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Article

Organic fertilizers diminish two important pests on collard green compared to synthetic fertilizer

Virginia Sousa[1]
Maurício Ursi-Ventura[2,§]
Fernando Teruhiko-Hata[3]
Leonel Vinícius-Constantino[2]

1 Universidade Rovuma-Extensión Niassa-Departamento de Ciencias Agrícolas y Alimentarias. Niassa, Lichinga, Mozambique.

2 Universidade Estadual de Londrina-Centro de Ciencias Agrícolas. Rodovia Celso García Cid, PR 445 km 380, PO Box 86055-900 Londrina-PR, Brasil.

3 Universidade Estadual de Maringá-Departamento de Agronomia. Av. Colombo, 5790 - Zona 7, PO Box 87020-900, Maringá-PR, Brasil.

Autor para correspondencia: mauricioursiventura@gmail.com

Abstract

Plant fertilization may impact directly on insect pest behavior and biology. Fertilization may be accomplished by using synthetic mineral or organic fertilizers. The effect of different doses of boiled hen chicken manure, Penergetic®, Bokashi compound and mineral fertilizer on the adult population of *Bemisia tabaci* and *Myzus persicae* on collards leaves were evaluated. The experiment was conducted in the greenhouse using the treatments: Bokashi, Penergetic® bioactivator, 2.5%, 5%, and 7.5% of solution of boiled manure, mineral fertilization and control. The number of insects was counted directly on the leaves, and the nitrogen, potassium content, and total phenolic compound in the leaves were determined. Higher populations of *Bemisia tabaci* and *M. persicae* were observed on NPK-fertilized plants compared to other treatments. Higher levels of nitrogen foliar were found for Bokashi-fertilized plants; and higher concentrations of total phenolic compounds were found in the control. A negative correlation between total phenolics and *B. tabaci* was established suggesting the importance of these compounds in the species development. The results indicate the importance of the source of the fertilization for sustainable pest management.

Keywords:

Bemisia tabaci, Brassica oleracea var. acephala, Myzus persicae.

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Introduction

Collard green (*Brassica oleracea* L. var. *acephala*) (Brassicaceae) is an excellent source of nutrients, phenolics, organosulfurs, carotenoids, and others bioactive substances associated with the prevention of many diseases (Ramirez *et al*., 2020). Due to these characteristics, the vegetable is being increasingly consumed. Among the principal limitations to collards production stands out the incidence of whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) and *Myzus persicae* Sulzer (Hemiptera: Aphididae) which may cause significant damages and losses in production. *B. tabaci* is assumed to be a complex of at least 40 morphologically similar cryptic species (Li *et al*., 2021).

Individuals cause direct damage as phloem feeders (de Barro *et al*., 2011) and indirect damage as vector of more than 100 different plant viruses (Hogenhout *et al*., 2008). The aphid complex: *M. persicae*; *Lipaphis pseudobrassicae* Davis and *Brevicoryne brassicae* L. (Hemiptera: Aphididae) feeding causes chlorosis and foliar wrinkling and by the secretion of sugar solution, both aphids and whitefly, induce the development of mold fungi, which hinder photosynthesis by covering leaf surface (Michereff Filho *et al*., 2021).

Synthetic chemical insecticides are generally used for control of these pests; however, despite their efficiency, negative implications on the environmental and human health are frequently reported (Alengebawy *et al*., 2021). Furthermore, insects eventually may develop resistant to insecticides in responses to systematic applications, making their control even more difficult. These constraints generated efforts to develop eco-friendly strategies to manage these and other pests.

Studies have suggested that fertilization of plants influences the biology of pests (Hosseini *et al*., 2015; Hata *et al*., 2019; Ramachandran *et al*., 2020; Sousa *et al*., 2021). Morales *et al*. (2001) observed a lower incidence of aphids on maize plants fertilized with organic fertilizer when compared to those fertilized with synthetic chemicals. A higher density of *B. tabaci* on tomato plants was observed using increasing doses of nitrogen (Žaniç *et al*., 2011) and similar results were found for *Aphis craccivora* Koch (Hemiptera: Aphididae) (Hosseini *et al*., 2015).

Higher amounts of nitrogen fertilizer applied in the soil results in increasing concentrations in plant tissues. A positive correlation between nitrogen/protein concentrations in leaves *vs* phloem feeders' insects and mites' population have been evidenced (Sousa *et al.*, 2021; Puspitarini *et al.,* 2021; Li *et al*., 2022). On the other hand, secondary metabolites as phenolic compounds (Hata *et al*., 2019; Sousa *et al.*, 2021), glucosinolates (Dar, 2021) and terpenoids (Zanin *et al.*, 2021) are negatively correlated with incidence.

