Original Article



Biodiversity Monitoring & Vegetable Pathogens Surveillance Mapping from Kazakhstan

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> Abstract: Advance disease monitoring systems are important to underpin food security and may support productivity increases by addressing adverse climatic mutations. To foster sustainable ecosystem management cost-effective feasible and uniform disease monitoring is needed to model the outcomes and alterations in vegetation diversity of Kazakhstan. A descriptive survey was carried out during the consecutive years (2021-23) leading diagnosis and incidence of disease-causing organisms on cabbage and tomato grown in the main vegetable production regions of Kazakhstan. Disease samples were collected, followed by pathogens isolation and morphological characterization. A total of 10 deteriorating pathogens (Alternaria brassica, Alternaria solani, Candidatus Phytoplasma solani, Cladosporium fulvum, Fusarium oxysporum, Pectobacterium carotovorum, Phoma lingam, Rhizoctonia solani, Rhizoctonia and Xanthomonas spp.,) were recorded during survey, where calcium deficiency was also reported effecting vegetable crops quality. Among various threatening rots identified F. oxysporum initiating fusarium wilt was significant on cabbage in each location, with up to 100% incidence. Whereas Alternaria solani causing Alternaria leaf blight was the most common and devastating disease affecting tomato crop, followed by A. brassicae, an important leaf spot pathogen of cabbage, that was only observed in Turkestan oblast. It is pertinent to mention that the options for fungicide and biocontrol agents as a disease management strategy in Kazakhstan are limited due to cost and societal standards. This survey initiates prior data provision on multi-pathogenic diseases in Kazakhstan vegetable growing areas and provides direction for adopting advance technologies viz remote sensing and spectral imaging to timely implement suitable management practices meeting future challenges.

Keywords: IPM, Disease Surveillance, Spectral Image Analysis, Vegetable Crops

Introduction

Cabbage (*Brassica oleracea*) and tomato (*Solanum lycopersicum* L.) are economically important vegetables grown in Kazakhstan, bringing over 8.4 billion dollars in farm-gate value annually (Artemyeva et al., 2018; Sasidharan, 2023). These vegetables are nutritious, used in local cuisine, and are important export crops. Greenhouse production of tomatoes has expanded in recent years to meet growing demand, including the construction of automated glasshouses with hydroponic systems to maximize yields (Sasidharan, 2023). The current public health focus in Kazakhstan and surrounding countries on increasing the consumption of fresh fruit and

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Fusarium wilt (F. oxysporum) is another important pathogen affecting both crops in the open ground. Resistant tomato lines were bred as a tool to combat the pathogen in Kazakhstan greenhouses (Sagitov et al., 2010). In the Russian Federation, research conducted to limit diseases of glasshouse crops including root rot and wilt of tomato demonstrated reduced disease incidence with soil sterilization and using clean seeds (Rudakov, 2000). Other pathogens affecting vegetable crops are not as well recorded. Statistical data on losses due to plant disease from the Soviet Union are often difficult to access (Medvedev, 1979), and more recent studies have focused on wheat and row crop diseases (Amanbetov and Azhbenov, 2004). In Kazakhstan, research on diseases affecting vegetable crops is limited due to low government funding and scientific interest (Toleubayev, 2009). The role of plant diseases in yield and quality loss in the region are poorly understood. Integrated pest management is an essential tool for managing diseases and pests of vegetables (van der Velden et al., 2012; Yano, 2004). Reducing reliance on specific pesticide classes is beneficial to growers and preventing environmental degradation. Fungicide resistance is of national concern for safe crop production and introducing biocontrol is a priority for ecologically focused production. Many growers have been reluctant to update methods due to remaining cultural and social complexes from collectivized agriculture that was present in the (FSR) former Soviet Union (Toleubayev, 2009). Disease surveys are important in developing countries reliant on agriculture as a major income source, as the magnitude of losses can be significant without proper identification and control measures (Lieberman, 2012). Vegetables are significantly important diet for people, due to rich contents of vitamins, dietary fiber, and phytochemicals, but their pathogens can affect humans by reducing crop yields. It is important to mention that soilborne plant pathogens including bacteria, fungi, viruses and nematodes are a major threat to many vegetables and other horticultural crops. These pathogens pose a serious challenge because intensive farming systems with narrow rotational crop practices frequently increase their population in the soil. Among various phytopathogens, fungal pathogens in general contribute significantly to the yield losses in agriculture crops including fruits & vegetables, respectively. Studies that have sought to improve IPM programs used data from survey studies to enable accurate disease thresholds to be developed (Bauske et al., 1998; Pennypacker et al., 1983). Due to the importance of successful production of vegetable crops in Kazakhstan, understanding the primary disease-causing organisms is essential. After the inputs of labor and agrichemicals losing a crop due to a plant disease outbreak is financially devastating, especially near harvest. Documentation of damaging diseases affecting these crops facilitates monitoring for resistance and population changes over time, as well as establishing thresholds. The major objective of this study was to elaborate disease mapping and surveillance of plant pathogenic diseases affecting cabbage and tomato crops in southern vegetable growing areas of Kazakhstan for development of disease management strategies enhancing economic stability.

Material & Methods Sampling and Identification

In the summer growing seasons of 2021-2023, an elaborative survey was conducted by Kazakh Research Institute of Plant Protection and Quarantine (KzRIPPQ), Almaty, of vegetable production farms in Almaty, Turkestan, and Zhambyl oblasts. The specific Almaty survey districts included were: Enbekshikazakhsky, Karasaysky, Zhambylsky; Turkestan: Tyulkubassky, Sayramsky, Kelessky, Zhetysaysky, Saryagashsky; and Zhambyl: Kordaysky, Baizaksky, Zhualinsky (Figure 1). These are the primary vegetable production regions of Kazakhstan. The soil type is a heavy loamy soil type with approx. 1% organic matter (Karimov et al., 2009). For cabbage the prior rotation crop was potato and buckwheat whereas for tomatoes grown in open ground, the previous rotation crops were buckwheat, fallow field and cabbage. Cabbage plants were sampled from at-heading until mature plant growth stage and included whole tops or midrib and wrapper leaves. Tomato plants were sampled from seedling stage until harvestable fruit maturity, and entire plants including roots were sampled. The cabbage cultivars surveyed in Almaty were Fresko, Zakaz, Vestri, Bucharest, Megaton, Peking; in Turkestan Champ, Rhinda, Centurion, Pharaoh, Peking; and cultivars surveyed Zhambyl were Fresko, Bucharest, Zakaz, Vestri, Rhinda. For tomatoes, Lodzheiin, Apache, Troya, Prostomech, and Novichok were the hybrids surveyed in all districts. At each location, farmers were visited for elaborating ecofriendly disease management practices, viz, biocides, biocontrol strategies, cultural control practices and use of synthetic fungicides. Farmers responses were recorded and summarized.

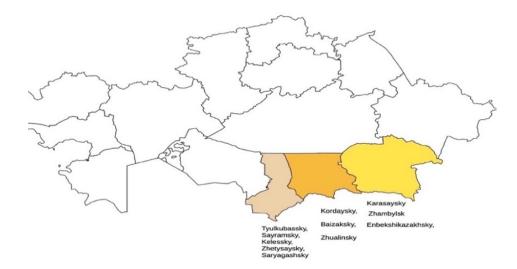


Figure 1: Map of surveyed areas of Kazakhstan for identification of Brassicaceae and Solanaceae Family Pathogens from Major Southern Vegetable Production Regions

Fungal Incidence

From each farm, approx. 20-25 plants were selected arbitrarily and observed regarding disease symptoms. Rotten plant and plant parts were carried to the laboratory of the Kazakh Research Institute of Plant Protection and Quarantine in plastic boxes containing ice to prevent wilting. The plants were quantified with respect to symptoms recorded for known diseases. Then individual leaves were removed and rinsed in tap water. Isolations were made from characteristic lesions by excising diseased tissue from the margin of the lesions, surface sterilizing in 70% ethanol for 5 sec and plating onto PDA (Difco Bacto, 39 g/L). Plants were incubated for short periods at $22\pm2^{\circ}$ C to observe additional symptom development. Tomato fruit with visible lesions were isolated directly onto PDA. Multiplication was carried out and morphological features followed by cultural studies were conducted, respectively (Khokhryakov et al., 1984; Sanin and Neklesa, 2004; Simmons, 2007).

Evaluation of Disease Severity

To assess disease severity, a scale was developed to represent the range of symptoms observed in the field. Plants were rated for severity during surveying in each oblast. Disease severity was recorded using a rating scale where all figures represent specific criteria viz, 0 = no disease, 1 = light spotting or chlorosis, 2 = moderate spotting, unthrifty plant, 3 = coalescing leaf spots, chlorotic and necrotic leaves, 4 = complete blight or wilt, and 5 = dead plant. Rows were arbitrarily selected and marked on each farm for rating.

Results Disease Incidence Recorded

A total of 10 species of plant pathogenic rots were recorded during the 2021-2023 seasons (Table 1). Fusarium wilt was significant in all years. Mosaic virus of tomato was identified based on visual symptoms in 2021-22 in Almaty and Turkestan oblasts (*data not shown*). Blossom end rot (calcium deficiency, commonly known as apical rot) was observed on tomatoes in 2021-22 and stolbur (mycoplasma-like organism) was observed in all years (Figure 2). Brown spot (*Cladosporium fulvum*) infected tomato leaves in each oblast. Bacterial soft rot (*Pectobacterium carotovorum*) commonly known as slimy rot) of cabbage was a significant problem each year, especially in Turkestan in 2021-22. The five most common pathogen genera recorded were *Alternaria, Fusarium, Cladosporium, Rhizoctonia* and *Phoma*. Incidence of the most common genera of insects observed causing damage in commercial fields in Almaty, Turkestan, and Zhambyl oblasts of Kazakhstan (Figure 3).

Various disease outbreaks were observed after extremes in precipitation. *Pectobacterium* was found infecting cabbage after heavy irrigation or precipitation and was often not uniform in the field. Fruiting structures of *Phoma* were observed on infected cabbage plants. The pycnidia developed in necrotic, dry lesions, and cracked in the center of the lesion. Most of the fungi collected from tomato were associated with leaf spots and blights (Figure 2). Brown rot could be severe, but usually appeared to be secondary to early blight. Root rots were less common but caused significant localized damage in affected fields. Physiological injuries to cabbage and

tomato leaves from high sun intensity were also observed and pathogens such as *Alternaria* spp. could be associated with the damaged tissue. Further, cabbage and tomato plants were observed affected by pests and insects attack (Figure 4).

Disease Severity & Type of Decay

Field type, disease incidence, and types of decay in fields and greenhouses from three oblasts of Kazakhstan were reported (Table 2). There were 62, 93, and 48 farm locations sampled in 2021, 2022, and 2023, respectively. Alternaria leaf spot of cabbage consisted of necrotic target spots on the lower leaves with a chlorotic halo. These leaves eventually withered as lesions coalesced. Cabbage plants with Fusarium wilt developed chlorotic and dark purplish lower leaves about 4 weeks after transplant. Eventually these leaves became chlorotic, and the plant did not mature completely.

In some varieties in Zhambyl in 2021, complete losses were observed (Table 2). The primary symptoms of Alternaria leaf blight of tomato were small necrotic spots that enlarged into larger coalescing lesions that eventually blighted the entire leaf. Disease was severe in Turkestan and Zhambyl oblasts each year. Stolbur was observed as thriftiness of the plants, curling and cupping of leaves, and general decline (Figure 1). Mature harvest stage fruits were not recovered from plants infected with stolbur. Disease severity on cabbage was observed more severe in 2022 across surveyed districts, whereas in 2021 major disease severity was recorded on tomato crop.

Disease Management Strategies

In each of the three years selective fungicides were applied against identified pathogens. Fundazol 50WP (Benomyl) was applied against cabbage Fusarium wilt. On tomato during vegetative growth, Amistar Top 325 (azoxystrobin + difenoconazole), Antrakol (propineb) and Luna Tranquility (fluopyram+ pyrimethanil) were applied to control early blight and Cladosporium leaf spot. Ridomil Gold SL (mefenoxam) was applied preventively to control southern phytophthora blight, and Kasumin 2L (kasugamycin) and Zerox (silver colloid) were applied to tomato and cabbage for bacterial canker and black rot control, respectively. Biocontrol's were not used for disease control in the open field. In the greenhouses, biocontrol agent (*Bacillus* spp.) was applied. Fungicides were applied during the vegetative growth only, and when conditions favored disease outbreak. Cultural strategic measures were limited against improving field drainage. Extension agents promoted cultural methods such as reduced irrigation, improved irrigation timing, and row spacing to support disease management.

 Table 1. Pathogenic fungi recovered from surveyed cabbage and tomato plants in vegetable growing regions of Kazakhstan

Genus-species	Common name	ommon name Year(s) recorded		Crop ^b	Type of	
					decay ^c	
Alternaria brassicae	Alternaria leaf spot	2022	Т	С	LS	
A. solani	Alternaria leaf blight	2021, 22, 23	A, T, Z	Т	LB	
Candidatus Phytoplasma solani	Stolbur	2021, 22, 23	A, T, Z	Т	LC	

	<u> </u>	2021 22	• T	T	D
Calcium deficiency	Apical rot	2021, 22	Α, Τ	1	Р
Cladosporium fulvum	Brown spot	2021, 22	A, T, Z	Т	LB
Fusarium oxysporum	Fusarium wilt	2021, 22, 23	A, T, Z	С	R
Pectobacterium carotovorum	Slimy rot	2021, 22	A, T, Z	С	LS, SR
Phoma lingam	Phomos	2022	Т	С	LB
Rhizoctonia solani	Root rot	2022, 23	A, T, Z	С	R
Rhizoctonia sp.	Root rot	2022, 23	A, T, Z	Т	R
Xanthomonas sp.	Black spot	2022	А	Т	LS

^a Location: A = Almaty, T = Turkestan, Z = Zhambyl.

^b Crop sampled: C = cabbage, T = tomato.

^c Type of decay: LS = leaf spot, LB = leaf blight, LC = leaf curling, P = physiological, R = root rot, SR = soft rot.

Table 2. Disease incidence and severity in fields of cabbage and tomato plants in three regions of Kazakhstan

Oblast ^a				Disease incidence (%)				Severity ^c	
	Year	Fields surveyed	Maturity ^b	Fusarium wilt	Bacterial slimy rot	Stolbur	Alternaria blight	Cab	Tom
Almaty	2021	23	M, CB	5	0	22	54	1	3.5
	2022	36	S, H, ES	1	3	5	10	2	1
	2023	11	S, F	5	0	0	0	0.73	1
Mean				3.6	1	9	21.3	1.24	1.83
Turkestan	2021	21	M, CB	6	6	5	60	1	3
	2022	28	S, H, ES	1	1	8	100	2	1
	2023	34	S, H, F	4	0	1	2	0.7	1
Mean				3.6	2.3	4.6	54	1.23	1.66
Zhambyl	2021	18	M, CB	100	0	25	75	2	1.5
	2022	29	S, H, ES	4	2	0	38	2.4	2
	2023	3	S, F	1	0	0	28	1	1.5
Mean				35	0.6	8.3	47	1.80	1.66
Average of 3 oblasts				14.1	1.3	7.3	40.7	1.43	1.72

^a Cabbage cultivars in oblasts A = Almaty, T = Turkestan, Z = Zhambyl were: Fresko (A, Z), Zakaz (A, Z), Vestri (A, Z), Bucharest (A), Megaton (A), Peking (A, T), Champ (T), Rhinda (T, Z), Centurion (T), and Pharaoh (T), and for tomato, Lodzheiin (A, T, Z), Apache (A, T, Z), Troya (A, T), Prostomech (A), Novichok (A, Z), and Hypinet (Z).

^b Maturity determined from planting date and visual appearance, where: S = seedling, H = heading (cabbage), M = mature plant, F = flowering (tomato), ES = early fruit set (tomato), CB = color break (tomato). ^c Severity recorded using scale where 0 = no disease, 1 = light spotting or chlorosis, 2 = moderate spotting, unthrifty plant, 3 = coalescing leaf spots, chlorotic and necrotic leaves, 4 = complete blight or wilt, and 5 = dead plant.



Figure 2: Disease symptoms on cabbage and tomato in southern Kazakhstan. (A) Cabbage Fusarium wilt (*Fusarium oxysporum*), showing wilt symptoms and discoloration. (B) Cabbage Fusarium wilt (*Fusarium oxysporum*). (C) Stolbur of tomato (MLO). (D) *Alternaria solani* affecting tomato leaves with fruit at mature green stage.

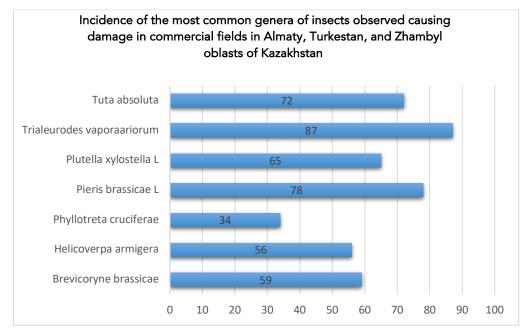


Figure 3: Incidence of the most common genera of insects observed causing damage in commercial fields in Almaty, Turkestan, and Zhambyl oblasts of Kazakhstan.

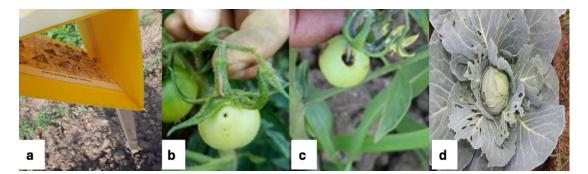


Figure 4: Insect monitoring and plant damage in Almaty oblast. (a) Monitoring trap located in tomato field with heavy capture of *Tuta absoluta* and *Helicoverpa armigera*. (b) Minor damage to immature tomato from *Tuta absoluta*, and fruit with larger feeding damage (c; d) Cabbage with heavy feeding damage from *Plutella xylostella* L.

Discussion

Determining the identity and importance of pathogens affecting vegetable crops is a significant initial step in developing integrated management programs. For vegetables that are required to be decay free during marketing and export, accurate disease management is essential. Severe disease outbreaks can result in total crop loss. The findings from this survey over three years in Kazakhstan have given researchers information about the disease pressure commonly observed in the southern growing regions. Fusarium wilt of cabbage was significant each year, and even resulted in complete losses on some farms in 2021 and 2022. The rapid onset of symptoms is difficult to predict and eradicating the pathogen from infested fields requires multiyear rotation and fumigation. Resistant varieties exist, however, resistance can break down at high temperatures (Rimmer et al., 2007). The main fungicide used to prevent Fusarium wilt was Fundazol. This compound has high systemic activity and very effectively controls susceptible pathogen populations. However, resistance of Fusarium spp. has been observed (Buga et al., 2000), which could limit effectiveness in field applications. Additional research is necessary to determine the sensitivity of Fusarium isolates from cabbage to this compound. Pathogens affecting tomato plants were also significant. Alternaria blight was observed each year. Similar studies by (Gannibal et al., 2014) support our research where Alternaria blight destroyed entire fields during severe outbreaks. Fungicides applied preventively are effective in reducing disease and pathogen population levels. Cladosporium leaf spot was also observed causing drying and withering of affected leaves. Application of fungicides (trifloxystrobin) against brown spot control in the open field and greenhouse, resulted significant results (Veloukas et al, 2007; Rakhimovaa et al., 2020). This disease can be very difficult to control in greenhouse conditions.

Applications of fungicides throughout the vegetative period reduced tomato leaf loss and improved plant health during the season. Timing fungicide sprays so that they coincide with environmental conditions conducive for disease has been shown to result in improved control of early blight (Louws et al., 1996). Diseases on the fruit were not frequently observed, however, many diseases can affect ripe fruit postharvest. Although this survey focused on field grown plants, postharvest rots are also significant in Kazakhstan. Losses of 15-70% have occurred in storage (B. K. Kopzhasarov, *Pers. Obs.*). Future research might include surveys of postharvest containers and storage facilities as specific surveys of disease affecting vegetable crops are limited, till date. In addition to fungal diseases, viruses were recorded during survey that lacks serological identification. Research to determine the presence of viruses in the tomato crop is an important next step in our research. Polsan et al., (1993) reported that insect populations monitoring for virus infected plants, and worker hygiene will undoubtedly help minimizing viral outbreaks (Polston et al., 1993).

The agriculture industry in Kazakhstan is facing significant challenges till date (Rushing and Christenbury, 1998; Toleubayev, 2009). Certain crops, such as wheat, has been widely studied due to its economic importance and widespread cultivation. However, for vegetable crops, limited government funding and scientific interest has resulted in reduced scope of research programs. Research has been published recently on diseases affecting vegetable crops (Amanbetov and Azhbenov, 2004). The importance of extension efforts in the FSR from both national and international organizations has been highlighted (Rushing and Christenbury,

1998). However, scientists' attention in dissemination of complicated research results is demanded. The significant socio-economic changes that occurred after the fall of the Soviet Union negatively influenced pest management practices and local knowledge sources, leading to increased pesticide use (Toleubayev, 2009). This has been termed a "paradox" as many growers had implemented intensive IPM programs in the past that were environmentally conscious. The increase in pesticide application has not always improved yield, as some traditional methods to improve plant health were lost. Reducing pesticide residues is an important goal worldwide (Palou et al., 2016). As European countries are important export partners for Kazakhstan, reducing the reliance on pesticides is essential to ensure safe export without quarantine measures. A recent study showed that residues of pesticides on tomato and cucumber from the Almaty region were present at high levels (Lozowicka et al., 2015). In Spain, over 50% of the pests of greenhouse grown tomatoes are controlled with IPM practices (van der Velden et al., 2012).

Conclusion

Pathogenic diseases are a major threat affecting vegetable crops grown in Kazakhstan as various deteriorating plant pathogenic diseases remain unidentified until advanced stages progress and hence cause significant economic losses. This study provides a baseline for future research in population changes, as well as improving on current IPM and eco-friendly disease management programs. Narrowing the timing and precision of pesticide and biocontrol applications on vegetable farms in southern Kazakhstan is dependent on accurate disease diagnosis. The improvement in detection and application efficacy will reduce unnecessary pesticide sprays and improve yields. The utmost recommendation of this study focuses on adopting advance disease forecasting technology viz remote sensing, hyperspectral image analysis, biosensors, machine and deep learning analysis to further adopt integrated management practices for growers regarding improved yield production and to mitigate reliance on synthetic pesticides.

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Author (s) Contribution: Bibigul Yertayeva: conducted survey, write-up, Bakyt Aitbayeva and Tynyshbek Yeszhanov: designed research, performed experimental work, Akmaral Shokanova data analysis, write-up and review.

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