



# Article Organic Production of Snap Bean in Bulgaria: Pests and Diseases Incidence and Control, Soil Fertility and Yield

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**Abstract:** Among factors affecting snap bean production in organic growing systems, pests and diseases are of paramount importance. The current study was aimed to determine the impact of organic production practices on snap bean pests and diseases infestation, soil fertility and yield. Five treatments of plants during the whole growing season with a Bordeaux mixture at a dose of 3000 g/ha, provided more than 50 percent protective effect against the development of the bacterial blight *Xanthomonas axanopodis* pv. *phaseoli* and anthracnose *Colletotrichum lindemuthianum*. In organic fields, commercial bioproducts containing pyrethrins or entomopathogenic fungi *Beauveria bassiana* can be successfully applied to control the black bean aphid (*Aphis fabae* Scop.). To limit the attack of bean weevil (*Acanthoscelides obtectus* Say), phytopesticide containing pyrethrins can also be used. Against the two-spotted spider mite (*Tetranychus urticae* Koch.), products containing the active ingredient azadirachtin were seen to be effective five days after treatment. Soil amendment with vermicompost at a dose of 2850 L/ha slightly increased the amounts of water-soluble nutrients; however, soil remained nutrient deficient across the growing season. Among the tested Bulgarian varieties, Evros possessed higher yield, and appear to be suitable for organic system than the Tangra variety.

Keywords: Phaseolus vulgaris L.; seed-borne pathogens; pest management; organic

# 1. Introduction

The snap bean (*Phaseolus vulgaris* L.) belongs to the *Fabaceae* legume family, and *Phaseolus vulgaris* L. is the only species that has distinct subspecies that are grown for snap beans. It is a unique vegetable that is enriched with protein content and is highly palatable [1]. In parallel, it also plays a synergistic role in organic farming systems due to its deep root system, and the ability to fix nitrogen from air, which makes it an ideal crop to consider for crop rotation [2,3].

A myriad of factors could affect snap bean growth and yield in organic systems. Undoubtedly pests and disease occurrences are of paramount importance as they could reduce the yield significantly and deteriorate the quality. Usually, it is seed transmitted diseases that mostly attack snap bean. Notably, bacterial blight *Xanthomonas axanopodis* pv. *phaseoli* [4] (Xap), halo blight *Pseudomonas savastanoi* pv. *phaseolicola* (Psp) and anthracnose *Colletotrichum lindemuthianum* [5–7] have been seen as the most harmful. These pathogens can persist in seeds for several years and reduce seed germination, cause rotting and seedlings death, as well reduce yield and quality [8]. Snap bean varieties developed at Maritsa Vegetable Crops Research Institute (MVCRI), Bulgaria, possess high productivity, good taste and relative tolerance to major diseases when grown conventionally from disinfected seeds [9]. Since seed disinfection is prohibited in organic systems, there is a need of effective alternative selection [10].

Aphids (Hemiptera: Aphididae) are a major pest group not only due to their ability of direct host damage, but also as a vector of several viruses [11]. Polyphagous black bean



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**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). aphid (Aphis fabae Scopoli) damages different parts of the plant [12] and can cause significant yield loss estimated to be about 37% [13]. Bean weevil (Acanthoscelides obtectus Say) is another important pest especially for bean seed production. The relatively short window of oviposition in the field is considered to be critical time for bean weevil control [14]. Since biological production is becoming more widespread in many countries, efforts have been directed towards the use of natural plant protection products such as phytopesticides. Many plants have insecticidal properties due to the presence of natural alkaloids, esters and other compounds [15]. Azadirachtin-based insecticides (Azadirachta indica A. Juss) are characterized as limonoids that act as antifeedant and deterrent [16–19]. Pyrethrins are extracted from flowers and seeds of the Asteraceae pyrethrum plant (Chrysanthemum cinerariaefolium Vis.). Worldwide, pyrethrum has been used as a safe and effective as pesticide for several years. Pyrethrum flowers contain pyrethrins that are synthesized from a mixture of esters. Pyrethrins have a broad-spectrum activity on most pests, causing quick knockdown effect as a contact and stomach poison. Simultaneously, they break down rapidly in the sunlight and, hence, do not cause harm to the environment and provide residue-free food [20–22]. Due to these unique properties of affecting only target pests, being highly effective when applied in a very small quantity, and demonstrating quick decomposition, pyrethrins and other botanical products have steered an interest among scientific community [23].

Besides plant derived insecticides, biological control using different predators, parasites and pathogens is another approach have been effective in organic systems. Entomopathogenic fungi produce metabolic compounds that may be toxic to insects and can be used to control pests as they are seen to be not detrimental against other non-target organisms [24]. For example, the fungal culture or culture filtrate of *Beauveria bassiana* (Bals.-Criv.) Vuill has potential for development as a mycopesticide to control the aphids [25].

Organic cultivation relies entirely on ecological practices such as bio-control and enhancement of plant health and soil fertility through natural processes. Maintaining good soil health by combining the use of naturally derived products to control pests and diseases is a key to obtaining high quality residue free snap bean production when grown on organic farms. In this respect, the aim of the current study was to determine the impact of organic production practices on snap bean pests and diseases infestation, soil fertility and yield.

## 2. Materials and Methods

The experiments were carried out during 2019–2022 at the Maritsa Vegetable Crops Research Institute, Plovdiv, Bulgaria, in a certified organic field at natural pest occurrence. Two snap bean varieties (Tangra and Evros, developed at MVCRI) were grown under different organic plant protection and fertilization treatments. Non-treated plants were used as controls. Each year, seeds were sown on 15–20 April (dates varied in different years) and were harvested until 25 July–19 August (dates varied in different years). Experiments included only one crop cycle each year.

#### 2.1. Disease Monitoring and Management

The degree of infection from bacterial blight and anthracnose was reported on a 4-point scale: 0—0–10% without or very weak symptoms; 1—from 11% to 25% of the surface with spots; 2—from 26 to 50% of the surface with spots; 3—from 51 to 75%; 4—over 75% of the surface with spots. The index of infestation was calculated according to McKinney's formula [26]. Biological activity of two fungicides Bordeaux Mix 20 WP 3000 g/ha (a.i., bordeaux mixture) and Funguran ON 50WP 3000 g/ha (a.i., copper hydroxide) was studied by two treatments in interval of 14 days and calculated according to Abbott [27]. Effectiveness of two chemical products containing Azoxystrobin and difenoconazole and Penconazole against anthracnose was evaluated under conventional growing system and results are used as comparison. Based on the better results obtained for Bordeaux Mix 20 WP, five subsequent treatments at 10–14 days' intervals were applied.

## 2.2. Pests Monitoring and Management

# 2.2.1. Black Bean Aphid (Aphis fabae Scop.)

Aphid-infested plants were sprayed with the appropriate concentration of two insecticides: Krisant EC (a.i., pyrethrins) at 750 mL/ha and Naturalis (a.i., *Beauveria bassiana* strain ATCC 74040) at 1000 mL/ha were applied once before flowering when the highest density of the pest was observed. The number of alive individuals were visually recorded on the field before treatment and after at the intervals of 1, 3, 5, 7, 10 and 14 days. Insecticide effectiveness (%) was calculated by the Henderson–Tilton formula [28].

## 2.2.2. Bean Weevil (*Acanthoscelides obtectus* Say)

Two insecticides, Krisant EC (a.i., pyrethrins) at a dose of 750 mL/ha and Neem Azal T/S 0.3% (a.i., azadirachtin), were applied twice at the intervals of 7 days. Representative samples of 100 pods were taken from each variant and the index of infestation (%) in seeds was calculated 50 days after harvesting according to the formula of McKinney [26]. Seed damages by bean weevil were classified from 0 to 4: 0—without holes; 1—with one hole from the weevil; 2—with two holes from the weevil; 3—with three holes from the weevil; 4—with four or more holes from the weevil. Pesticide effectiveness (%) was calculated based on the index of infestation by Abbott's formula [27].

## 2.2.3. Two-Spotted Spider Mite (Tetranychus urticae Koch.)

One plant protection product Neem Azal T/S 0.3% (a.i., azadirachtin) was tested. It was applied once during pods formation. The number of pest larvae, nymphs and adults was counted on four infested leaves per plant under stereomicroscope before the treatment and at 1, 3, 5, 7, 10 and 14 days post spraying. Effectiveness was calculated by the Henderson–Tilton formula.

#### 2.3. Organic Fertilization and Soil Properties Assessment

Commercial vermicompost manure was used to supply the nutrients needed for growth of bean plants. The vermicompost was made from 95% cow and 5% pig + horse dung. It contains 77.5% organic matter, 2192 mg/100 g extractable  $P_2O_5$  and 10,500 mg/100 g extractable  $K_2O$ . The soil amendment was applied twice during the growing season. The first application (1700 L/ha) was made a month after sowing and the second (1150 L/ha)—40 days later. Before sowing and throughout the vegetation, soil samples from all plots were taken and following parameters were assessed in water extract 1:2 according to Sonneveld [29]: pH, EC,  $NO_3^-$ , P, K, Ca, Mg.

## 2.4. Plant Growth and Productivity

At two maturity stages of technological maturity (fruit development when beans begin to fill out) and botanical maturity (fully ripen stage when beans are hard), several indexes were assessed in three replications with five plants per replication. Measured indexes were: plant height (cm), fresh weight (g), number of pods per plant and pods weight per plant (g). Additionally, number of seeds per plant and seeds weight (g) was assessed at botanical maturity.

# 2.5. Data Analyses

The trial was set up in randomized complete blocks (RCB) design. The experimental setup consisted of 12 plots of ~167 square meters each. Total area experimental area was 0.2 ha. Each variant (treatment) consisted of three replications, and each replication was composed by 10 plants. The variants are spatially separated from each other by plants treated accordingly but not evaluated. A two-way analysis of variance (ANOVA) was used to test the effects of the year and treatment on treatment effectiveness. Since no effect of the year was established, nor any interaction between the two factors, data are presented as mean of three replications and processed using Duncan's multiple range test at p < 0.05 [30].

## 3. Results

#### 3.1. Diseases

During the three experimental years, bacterial blight was reported as the main bacterial disease affecting snap beans. First symptoms usually occurred after plant emergence of and developed massively during flowering. Observed infection appeared as water-soaked spots on the lower leaf surface and lesions were usually surrounded by a narrow yellow halo those, turned brown. Over time, the lesions enlarged and ran together resulting in extensive necrotic areas. Leaves often appeared burned when the infection was severe. Meanwhile, leaves became ragged and sparsely dropped prematurely. Disease symptoms on pods were observed with variable size lesions that appeared circular, sunken and reddish-brown. Infected seeds shriveled, discolored and exhibited poor germination and vigor.

Anthracnose *Colletotrichum lindemuthianum* is the most common fungal disease that occurred throughout the experimental years. Anthracnose symptoms appeared on leaves, stems, pods and seeds. The most easily recognized anthracnose symptom were seen on the pods. Small, reddish-brown elongated spots were formed initially. During wet weather, a mass of pinkish colored spores was seen on the lesions. Infection started with the pod end up infecting developing seeds. Infected seeds had dark, sunken lesions of various sizes, which extended through the seed coat. When infected seeds were planted, the fungus multiplied and formed lesions on the cotyledons, and eventually spread to other plant parts throughout the season.

Other bean diseases observed during the growing seasons were of varying importance and frequently occurred diseases were Bean common mosaic virus (BCMV) that caused damage almost every year, while fusarium root rot (*Fusarium solani* f. *faseoli*) showed significant damage in some years.

The choice of plant protection products depends on their biological effectiveness, stability and its impact on key economic indicators. Considering these indicators, a first step in the plant protection program was to assess the efficacy of several fungicides (Table 1).

Treatment	Bacterial Blig	ht	Anthracnose		
	Infestation Index, %	Effect, %	Infestation Index, %	Effect, %	
Control	30.3 <sup>a</sup>	-	25.4 <sup>a</sup>	-	
Bordeaux mixture	11.3 <sup>a</sup>	62.7 <sup>c</sup>	7.7 <sup>b</sup>	69.7 <sup>ab</sup>	
Copper hydroxide	12.3 <sup>a</sup>	59.4 <sup>c</sup>	7.3 <sup>b</sup>	71.3 <sup>ab</sup>	
Azoxystrobin and difenoconazole *	-	-	5.8 <sup>c</sup>	77.2 <sup>a</sup>	
Penconazole **	-	-	12.5 <sup>c</sup>	50.8 <sup>c</sup> *	

**Table 1.** Influence of fungicides treatments on bacterial blight and anthracnose development on snap bean.

\* Means, followed by the same letter ( $^{a}$ ,  $^{ab}$ ) do not differ significantly; different letters ( $^{a}$ ,  $^{b}$ ,  $^{c}$ ) in each column show significant difference according to Duncan's multiple range test (p < 0.05). \*\* Effectiveness of two chemical products containing Azoxystrobin and difenoconazole and Penconazole, evaluated in conventional snap bean field, were used as comparison.

As a result, the effect of double application of Bordeaux Mix 20 WP 3000 g/ha and Funguran ON 50 WP 3000 g/ha against bacterial blight and anthracnose was 65% and 66%, respectively. Against anthracnose, a comparison with two synthetic fungicides Ortiva Top 1000 mL/ha (a.i., azoxystrobin + difenoconazole) and Topaz 100 EC 500 mL/ha (a.i., penconazole), applied at flowering to pod formation in conventional field, showed 77.2% and 50.8% effectiveness, respectively. These results suggest that the Cu-containing products Bordeaux Mix 20 WP and Funguran ON 50 WP were equally effective to chemical fungicides against the main seed-borne diseases and, hence, can be used to control blight and anthracnose in snap bean production in organic system (Figure 1).

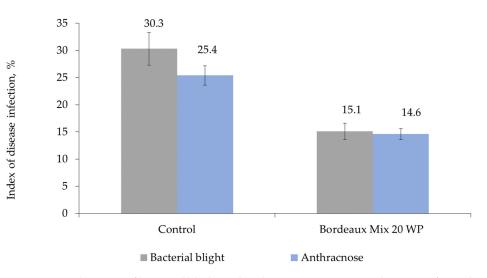


Figure 1. Development of bacterial blight and anthracnose in organic cultivation of snap bean.

The relatively lower effect of the applied plant protection products during the growing season was likely because the used seeds were non-treated before sowing. Hidden asymptomatic form of the infection resulted in a sustained increase in disease incidence in the crop over the years. The rate of seed infection increased over the years of the experimental period (Figure 2).

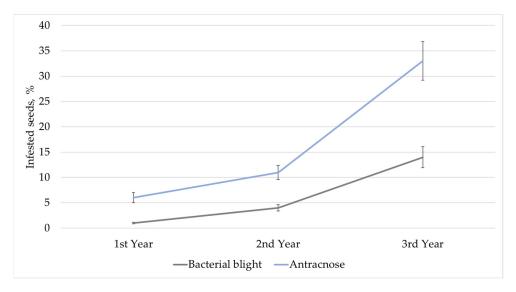


Figure 2. Dynamic of bacterial blight and anthracnose infection in bean seeds.

# 3.2. Pests

Biological activity assessment of two products against *A. fabae* showed that the product Krisant EC (750 mL/ha) had very good effectiveness (85.8%) within days of treatment, which was probably due to its good contact action inherent in pyrethrins. Naturalis (1000 mL/ha) also demonstrated good effectiveness against this pest, with maximum value recorded after the 7th day of the treatment (E = 83.5%). With Naturalis, no elevated initial activity was reported, but a good effectiveness of over 74% was observed in the interval 5–10 days after treatment. This is due to the mechanism of action of microbial products (Table 2). Against the bean weevil, phytopesticide Krisant EC showed relatively better biological activity in both varieties studied here, with an overall effectiveness of 75% (Table 3).

Days after Treatment	Krisant EC 750 mL/ha	Naturalis 1000 mL/ha
1	72.6 <sup>bc</sup>	58.4 <sup>c</sup>
3	85.8 <sup>a</sup>	71.0 <sup>ab</sup>
5	84.9 <sup>a</sup>	74.5 <sup>ab</sup>
7	80.1 <sup>a</sup>	83.5 <sup>a</sup>
10	70.7 <sup>bc</sup>	75.6 <sup>ab</sup>
14	60.6 <sup>c</sup>	64.4 <sup>c</sup> *

Table 2. Effectiveness (%) of plant protection products against black bean aphid (Aphis fabae Scop.) in organic bean fields.