The organic fertilizers as Bokashi compost, boiled hen chicken manure and the bioactivator Penergetic® have been used as options to produce greens, vegetables, and fruits (Xavier *et al*., 2019; Maass *et al*., 2020; Hata *et al*., 2021a; Hata *et al.,* 2021b) for organic and conventional farming systems. However, information concerning the impact of these organic fertilizers and amendments on pest incidence are still scarce. Therefore, in this research, we evaluated the *B. tabaci* and *M. persicae* populations fertilized with Penergetic® bioactivator, Bokashi compound, NPK mineral fertilizer and doses of boiled hen chicken manure on collard plants in a greenhouse.

Materials and methods

Seedlings of collards Georgia cv. were sown in trays (December 23, 2016) and transplanted (January 24, 2017) to 5 L pots containing soil (Red Latosol, clay texture) in a greenhouse of the Universidade Estadual de Londrina-UEL, (23° 20' 28" S, 51° 12' 34" W, 548 m). In the second cycle, plants were sown in trays on April 15, 2017, and transplanted on May 15, 2017. Each pot corresponded to a plot. Plants were irrigated twice a day.

Phytosanitary measures were not accomplished. The treatments were: control (no fertilization), doses of boiled hen chicken manure (2.5%, 5% and 7.5%), EM Bokashi, Penergetic® bioactivator and mineral fertilizer (15-10-15; 15% nitrogen, 10% phosphorous and 15% potassium).

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Manure was prepared boiling 30 kg of fresh hen chicken during four hours. The solution was then filtered and diluted to the concentrations used in the experiment. The boiled manure treatments were applied twice a week (50 ml of solution per plant) from 38 days after sowing (DAS) to the last evaluation. For Penergetic treatments, 3 g of bioactivator Penergetic® k or p were diluted in 1.5 L of water and then applied on the soil (Penergetic® k) 38 DAS and sprayed on the plant (Penergetic® p) 68 DAS. Mineral fertilization was applied 38 and 48 DAS (10 and 5 g per plant, respectively). Bokashi was applied at 38, 48 and 60 DAS (25 g per plant in each stage).

The insects infesting plants were assessed twice a week in the morning, when *B. tabaci* adults were motionless compared to the warmer periods of the day. Seven leaves of the same size were randomly selected for each plot, carefully observing the abaxial and adaxial surfaces of the leaves. The same procedure was performed for *M. persicae* assessing three leaves per plot (120 samples per plot).

On March 24, 2017, sixteen leaf samples were collected from each treatment, cleaned with running water and distilled water. Next, the samples were dried in a forced air stove at 65° C for 72 h and leaves ground in a mill.

For nitrogen analysis, 0.1 g of each sample was digested at 350 °C which was gradually increased by 50 °C every 30 min, according to Kjeldahl's analytical method. For potassium determination, flame photometry method was used.

For total phenolic determination, the methodology from (Stratil *et al.*, 2006) with modifications was used. Extraction of 1 g of fresh leaf with 10 ml of absolute ethanol at 80% (v/v), stirring for 30 min at 120 rpm (Orbital-New Organic) was performed. After, the extract was then separated by centrifugation at 2 500 rpm (Excelsa 2 Fanem model 205 N) for 5 minutes.

For the analysis, an aliquot of 1 ml of extract, 1 ml of distilled water, 1 ml of Folin-Ciocalteau reagent 0.9 N and 1.0 of sodium carbonate 10% (m/v) were used. The mixture remained during 30 min in the dark under indoor environment temperature. Subsequently, the absorbance was measured at 720 nm in a Micronal spectrophotometer, AJX 1600. Gallic acid was used as the standard at 0.5, 20, 40, 60, 80, 100, 150 µg ml^{-1}. The results were shown as mg of Gallic acid equivalents in g per sample (mg EAG g^{-1}). Analysis was performed in triplicate.

A randomized block design with seven replications was used. To verify the assumptions for the analysis of variance, tests of the variance homogeneity and normality were performed. If the assumptions were met, the means were compared by the Tukey test (5%). Otherwise, the Friedman's test (5%) was used. A Pearson correlation matrix (1%) was used in each of the variables studied to infer the relationship between them. Statistical packages Past (Hammer *et al.*, 2001) and Bioestat 5.0 (Ayres, 2007) were used.

Results

In the first evaluation, by using NPK fertilization, a higher number of adults of *B. tabaci* per leaf ($F= 3.17$, $p< 0.05$) compared to treatments non fertilized and boiled manure 2.5% was found (Table 1). Other treatments (5%, 7.5%, Bokashi and Penergetic®) were intermediate. In the second and third evaluations, higher number of *B. tabaci* adults was found for the mineral fertilization treatment than the other treatments ($F= 61.27$ and 18.1, $p< 0.05$) (Table 1).

Table 1. Adults of *Bemisia tabaci* populations on collards greens leaves under different fertilizers. Londrina, Paraná, Brazil. Feb. to Mar. aw23 4 aw23 4 2017.

Treatments	Feb. 24	Feb. 28	Mar. 07	Mar. 10	Mar. 14	Mar. 18	Mar. 24	Mar. 28	Mean
Treatments	Feb. 24	Feb. 28	Mar. 07	Mar. 10	Mar. 14	Mar. 18	Mar. 24	Mar. 28	Mean
Control	0.25 ±0.41b	0.34 ±0.41b	0.23 ±0.16b	0 ±0b	0.07 ±0.08b	0.43 ±0.31a	0.29 ±0.12a	0.04 ±0.07a	0.3 ±0.29b

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Treatments	Feb. 24	Feb. 28	Mar. 07	Mar. 10	Mar. 14	Mar. 18	Mar. 24	Mar. 28	Mean
BM 2.5%	0.31 ±0.27b	0.54 ±0.34b	0.46 ±0.4b	0.25 ±0.14ab	0.21 ±0.08ab	0.46 ±0.25a	0.39 ±0.39a	0.3 ±0.36a	0.45 ±0.25b
BM 5%	0.77 ±0.7ab	0.31 ±0.29b	0.29 ±0.34b	0 ±0b	0.14 ±0.16ab	0.61 ±0.46a	0.32 ±0.24a	0.13 ±0.22a	0.29 ±0.22b
BM 7.5%	0.98 ±1.21ab	0.08 ±0.17b	0.2 ±0.24b	0.43 ±0.33ab	0.43 ±0.35ab	0.35 ±0.32a	0.12 ±0.16a	0.12 ±0.16a	0.34 ±0.31b
Penergetic®	0.88 ±0.7ab	0.49 ±0.44b	0.2 ±0.24b	0.23 ±0.1ab	0.18 ±0.27ab	0.46 ±0.38a	0.18 ±0.07a	0 ±0a	0.45 ±0.25b
Bokashi	1.12 ±1ab	0.46 ±0.46b	0.43 ±0.58b	0.64 ±0.41ab	0.93 ±0.76ab	1.29 ±0.9a	1.85 ±0.59a	2.81 ±1.54a	1.51 ±0.71b
Mineral (NPK)	2.15 ±0.87a	4.48 ±0.19a	2.98 ±0.98a	3.92 ±0.41a	5.19 ±2.74a	5.8 ±1.85a	3.26 ±1.54a	3.46 ±0.54a	3.82 ±0.99a
CV (%)	87.29	97.84	9.18	35.21	104.52	64.95	66.58	65.93	77.01
F or Fr*	3.17	61.27	18.1	18.75*	14.14*	12.18	15.86	16.26	11.12

BM= boiled manure; N= nitrogen; P= phosphorous; K= potassium. Means ± SD within a column followed by the same letter is not significantly different based on Tukey's test ($p > 0.05$) or the Friedman test (*) ($p > 0.05$).

In the fourth evaluation, higher adults of *B. tabaci* on the NPK fertilized plants than 5% of boiled manure and control were assessed. On the fifth evaluation, higher number was observed on NPK than the control treatment (74 times more insects). The means of all assessment indicated 6.82 times more insects in the NPK treatments than the means of the remaining ones.

In the fifth, sixth, and general evaluations for *M. persicae* in the first cycle, differences between treatments were observed (F= 17.41, $p < 0.05$; F= 14.94, $p < 0.05$; F= 61.03, $p < 0.05$). In the fifth and sixth evaluations, higher number of *M. persicae* adults was found in the NPK than the remaining treatments (Table 2). For the second cycle, higher magnitude of differences was observed for the fourth assessment in which NPK aphid treatments were higher than all other treatments (means 5.48 times more). The general means of the assessments, in general, higher populations were also found for NPK treatment (Table 3) (two times more insects).

Table 2. Adults of *Myzus persicae* populations on collards greens leaves under different fertilizers first cycle. Londrina, Paraná, Brazil. Feb. to Mar. 2017.

Treatments	Feb. 24	Feb. 28	Mar. 07	Mar. 10	Mar. 17	Mar. 24	Mar. 28	Mean
Control	1.17 ±1.37a	0.5 ±1a	3.75 ±4.84a	0.22 ±0.31a	6 ±4.25b	0.78 ±0.42b	9.33 ±13.29a	3.11 ±2.23b
BM 2.5%	1.67 ±1.33a	0 ±0a	2.25.8 ±4.5a	0.25 ±0.5a	2.5 ±3b	0 ±0b	0 ±0a	0.81 ±0.91b
BM 5%	0.33 ±0.47a	0 ±0a	0 ±0a	0. 42 ±0.5a	10.58 ±13.02b	12.83 ±16.19a	10.58 ±19.64a	1.79 ±2.4b
BM 7.5%	2 ±2.31a	3.75 ±3.28a	3.67 ±3.71a	2.67 ±4.49a	17.75 ±21.24b	0.68 ±0.94b	14.33 ±26.49a	3.06 ±2.29b
Penergetic®	0.33 ±0.67a	4.67 ±5.16a	1.67 ±3.33a	0.25 ±0.32a	0.5 ±1b	3.17 ±2.38a	2.42 ±2.82a	1.86 ±1.36b
Bokashi	0.25 ±0.5a	3.92 ±6.18a	8.42 ±16.17a	1.67 ±2.91a	2.75 ±3.77b	0 ±0b	6.08 ±7.32a	1.59 ±2.24b
Mineral (NPK)	0 ±0a	4.33 ±3.41a	15.11 ±19.52a	28.58 ±42.13a	68.79 ±16.72a	43.92 ±14.84a	23.08 ±15.83a	22.06 ±1.71a
CV (%)	175.4	144.91	202.15	330.01	74.61	95.35	159.55	39.8
F	1.35	1.48*	1.06	1.71	17.41*	14.94*	1.07	61.03

BM= boiled manure; N= nitrogen; P= phosphorous; K= potassium. Means ± SD within a column followed by the same letter is not significantly different based on Tukey's test ($p > 0.05$) or the Friedman test (*) ($p > 0.05$).

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Table 3. Adults of *Myzus persicae* populations on collards greens leaves under different fertilizers second cycle. Londrina, Paraná, Brazil. July 2017.

Treatments	Jun. 11	Jun. 16	Jun. 20	Jun. 24	Jun. 28	Mean
Control	2.08 ±3.95 a	4.25 ±2.95 ab	9.33 ±5.53 a	7 ±3.63 b	6.38 ±4.71 a	5.81 ±0.73 b
BM 2.5%	0.92 ±0.2 a	3.67 ±2.58 ab	16.08 ±4.76 a	6.5 ±4.43 b	6.83 ±3.18 a	6.8 ±0.73 ab
BM 5%	1.92 ±2.78 a	7.33 ±0.98 a	7 ±8.29 a	3.58 ±2.66 b	9.08 ±3.46 a	5.78 ±1.95 b
BM 7.5%	4.58 ±3.04 a	4.75 ±2.44 ab	6.33 ±5.57 a	3.33 ±2.74 b	9.42 ±4.79 a	5.68 ±2.48 b
Penergetic®	0.33 ±0.47 a	5.5 ±1.91 ab	12.75 ±7.41 a	6.08 ±3.68 b	4.58 ±4.57 a	5.85 ±2.34 b
Bokashi	2.58 ±0.42 a	1.83 ±1.29 b	9.72 ±6.33 a	6.33 ±6.72 b	5.22 ±2.86 a	5.14 ±2.66 b
Mineral (NPK)	1.67 ±0.38 a	5 ±1.25 ab	12.17 ±5.59 a	30 ±12.84 a	10.67 ±5.94 a	11.9 ±3.48 a
CV (%)	110	44.19	50.38	30.57	58.19	39.8
F or Fr*	1.5*	2.74*	1.68*	7.4*	1.12	61.03

BM= boiled manure; N= nitrogen; P= phosphorous; K= potassium. Means ± SD within a column followed by the same letter is not significantly different based on Tukey's test ($p > 0.05$) or the Friedman test (*) ($p > 0.05$).

Higher nitrogen contents were observed in plants fertilized using Bokashi than control, boiled manure 2.5% and Penergetic (Figure 1A). Higher potassium levels were found in NPK fertilized plants than boiled manure 2.5% and control (Figure 1B). For total phenolics, higher contents were found for control treatment than NPK, