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# Screening of pepper (*Capsicum annuum* L.) genotypes for response to pathogens and pests under conditions of conventional and organic field production

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## Abstract

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Monitoring of eight pepper genotypes is conducted at Maritsa Vegetable Crop Research Institute, Plovdiv, Bulgaria, during two successive years, under different diseases and pests'management systems. The surveys is carried out, using the standard field inspection methods to detect virus diseases (*Tobacco mosaic virus* – TMV, *Cucumber mosaic virus* – CMV and *Tomato spotted wilt virus* – TSWV, stolbur (*Phytoplasma solani*), verticillium wilt (*Verticillium dahlia* Kleb.), brown leaf spots (*Alternaria solani* Ellis & Martin) the pests: cotton bollworm (*Helicoverpa armigera* Hubn.), aphids (*Homoptera:Aphididae*) and thrips (*Thysanoptera:Thripidae*). Among observed pathogens *Alternaria solani* Ellis & Martin and *Phytoplasma solani* were most important for all varieties and breeding lines in all studied production systems as their infestation were on average 27.37% and 31.79%, respectively. During the survey, no plants with symptoms of verticillium wilting were reported in all studied genotypesunder all growing systems. The mean score of virus attack was below 12%. The average rate of brown leaf spots ranged between 29.05% for breeding line K992 to 36.26% for line K995. The established highest *Phytoplasma solani* attack averaged from 24.10% for Stryama to 32.15% for Kapia UV. Single plants with thrips damage were observed during seedling production and immediately after transplanting in the field. At maturity stage, an infestation of cotton bollworm (*Helicoverpa armigera* Hubn.) was observed, while aphids' population was low with no colonies established. The lowest infestation rates by *H. armigera* were reported in pepper genotypes K992 (0.92%) and K995 (0.49%) grown under organic production conditions.

*Keywords: Capsicum annuum* L.; screening; diseases; insects; infestation

## Introduction

Pepper is grown as an annual crop due to its sensitivity to frost and is an herbaceous perennial and yield for several years in tropical climates (Kelley et al., 2009). Pepper fruits are an important source of antioxidants, such as ascorbic acid, carotenoids, tocopherols (Sreeramulu & Raghunath, 2010), phenolic compounds, particularly flavonoids (Chen & Kang, 2013).

Conventional and organic agricultural practices represent dynamic systems that can vary greatly depending upon region, soil quality, and prevalence of pests, crop, and climate and farm philosophies. This makes comparisons very difficult, and may affect the nutritive composition of plants, including secondary plant metabolite (Sander & Heitefuss, 1998; Mitchell et al., 2007). The chemical, physical, and biological properties of fresh manure vary tremendously due to specific animal feeding and manure management practices.

Conventional farms utilize fertilizers containing soluble inorganic nitrogen and other nutrients, which are more directly available to plants (Mitchell et al., 2007).

Organic foods have a nutritional and/or sensory advantage when compared to their conventionally produced counterparts. Advocates for organic produce claim it contains fewer harmful chemicals, is better for the environment and may be more nutritious (Mitchell & Chassy, 2005). Additionally, there seems to be a widespread perception among consumers that organic farming results in products of higher nutritional quality (Williams, 2002). It was concluded that organic products contained significantly more vitamin C, Fe, Mg and P, and significantly less nitrates and heavy metals when present, than products from conventional agriculture (Worthington, 2001).

Organic fertilization typically does not provide nitrogen in a form that is as readily accessible to plants as conventional fertilizers (Doll et al., 1994). Most well-designed studies comparing nutrient density (milligrams of a given nutrient per kilogram of food) in organically and conventionally produced fruits and vegetables, show simpleton moderately higher concentrations of nutrients in organic products (Benbrook, 2009). Furthermore, the evaluation of the mineral content of several edible products or even food supplements is paramount, since it allow us to monitor the enrichment or the poorness of essential elements to human nutrition and metabolism, or even detect possible contamination by heavy metals such as Cd, Pb, and Hg, among others (Reboredo et al, 2018; Reboredo et al., 2019; Reboredo et al., 2020).

One of the most important functions of different agricultural production systems is to provide almost all essential mineral and organic nutrients to humans (Wang et al., 2008). In the view of non-polluting strategies, research on interactions between fertilization and beneficial microorganisms on vegetable production and quality as well as on biotic and abiotic stress prevention have been carried out by several authors (Del Amor et al., 2008).

Environmental protection and human health have recently become important factors when selecting food production systems. The wide usages of pesticides and synthetic fertilizers in conventional production cause environmental pollution (Robacer et al., 2016).

The thrips and aphids, and the virus diseases transmitted by them along with fungal and bacterial diseases are the limiting factors in pepper productivity. The solution for managing these pests on a sustained basis exists in adopting eco-friendly approaches like using resistant cultivars. Fortunately, huge natural genetic diversity exists in pepper and therefore, essential research efforts in finding out resistant sources and their utilization have been by and large dynamic and successful. Despite continuous scientific efforts there is a dire need for new cultivars with resistant traits for various pests suitable to varied climatic conditions, consumption and quality preferences all over the world. Efforts need to be intensified to find out useful genetic material and to introduce genes of resistance against insects, fungal and virus diseases into commercial cultivars (Babu et al., 2011).

The aim of this study is to investigate the reaction of pepper genotypes to phytopathogenic and enthomogenic factors under different agricultural systems.

## Material and Methods

The experimental work is carried out with eight genotypes: the varieties Sivria 600, Stryama, Milkana F1, Kapia UV, three breeding lines type Kapia K992, K993, K995 and variety Bulgarskiratund (Figure 1). The first three are suitable for forced, early and medium early field growth, and the production is intended for consumption at technical maturity of the fruit, while for the rest the consumption is mainly of botanically ripe fruits.

The reaction of pepper genotypes in the studied production systems is investigated: 1 – control, conventional production; 2 – natural fertility without plant protection; 3 – fertilization with bio humus and protection with biopesticides, against attack by pathogens and pests. The surveys are carried out, using the standard field inspection methods to detect virus diseases (*Tobacco mosaic virus* – TMV, *Cucumber mosaic virus* – CMV and *Tomato spotted wilt virus* – TSWV, stem rot (*Phytoplasma solani*), verticillium wilt (*Verticillium dahliae* Kleb.), brown spots (*Alternaria solani* Ellis & Martin), the pests cotton bollworm (*Helicoverpa armigera* Hubn.) and aphids (*Homoptera:Aphididae*). During the growing season, regular surveys are carried out using the route method. Monitoring of the attack by phytopathogenic and enthomogenous factors is carried out based on a percentage of attacked plants.

The software programs used in data processing are “MS Excel Analysis ToolPak Add-Ins” 2019 and “R-4.0.3” in combination with “RStudio-0.98” and installed package “agricolae 1.2-2” (De Mendiburu, 2021).

## Results and Discussion

When examining the pepper plants grown under a system of conventional production, the strongest attack of stolbur (*Phytoplasma solani*) is registered in cultivar Bulgarski ratund (20.84%), genotypes K993 (20.42%), K992 (19.59%) and K995 (19.17%). The weakest attack in this stolbur cultivation system is observed in the variety Milkana F1. Against

**Sivria 600**

**Stryama**

**Milkana F1**

**K992**

**Fig. 1. Pepper genotypes grow in 3 different systems**

**K993**

**K995**

**Kapia UV**

**Bulgarski ratund**

**Fig. 1. Continued**

the causative agent of brown leaf spots again the strongest is the prevalence in Bulgarski ratund (30.84%) and Sivria 600 (26.67%), and the least is the attack in Stryama and Milkana F1 – 20%. The genotypes with weak viruses' attack are detected (about 5%) are K995, K992 and Kapia UV. A high percentage is registered in Milkana F1 – 12.5% and Sivria 600 – 11.67%. The percentage of cotton bollworm attack in conventional production is below 7% and is strongest in Sivria 600 – 6.67% (Table 1). This is probably due to the insufficiently good efficacy of the chemicals included in the study and the weather conditions that affect both the development of the enemy and the biological activity of the insecticides.

Monitoring to establish the degree of damage from phytopathogenic and enthomogenic factors in system 2 – natural fertility without plant protection when growing pepper shows a strong stolbur attack in genotypes K993 – 41.08% and Kapia UV – 39.57%. The lowest damage rate is found in the Bulgarski ratund – 30.63% and Stryama 31.57%. The highest percentage of infection by brown leaf spots is recorded in K995, and the lowest in Sivria 600 and K992. The virus attack is less than 15% for all genotypes studied and the lowest symptoms are detected in Milkana F1 – 4.63%. There is zero attack by cotton bollworm in this system of cultivation in K995 and Bulgarski ratund – 0.38% (Table 1).

During the reporting of the stolbur degree attack in system 3 – fertilization with bio humus and protection with biopesticides it is found to be the weakest in Milkana F1 – 23.96% and Stryama 23.96%. Genotypes K993 and K992 show a slight attack by the brown leaf spots. A strong degree of infection by viruses is recorded in Bulgarski ratund (18.34%), and the weakest is in Milkana F1 (5.49%). A very low attack by the cotton bollworm is recorded in all genotypes examined (0%-6.67%), being 0% in K992 and K995 (Table 1).

No plants with symptoms of verticillium wilting are detected when crops were examined for attack by phytopathogenic factors. No statistical differences are demonstrated between the examined genotypes in stolbur attack and brown leaf spots in total for all cropping systems. During the pe-

**Table 1. Evaluation reaction the pepper genotypes growth under different conditions to pathogens and pests**

| Genotype | System of growth | *Phytoplasma solani* | *Alternaria solani* | *Viruses* | *H. armigera* |
|---|---|---|---|---|---|
| 1. Sivria 600 | System 1 | 15 | 26.67 | 11.67 | 6.67 |
| | System 2 | 33.07 | 31.72 | 9.66 | 2.18 |
| | System 3 | 26.76 | 34.09 | 8.71 | 1.6 |
| 2. Stryama | System 1 | 16.25 | 20 | 10 | 3.75 |
| | System 2 | 31.57 | 36.48 | 8.03 | 4.5 |
| | System 3 | 24.48 | 35.62 | 7.11 | 1.13 |
| 3. Milkana F1 | System 1 | 13.75 | 20 | 12.5 | 3.34 |
| | System 2 | 35.02 | 33.36 | 4.63 | 3.73 |
| | System 3 | 23.96 | 38.16 | 5.49 | 2.94 |
| 4. K992 | System 1 | 19.59 | 21.67 | 5.42 | 1.67 |
| | System 2 | 33.54 | 31.73 | 5.41 | 1.09 |
| | System 3 | 29.49 | 33.75 | 7.29 | 0 |
| 5. K993 | System 1 | 20.42 | 22.5 | 8.75 | 2.29 |
| | System 2 | 41.08 | 33 | 8.13 | 1.36 |
| | System 3 | 31.38 | 32.74 | 8.93 | 3.3 |
| 6. K995 | System 1 | 19.17 | 24.58 | 5 | 1.46 |
| | System 2 | 34.51 | 43.36 | 13.39 | 0 |
| | System 3 | 31.79 | 40.84 | 6.8 | 0 |
| 7. Kapia UV | System 1 | 17.92 | 25 | 5.83 | 2.09 |
| | System 2 | 39.57 | 37.55 | 11.31 | 4.85 |
| | System 3 | 38.97 | 34.21 | 14.73 | 1.8 |
| 8. Bulgarski ratund | System 1 | 20.84 | 30.84 | 10 | 2.5 |
| | System 2 | 30.63 | 34.83 | 5.58 | 0.38 |
| | System 3 | 28.05 | 40.27 | 18.34 | 1.38 |

System 1 – conventional production; System 2 – natural fertility without plant protection; System 3 – fertilization with bio humus and protection with biopesticides riod, a strong stolbur attack is found, which averaged from 24.10% for Stryama to 32.15% for Kapia UV. The brown leaf spot attack averaged between 29.05% for line K992 to 36.26% for line K995. The data prove that the lowest virus attack is studied – less than 12% (Table 2).

In recent years, there have been changes in the density and species composition of pepper's pests.Green peach aphid (*Myzus persicae* Sulz.), thrips (*Frankliniella occidentalis* Perg., *Thrips tabaci* Lindeman) and cotton bollworm (*Helicoverpa armigera* Hubn.) are common and have become economically important pests. Damage from these enemies leads to deterioration of production qualities and reduction of yields. Aphids and thrips are vectors of viral diseases (Yankova et al., 2021).

Entomological surveys and observations were carried out to report the degree of attack by pests in the different genotypes of pepper included in the study. During seedlings and immediately after transplanting, single plants with thrips (*Thysanoptera:Thripidae*) damage were observed. During the growing season, an attack of cotton bollworm (*Helicoverpa armigera* Hubn.) was found, but no aphid colonies (*Homoptera*: *Aphididae*). The attack by the cotton bollworm in the genotypes studied in the different production systems had statistically proven differences in the least attacked genotype K995 and the most heavily attacked Sivria 600 (Table 2).

Phytoplasmas are pathogens of important annual crops as well as perennial cultures, causing different symptoms that ranges from yellowing to death of infected plants. These pathogens multiply within the phloem cells of the host plant and are transmitted from plant to plant by phloem-feeding insects and by vegetative multiplication of infected plant material (Weintraub & Beanland, 2006). Phytoplasmas spread through the plants by insect vectors during feeding activity and survive and multiply in the plant phloem and insecthemolymph (Bertaccini et al., 2014).

*Candidatus* Phytoplasma solani' is the most common phytoplasma, which has a wider range of host plants (Balakishiyeva et al., 2010). '*Ca.* P. solani', is widespread in all European and Mediterranean areas, and itscan seriously affect quality and quantity of production (Quaglino et al., 2013). This phytoplasma induces stolburdisease and infected plants show leaf discoloration, stunting and especially flower malformations, such vitrescence and phyllody, leading to sterility (Bertaccini & Duduk, 2009).

In our study, it is found that genotypes of pepper grown in different systems showed a strong stolbur attack, which is probably due to the strong prevalence of the pest transfer disease during the observed period.

*Alternaria* species can be broadly split into two categories: large spored species, which are relatively simple to diagnose due to their distinctive morphologies and stable host ranges, and small spored species, which are not (Peever et al., 2004; De Hoog & Horré, 2002). Fungi are widespread and exhibit broad variation in nutritional mode, living both as symbionts and as saprophytes on organic matter (Arnold & Lutzoni, 2007; Taylor et al., 2014; Sánchez-García et al., 2020). However, fungal ecology and evolution interface in varied ways; nutritional mode does not appear to be correlated with phylogeny for most fungi (Arnold et al., 2009; Chaverri & Samuels, 2013; Delaye et al., 2013), while other lineages seem constrained to a single lifestyle (Delaye et al., 2013; Wheeler et al., 2019).

During the study period, it was found that the examined peppers showed a high degree of attack by the causative agent of brown leaf spots.

The number of virus species infecting pepper (*Capsicum* spp.) crops and their incidences has increased considerably over the past 30 years, particularly in tropical and subtropical pepper production systems. This is probably due to a combination of factors, including the expansion and intensification of pepper cultivation in these regions, the increased volume and speed of global trade of fresh produce (including peppers) carrying viruses and vectors to new locations, and perhaps climate change expanding the geographic range suitable for the viruses and vectors. The main virus groups infecting peppers are transmitted by aphids, whiteflies, or thrips, and a feature of many populations of these vector groups is

**Table 2. Duncan test for reaction the pepper genotypes growth under different conditions to pathogens and pests**

| Genotype | *Phytoplasma solani* | | *Alternaria solani* | | Viruses | | *H. armigera* | |
|---|---|---|---|---|---|---|---|---|
| 1. Sivria 600 | 24.94 | a | 30.83 | a | 10.01 | a | 3.48 | a |
| 2. Stryama | 24.10 | a | 30.70 | a | 8.38 | a | 3.13 | ab |
| 3. Milkana F1 | 24.24 | a | 30.51 | a | 7.54 | a | 3.34 | ab |
| 4. K992 | 27.54 | a | 29.05 | a | 6.04 | a | 0.92 | ab |
| 5. K993 | 30.96 | a | 29.41 | a | 8.60 | a | 2.32 | ab |
| 6. K995 | 28.49 | a | 36.26 | a | 8.40 | a | 0.49 | b |
| 7. Kapia UV | 32.15 | a | 32.25 | a | 10.62 | a | 2.91 | ab |
| 8. Bulgarski ratund | 26.51 | a | 35.31 | a | 11.31 | a | 1.42 | ab |

that they can develop resistance to some of the commonly used insecticides relatively quickly. This, coupled with the increasing concern over the impact of over- or misuse of insecticides on the environment, growers, and consumers, means that there should be less reliance on insecticides to control the vectors of viruses infecting pepper crops. Instead, integrated and pragmatic virus control measures should be sought that combine (1) cultural practices that reduce sources of virus inoculum and decrease the rate of spread of viruliferous vectors into the pepper crop, (2) synthetic insecticides, which should be used judiciously and only when the plants are young and most susceptible to infection, (3) appropriate natural products and biocontrol agents to induce resistance in the plants, affect the behavior of the vector insects, or augment the local populations of parasites or predators of the virus vectors, and (4) polygenic resistances against viruses and vector insects with pyramided single-gene virus resistances to improve resistance durability (Kenyon et al., 2014).

The least attack of viruses on the observed genotypes compared to other phytopathogenic factors is recorded.

## Conclusion

When examining the plantation for attack by phytopathogenic factors, the attack by stolbur and brown leaf spots in total for all systems of cultivation is the highest degree. During the period, a strong stolbur attack was found, which averaged from 24.10% for Stryama to 32.15% for Kapia UV. The brown leaf spot attack averaged between 29.05% for line K992 to 36.26% for line K995. The least virus attack has been shown to be less than 12%. The attack by the cotton bollworm in the genotypes studied in the different production systems was lowest in system 2 – K995 (0%) and in system 3 – K992 and K995.

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