\* Means, followed by the same letter (<sup>a</sup>, <sup>ab</sup>; <sup>b</sup>, <sup>bc</sup>) do not differ significantly; different letters (<sup>a</sup>, <sup>b</sup>, <sup>c</sup>) in each column show significant difference according to Duncan's multiple range test (p < 0.05).

<b>Table 3.</b> Effectiveness of plant protection products against bean weevil ( <i>Acanthoscelides obtectus</i> Say) in two bean varieties, Tangra and Evros, grown in organic field.								
Treatment/Variety	<b>T</b> . 1	Infested Seeds						
	Total Number of Seeds	Number	%	<ul> <li>Index of Infestation, %</li> </ul>	Effectiveness, %			
Krisant EC/Tangra	452	10	2.2	1.1	77.1 <sup>ns</sup>			
Krisant EC/Evros	512	32	6.3	3.5	75.2 <sup>ns</sup>			
Neem Azal/Tangra	448	14	3.1	1.3	72.9 <sup>ns</sup>			
Neem Azal/Evros	506	39	7.7	3.7	73.7 <sup>ns</sup> *			
Control/Tangra	408	38	9.3	4.8	-			
Control/Evros	447	114	25.5	14.1	-			

\* <sup>ns</sup>—non-significant difference according to Duncan's multiple range test (p < 0.05).

In recent years, unpredictable weather events caused by climatic changes seem to have increased the occurrence of the two-spotted spider mite (Tetranychus urticae Koch.) in bean crops. The highest population density was found in the period from the second ten days of July to the second ten days of August, when average daily temperatures were highest [31]. In our experiments, we observed good biological activity of Neem Azal against the two-spotted spider mite. The maximum value of effectiveness against the mobile forms of *T. urticae* was 84% and was reported on the fifth day after treatment (Table 4).

Table 4. Effectiveness of the product Neem Azal T/S 0.3% against two-spotted spider mite (Tetranychus urticae Koch.) in organic bean field.

	Populat					
Days Post-Treatment	Control Pre-Treatment	Control Post-Treatment	Neem Azal T/S Pre-Treatment	Neem Azal T/S Post-Treatment	Effectiveness, %	
1	35.3 <sup>a</sup>	40.3 <sup>c</sup>	41.5 <sup>a</sup>	19.0 <sup>ab</sup>	59.9 <sup>c</sup>	
3	35.3 <sup>a</sup>	43.8 <sup>c</sup>	41.5 <sup>a</sup>	13.8 <sup>bc</sup>	73.3 <sup>ab</sup>	
5	35.3 <sup>a</sup>	49.0 <sup>bc</sup>	41.5 <sup>a</sup>	9.3 <sup>c</sup>	84.0 <sup>a</sup>	
7	35.3 <sup>a</sup>	52.3 <sup>ab</sup>	41.5 <sup>a</sup>	12.0 <sup>c</sup>	80.5 <sup>a</sup>	
10	35.3 <sup>a</sup>	54.8 <sup>a</sup>	41.5 <sup>a</sup>	16.8 <sup>b</sup>	74.0 <sup>ab</sup>	
14	35.3 <sup>a</sup>	59.5 <sup>a</sup>	41.5 <sup>a</sup>	24.0 <sup>ab</sup>	65.7 <sup>c</sup> *	

\* Means, followed by the same letter (<sup>a</sup>, <sup>ab</sup>; <sup>b</sup>, <sup>bc</sup>) do not differ significantly; different letters (<sup>a</sup>, <sup>b</sup>, <sup>c</sup>) in each column show significant difference according to Duncan's multiple range test (p < 0.05).

#### 3.3. Monitoring Soil Nutrients

The experiment trial was initiated at a low level of soluble soil nutrients, and this deficiency remained constant during whole growing season irrespective of vermicompost application (Table 5).

Sampling Date	Variety	pН	EC, mS/cm	N, mg/L	P, mg/L	K, mg/L	Ca, mg/L	Mg, mg/L
Mid-April	Before sowing	$6.55\pm0.15$	$0.05\pm0.01$	$10\pm5$	$8.1\pm2.3$	$13.3\pm2.5$	$4.0\pm1.5$	$9.6\pm2.5$
End of April	Evros	$6.52\pm0.12$	$0.04\pm0.01$	$10\pm7$	$1.8\pm0.5$	$8.3\pm1.5$	$4.0\pm1.5$	$12.0\pm2.8$
	Tangra	$6.44\pm0.11$	$0.04\pm0.01$	$10\pm 8$	$2.9\pm0.4$	$8.3\pm1.2$	$4.0\pm1.2$	$9.6\pm2.2$
Beginning of June	Evros	$5.76\pm0.13$	$0.05\pm0.02$	$0\pm 5$	$0.9\pm0.1$	$5.0 \pm 1.0$	$4.0\pm1.3$	$7.2\pm2.1$
	Tangra	$5.60\pm0.10$	$0.04\pm0.01$	$0\pm 5$	$0.0\pm0.3$	$5.0 \pm 1.1$	$4.0\pm1.5$	$7.2\pm2.0$
End of June	Evros	$7.20\pm0.16$	$0.09\pm0.02$	$25\pm10$	$2.2\pm0.3$	$13.3\pm2.6$	$4.0\pm1.5$	$12.0\pm2.6$
	Tangra	$7.14\pm0.12$	$0.10\pm0.03$	$25\pm 8$	$0.0\pm0.8$	$13.3\pm2.3$	$4.0\pm1.1$	$9.6\pm2.5$
Beginning of August	Evros	$7.00\pm0.06$	$0.10\pm0.01$	$25\pm5$	$1.6\pm0.5$	$13.3\pm2.4$	$4.0\pm1.0$	$9.6\pm2.3$
	Tangra	$7.02\pm0.10$	$0.11\pm0.03$	$25\pm5$	$1.4\pm0.7$	$16.6\pm2.6$	$4.0\pm2.0$	$12.0\pm2.5$

**Table 5.** Soil analyzes during growing seasons of two bean varieties grown in organic field. Data arepresented as average from three years  $\pm$  standard deviation.

Before sowing (sample from mid-April), electrical conductivity (EC) of soil solution was very low (0.05 mS/cm), which corresponded to the low macronutrients content for all tested macronutrients, except P. Nutrient analysis of soil samples collected after ten days of sowing (end of April), which showed that the nutrients in soil were also low in both tested varieties. Right after the first application of vermicompost (beginning of June), the amounts of N, K and Mg, as well as pH, became lower, while Ca content and EC had not changed. After the second application of vermicompost (end of June), a slight increase in tested soil parameters was observed, generally N and EC, which was retained until the end of experiment.

# 3.4. Plant Growth and Productivity

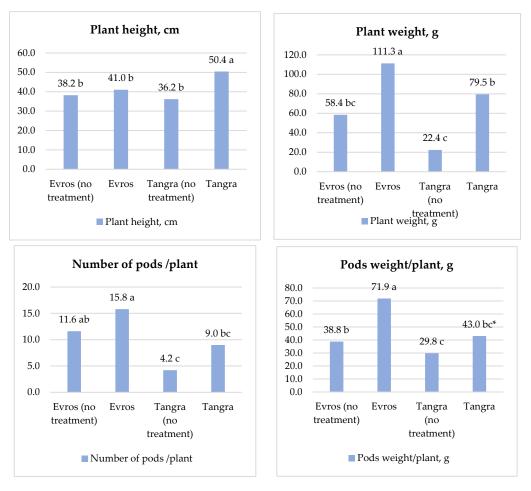
The highest plants were produced by the Tangra variety in both plant development stages with height of 50.4 cm and 47.4 cm (Figure 3 and Table 6). At technological maturity, plant weight was highest in the Evros variety (111.3 g), while at botanical maturity, Tangra had heaviest plants (9.1 g). At technological maturity, the Evros variety formed the highest number and the heaviest green pods (15.8 and 71.9 g, respectively), which were significantly different from the Tangra variety. At botanical maturity, no differences between the varieties were observed for both traits.

**Table 6.** Biometric measurements of green bean varieties grown in organic field at botanical maturity (fully ripe stage when beans are hard).

Varieties	Plant	Plant	Number of	Pods	Number of	Seeds	Seed
	Height, cm	Weight, g	Pods/Plant	Weight/Plant, g	Seeds/Plant	Weight/Plant, g	Yield, kg/da
Evros (no treatment)	36.6 <sup>b</sup>	3.9 <sup>c</sup>	7.4 <sup>ns</sup>	11.2 <sup>ns</sup>	35.8 <sup>ab</sup>	8.1 <sup>a</sup>	161.0 <sup>a</sup>
Evros	37.4 <sup>b</sup>	6.3 <sup>b</sup>	7.2 <sup>ns</sup>	9.4 <sup>ns</sup>	41.6 <sup>a</sup>	8.9 <sup>a</sup>	178.8 <sup>a</sup>
Tangra (no treatment)	38.2 <sup>b</sup>	2.9 <sup>c</sup>	5.6 <sup>ns</sup>	7.2 <sup>ns</sup>	19.4 <sup>c</sup>	4.4 <sup>b</sup>	87.6 <sup>b</sup>
Tangra	47.4 <sup>a</sup>	9.1 <sup>a</sup>	7.2 <sup>ns</sup>	11.1 <sup>ns</sup>	29.0 <sup>bc</sup>	8.2 <sup>a</sup>	164.6 <sup>a</sup> *

\* Means, followed by the same letter ( $^{a}$ ,  $^{ab}$ ;  $^{b}$ ,  $^{bc}$ ) do not differ significantly;  $^{ns}$ —non significant difference; different letters ( $^{a}$ ,  $^{b}$ ,  $^{c}$ ) in each column show significant difference according to Duncan's multiple range test (p < 0.05).

The number of seeds per plant significantly differed between the two varieties—41.6 for Evros versus 29.0 for Tangra. Both varieties had statistically similar values for seed weight per plant and seed yield (Table 6). For most plant growth and productivity traits, higher values were recorded in treated with organic pesticides plants, suggesting that the applied plant protection measures can limit pest attacks and therefore can reduce the risk of yield losses.



**Figure 3.** Biometric measurements of snap bean varieties grown in organic field at technological maturity (fruit development when beans begin to fill out). \* Means, followed by the same letter (a, ab; b, bc) do not differ significantly; different letters (a, b, c) in each chart show significant difference according to Duncan's multiple range test (p < 0.05).

#### 4. Discussion

Plant protection in organic fields entirely depends on adoption of preventive measures and techniques encouraging natural pest control. Often, an outbreak of pests imposes direct regulatory measures including application of approved biopesticides. Bordeaux mixture, containing copper sulfate, calcium hydroxide and water, is one of the first fungicides. It is commonly used in organic systems pest management and it is highly effective against bacterial and fungal diseases and used to manage several plant diseases [32]. The current study examined two Cu-containing substances (Bordeaux mixture and Copper hydroxide), both are authorized to be used in organic fields under specific conditions and limits. Similar biological effectiveness of the two products against bacterial blight and anthracnose observed in this study suggests that they can be successfully used to control seed-borne diseases in organic cultivation of snap bean. Similarly, other authors observe good performance of copper-containing products in controlling anthracnose and blight diseases and increasing yield of bean and other crops [33,34]. In this study, use of non-pre-treated seeds resulted in a continued increase in diseases incidence over three years. This suggests that preventive control measures might be most important in organic snap bean growing and these measures are: use of disease-free seeds, crop rotation and weed control. Fungicide treatments should be applied if disease is present and if weather conditions would favor its development.

Pest management includes the use of plant insecticides as a suitable alternative for plant protection [16,35], since they have minimal residual effects and no pest resistance has

been established against them. Most widely distributed and used commercial botanical pesticides include pyrethrum and neem products [36]. Botanical pesticides are subject to many tests, performed mainly in laboratories [37], but very few studies have shown their practical usefulness. Moreover, there is a lack of comparisons of biological effectiveness of a product on different pests' species. The current study presents the biological activity of two commercial formulations of plant insecticides against the widespread pests of bean grown in organic field. Our findings showed a very good effectiveness of 85.8%, three days after treatment of the product with active ingredient pyrethrins against black bean aphid. These results are in agreement with Pavela [38], who reported that extracts obtained from *Chrysanthemum cinerariifolium* caused 98.3% mortality against *Myzus persicae* Sulzer after two days of treatment. This was due to the rapid contact action that pyrethrins have, exerting their insecticidal effect by modulating the activity of voltage-gated sodium channels in the insect's nervous system [39].

Entomopathogenic fungus *Beauveria bassiana* is a biocontrol agent that infects insect body by contact and is widely distributed in nature with wide range of hosts. It is safe for non-target organisms such as predators and parasitoids [40]. After invading the insect host, *B. bassiana*, produces many different secondary metabolites, such as beauvericin, bassianin, bassianolide, beauverolides, tenellin, oosporein and oxalic acid. These toxins induce a range of symptoms in the host, including severe dehydration, abnormal behavior, lack of coordination, convulsions, hindered feeding and metabolic disorders that eventually cause insect death [41]. Our data showed that the product Naturalis, containing *B. bassiana*, exhibited 83.46% effectiveness against *Aphis fabae* after seven days of treatment. Similarly, Omar and co-authors [42] investigated three different concentrations  $(1 \times 10^2; 1 \times 10^4; and$  $1 \times 10^8$  spores/mL) of *B. bassiana* on adults of black bean aphid and reported 80% mortality on third day at the highest concentration  $(10^8 \text{ spores/mL})$ .

Azadirachtin, a triterpenoid found in seeds of neem trees is one of most successful botanical pesticides, effective against a broad range of insect pest species. Azadirachtin- and neem-based products have also been evaluated against the spider mite [43] and considered as valuable tool to control it [44]. Azadirachtin causes several negative effects on arthropods, such as repellency, feeding inhibition, decreased oviposition, reduced fertility and fecundity, changes in behavior and increased mortality [15,45,46]. In the current study, the maximum *T. urticae* population reduction was observed five days after treatment. Normally, the highest peaks of neem-derived products that translocate in the plant occur on the fifth day after treatment and are stored in the roots, stems and leaves of plants up to a maximum of eight days. Afterwards, the effectiveness of the products declines [47].

In organic fields, the only way to add more nutrients to the soil is the application of composts or vermicomposts as soil amendments. Vermicompost is considered a better alternative to the conventional compost due to better physical properties, higher microbial and enzymatic activity, and higher content of available nutrients [48,49]. In the current study, the application of 2850 L/ha vermicompost assured normal plant growth and satisfactory yield, despite of the low content of soluble soil nutrients throughout the experiment. As suggested by other authors, nutrients in vermicompost are not the sole factor that influence plant growth. It is well known that it can improve soil physical properties, nutrient turnover and increase the diversity and activity of soil microorganisms [49].

The current study describes several techniques that can be successfully applied for organic growing of snap bean in open fields. They are in accordance with the current legislation adapted in Bulgaria, proved to be effective in a certified organic field and can contribute to increase the profitability of organic production. Hence, more research is needed to examine the long-term effect as well as the integration of other practices that can increase further benefits.

## 5. Conclusions

Bacterial blight (Xap) and anthracnose were the main diseases in snap beans in organic fields. The use of organic seeds resulted in a sustained increase in bacterial and fungal

infection in the snap bean over three experimental years. The two tested commercial Cu-containing fungicides had comparable efficiency compared to chemical and can be used to control blight and anthracnose in organic cultivation. The black bean aphid can also be successfully controlled by products containing pyrethrins or entomopathogenic fungi *Beauveria bassiana*. To limit the attack of the bean weevil (*Acanthoscelides obtectus* Say), phytopesticide containing pyrethrins can be used. Against the two-spotted spider mite (*Tetranychus urticae* Koch.), the product with active ingredient azadirachtin showed 84% effectiveness on the fifth day after treatment.

Vermicompost slightly increased the amounts of water-soluble nutrients in the soil after the second application; however, organic maintained soil remained nutrient deficient throughout the growing season. The Evros variety produced a higher yield, which makes it more suitable for organic growing than the Tangra variety.

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