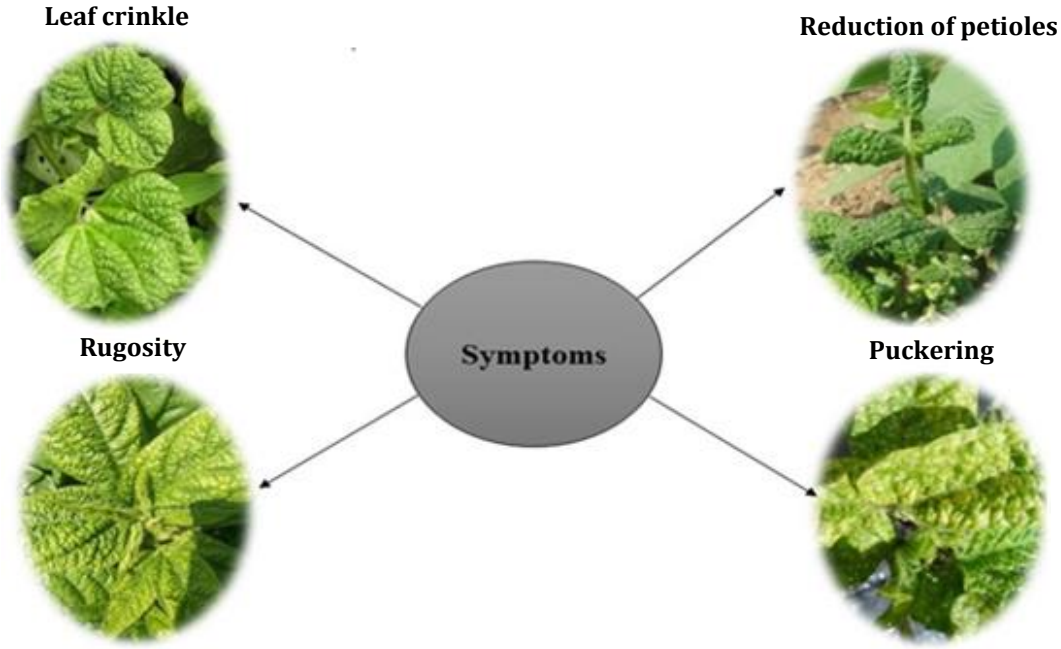

Figure 1: Characteristic diseased symptoms of Urdbean leaf crinkle virus.

The incubation period was short, and when the plants were inoculated at the early stage, then the development of symptoms occurred on the second trifoliate leaf stage as compared to older plants. The plant had no infection when inoculated preceding to flowering stage. In infected plants, leaflets become large from third trifoliate onward. Size of stipules in infected plants increased earlier to symptoms development. Kolte and Nene (1979) inoculated many plant species with *Urdbean leaf crinkle virus,* but infection appeared on *Vigna rediata* (mungbean), *Vigna mungo* (black gram), *Vigna unguiculate* (cowpea), *Nicotiana tabacum* (tobacco) and *Phaseolus vulgaris* (french bean). *Cyamopsis tetragonoloba* (guar) and *Arachis hypogea* (peanut) were found to be the supplementary hosts of *Urdbean leaf crinkle virus*. Two more supplementary hosts i.e. bottle gourd and cucumber were also reported by Beniwal et al. (1983). Kadian (1983) studied the host range of ULCV on weed plants and reported that *Datura stamonium* (jimsonweed), *D. metel* (datura), *D. metaloides* (desert thorn-apple) and *Convolvulus arvensis* (L) (field bindweed) were susceptible to ULCV. The plant species like *Amaranthus* spp., *Achyranthes* spp., *Cannabis sativa* (L), *Boerhavia diffusa* (punarnava), *Chenopodium giganteum* (tree spinach), *Chenopodium quinoa* (quinoa), *Celosia argentea* (silver cock's comb), *Cyperus rotundus* (nut grass), *Euphorbia hitra* (asthma-plant), *E. microphylla* (Philippine tea tree), *Digera arvensis* (Forsk.). *Nicotiana plumbaginifolia* (tex-Mex tobacco), *Phyllanthus niruri* (gale of the wind), *Portulaca oleracea* (common Purslane), *Polygala chinensis* (Indian milkwort), *Trianthema monogyna* (giant pigweed) and *Xanthium strumarium* (rough cocklebur) were resistance against *Urdbean leaf crinkle virus* out of 23 plant species. Infection was localized in *Datura* species and systemic in *C. arvensis*. It was also observed that disease incidence was less in those fields where *C. arvensis* was not prevalent. Nawaz and Narayanasamy (1983) examined that mash bean plants infected by *leaf crinkle virus* had no effect on the development of powdery mildew.

Disease incidence was marginally higher in virus-infected than in healthy plants.

Transmission and disease cycle: *Urdbean leaf crinkle virus* is transmitted through insect, sap inoculation and seeds (Kadian, 1982; Nene, 1972). Nene (1972) and Narayanasamy and Jaganathan (1975) reported the seed born nature of *Urdbean leaf crinkle virus*. They also reported the transmission of *Urdbean leaf crinkle virus* by two aphid species (*Aphis gossypii* and *Aphis craccivora*), whitefly (*Bemisia tabaci*) and leaf-feeding beetle (*Henosepilachna dodecastigma*). Chowdhary and Saha (1985) confirmed a quick method of injection of ULCV. According to Sravika et al. (2018), transmission of *Urdbean leaf crinkle virus* by *hystero-neurasthenia* and *Lipaphiserysime* with an acquisition period of 1 minute highly percentage of transmission occurred. Brar and Rataul (1986) described that transmission of *Urdbean leaf crinkle virus* was not due to *Aphis gossypii, Bemisia tabaci* and *A. craccivora*. They also stated that either in laboratory or field there was no insect vector which was responsible for *Urdbean leaf crinkle virus*. They concluded that there was no specific pattern of disease spread, and disease did not transfer from infected to healthy plants. They also stated that 45% moderately infected plant and 77.84% in severity were caused due to seed transmission of virus to different cultivars of urdbean having a rate of transmission 0 to 15%. Ahmad et al. (1997) described the transfer of urdbean leaf crinkle virus through seed at the ratio of 2.7-46%. Reddy et al. (2006) reported that plant height, seed weight, nodulation, length of pod and root length reduce due to Urdbean leaf crinkle virus. They examined that losses of urdbean are 41.1-69.3%. Ravinder et al. (2005) conducted field trials on the yield of urdbean and rate of seed born transmission to check the effect of *Urdbean leaf crinkle virus*. Diseased seeds showed losses up to 31.25% at one side of the field, but at the other two sites of the area, losses were 2.0 and 4.4 per cent. Pushpalatha et al. (1999) used cucumber and cowpea plants as indicator hosts for *Urdbean leaf crinkle virus*. They also screened many cultivars of urdbean and reported that the disease incidence was 1-83%. Reddy et al. (2005) concluded that at the base of urdbean plant, the rate of speed transmission of *Urdbean leaf crinkle virus* was maximum.

Epidemiology: Weather is one of the crucial parameters that influence plant disease epidemics. Hence, understanding the weather data and climatic conditions is required to provide baseline information for developing a simple and reliable disease prediction system (Mubeen et al., 2017). 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 concerning a buildup of inoculums and potential spread of the disease has not been studied quantitatively. Prediction of ecological factors that have a vital role in disease spread is 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 of urdbean germplasm against ULCV and to identify adverse environmental conditions under which the virus causes severe crop losses. The ecological factors that play essential 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 (Binyamin et al., 2011). In northern areas of India, there is more prevalence of *Urdbean leaf crinkle virus* disease than southern regions in Haryana India (Kadian, 1982). *Urdbean leaf crinkle virus* perpetuate at a minimum temperature 25 ^{o}C, maximum temperature 35 ^{o}C and evening relative humidity (RH) less than 60% and minimum and relative humidity (RH) above 70%. The symptoms remained masked at 38-45 ^{o}C in summer when the relative humidity of evening and morning remained 40 to 60% respectively. Above 47 ^{o}C for a day, the disease symptoms did not appear or even at 35 ^{o}C for a week with evening and morning relative humidity 20 and 45% (Kadian, 1983).

Management: Sowing infected seed may lead to severe crop damage and high incidence of ULCV in harvested seed. This is due both to the effect of seed-borne infection and to the possibility of early onset of the spread of the vector field. The planting of disease-free seedlings appears to be an essential measure for controlling the disease to avoid initial introduction into the crop (Binyamin et al., 2011). Seed testing for ULCV should be included in the *Vigna* susceptible species fixed patterns. The first elimination of symptomatic plants or possible collateral weed hosts could reduce the probability of further field spread and reduce further infected seed production. Heat treatment of seeds has been reported to remove/decrease infection with a virus (seed-borne aspects, seed treatment). The following methods help to manage the ULCV (Figure 2).

Screening: Haq (1991) observed forty nine cultivars against *Urdbean leaf crinkle virus* and proved that lines AARI M-35 and AARI M-1 were found to be highly resistant and immune, respectively. Two lines were susceptible, four moderately sensitive, ten resistant and thirty one were moderately resistant. He also observed the growth response against different urdbean cultivars and described that due to the infection of *Urdbean leaf crinkle virus,* both reproductive and vegetative components were reduced. Iqbal et al. (1991) screened nineteen genotypes of urdbean for two consecutive years (1988-1989) against *Urdbean leaf crinkle virus* under natural infection condition. Due to seed transmission of the virus during second-year disease, intensity was very high. Four cultivars viz. S-250, S-210, Mash SKT and MM 5-60 remained resistant while other varieties showed moderate reaction against *Urdbean leaf crinkle virus*. Reaction of eighty lines of urdbean against *Urdbean leaf crinkle virus* and *Mungbean yellow mosaic virus* was determined by Bashir et al. (1996). Many varieties (9011, 9080, 92048, 92054, 92055, 92011, 92013, 92014 and 92050) were utterly free of ULCV and Mungbean yellow mosaic virus. Thirty lines were resistant against *Mungbean yellow mosaic virus* while sixty lines were resistant against *Urdbean leaf crinkle virus*. The remaining lines were either susceptible or tolerant to ULCV and MYMV. Under field conditions, thirty two varieties were screened against *Urdbean leaf crinkle virus* and *Mungbean yellow mosaic virus* Bashir et al. (1996). They found that 26% of urdbean varieties were highly resistant to *Urdbean leaf crinkle virus* and 53% to *Mungbean yellow mosaic virus*. Above 60% varieties expressed multiple disease resistance to both ULCV and MYMV.

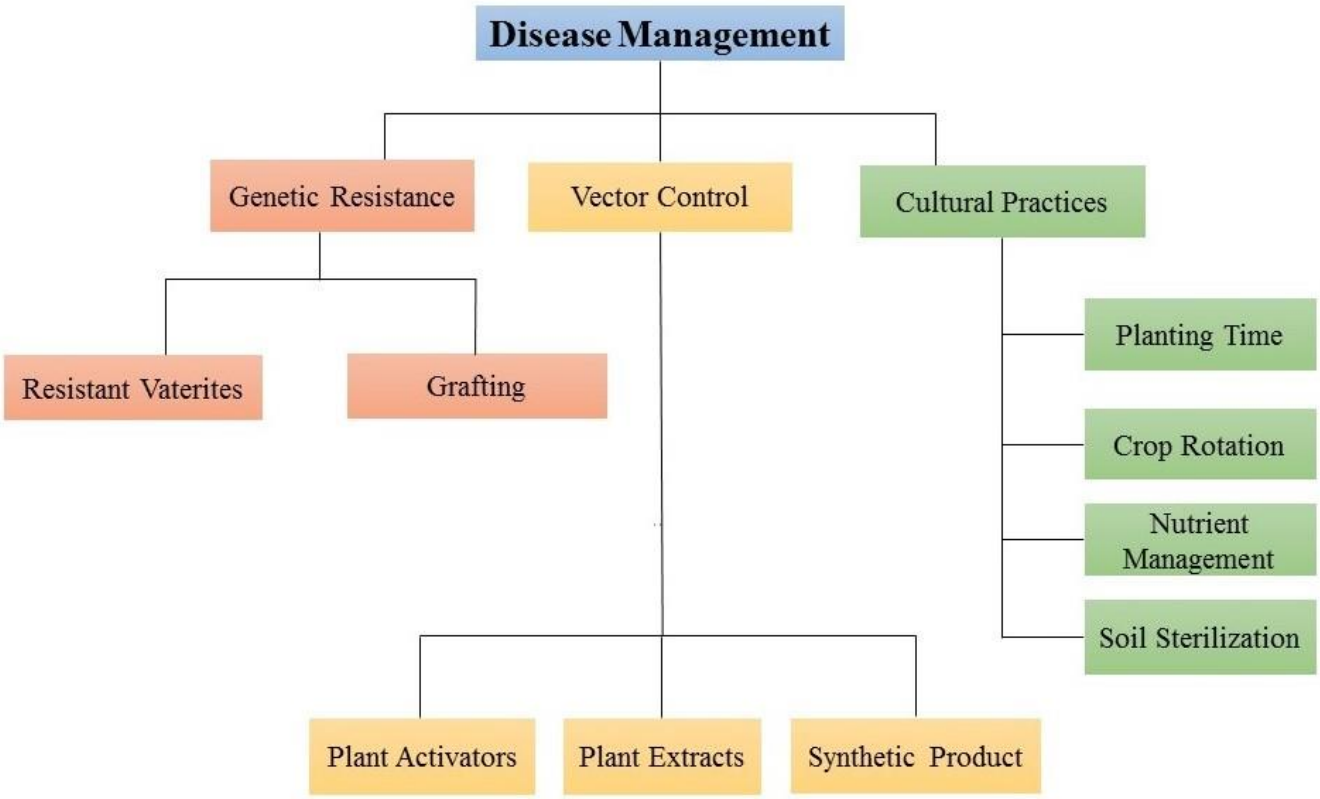

Figure 2: Disease management practices to manage the ULCV.

Plant-based products: Chowdhary and Saha (1985) reported that extract of turmeric and ginger reduced *Urdbean leaf crinkle virus* infection more than 50% and pre-inoculation spray of onion extract exhibited a maximum reduction of *Urdbean leaf crinkle virus* in *vivo* and *in vitro*. Bhardwaj and Dubey (1986) tested the effectiveness of different plant oils to prevent transmission of ULCV by *Aphis craccivora*. Emulsion of groundnut (2.0-2.5%), mustard (2.0-2.5%), coconut and castor oils (both at 1.0-2.1and 2.5%) have the phytotoxic

effect to plants. In contrast, rapeseed, mustard, groundnut and sesame at 1.0% reduced the transmission of the virus, and 2% emulsion of sesame and rapeseed oils prevented from the transmission of the virus completely. The oils showed a marked effect on virus acquisition. The aphid failed to acquire the virus from diseased plants sprayed by the rapeseed, mustered, castor and sesame oils at 2.5%. Similarly, fewer viruses were acquired from diseased plants sprayed with all other concentration of all these oils. The virus was completely lost by the viruliferous aphids feeding on plants pre-sprayed with rapeseed oil @ 2.0% and 2.0-2.5% emulsion of sesame and rapeseed oil. According to Chowdhary and Saha (1985) neem extract reduced 30% of disease incidence over control but according to Reddy et al. (2006) neem *(Azadirachta Indica)* extract reduced 40% of disease incidence over control. According to Binyamin et al. (2011), neem extract gave recovered results to overcome vector population as well as disease prevalence. Reddy et al. (2006) reported that six plants extract viz: *Datura metel* (datura)*, Mirabilis jalapa* (Marvel of Peru), *Bougainvillea spectabilis* (Bougainvillea), *Azadirachta Indica* (Neem), *Catharanthus roseus* (Bright eyes) and *Boerhavia diffusa* (Red spiderling) suppressed the disease incidence at field level. Thirumalaisamy et al. (2003) conducted a greenhouse experiment to evaluate different methods of application of plant extracts and identified them as potential inhibitors of *Urdbean leaf crinkle virus*. The study was conducted on physical properties of selected potential plant extracts against ULCV. The plants' parts and species used for the extracts comprised of *Allium sativum* cloves, *Allium cepa* bulbs, *Aloe vera* leaves, Neem leaves, Bougainvillea leaves, *Chenopodium giganteum* leaves, *Callistemon citrinus* leaves, *Curcuma longa* rhizome, *Datura stramonium* leaves, *Eucalypts globules* leaves, *Eclipta Alba* leaves, *Lantana camara* leaves, *Mentha arvensis* leaves, *Ocimum sanctum* leaves, *Phyllanthus niruri* leaves, *Piper nigrum* fruits, *Piper longum* leaves, *Polyalthia longifolia* leaves, *prosopis juliflora* leaves, *Solanum nigrum* leaves and *Zingiber officinale* rhizome. The extracts from *Piper longum*, *Zingiber officinale*, and *prosopis juliflora* possessed the most potent anti-ULCV property and used for further studies. The extracts of *Prosopis juliflora* and *Zingiber officinale* diluted from 1:5 and 1:5, and *Piper longum* diluted from 1:1 to 1:10 showed high percentage of disease inhibition. The thermal inactivation points of *Piper longum*, *Zingiber officinale, Prosopis juliflora* was 65, 45 and 55°C respectively. The aqueous extracts of *Piper longum* were remained effective up to 15 days while *Prosopis juliflora* and *Z. officinale* remained effective up to 9 days when stored at room temperature. Ali et al. (2010) concluded that extracts reduced the whitefly population and played an important role to minimize the disease progression. Similarly, Saleem et al. (2018) also used garlic and neem extracts and found significant results. Thirumalaisamy et al. (2003) also used many plants extracts against *Urdbean leaf crinkle virus* (ULCV). They concluded that neem and garlic extracts gave the best results and minimized the disease and whitefly population. Similarly, Ali et al. (2010) also used garlic, neem, and mint extracts to minimize the whitefly population. They concluded that these extracts had significant results against the whitefly population and also overcame the disease incidence.

Chemotherapy: Disease severity was reduced up to 18.31% by Boron and Zn (Islam et al., 2002). Ganapathy and Karuppiah (2004) evaluated seven sprays and two seed treatment against whitefly for two consecutive years against *Urdbean leaf crinkle virus* and *Mungbean yellow mosaic virus* incidence under field condition. Whitefly population, *Mungbean yellow mosaic virus* and *Urdbean leaf crinkle virus* reduced significantly and in comparison with unsprayed control higher yield in all treatment were recorded. Seed treatment with thiamethoxam @ 5g/kg reduced disease incidence of *Urdbean leaf crinkle virus* by 5.8 % after 15 days. The incubation period of the virus in urdbean plant was increased by chemical at maximum extents. Zeshan et al. (2012) used nutrients against *Urdbean leaf crinkle virus* on four urdbean varieties (AZRI-2006, M-6, M-97001 and NM-2006). NPK was confirmed more effective against ULCV and decreased the disease severity up to 60%. Boron and zinc reduced the disease severity up to 18.31%. Urea reduced the disease severity up to 58.57% and Naphthalene acetic acid (NAA) reduced the disease concentration up to 60.33%. Nutrients also played an important role to suppress the disease because it supports the defence mechanism of plants. Micronutrients are necessary for sugar translocation in plants, and they also affect carbohydrates and nitrogen. Islam et al. (2002) used boron and zinc against leaf crinkle disease of mungbean and found good results. They concluded that boron and zinc minimize disease severity.

Conclusion and future aspects: Plants extracts and micronutrients have been found useful for the

nutritional management of *Urdbean leaf crinkle virus*. These are eco-friendly approaches. Pesticides and insecticides were harmful to the environment, so the replacement with natural products is the dire need of the time. Different insects can transmit this disease; therefore, integrated management should also include different non-synthetic insecticides and biological control agents. Boron and zinc have been used by various scientists as a treatment application on leaf crinkle disease and found the best results. This literature helps design appropriate methodologies for eco-friendly management of *Urdbean leaf crinkle virus* and its vector.

Author contribution

All the authors equally contributed in the collection of the literature, compilation of this review, write up and editing the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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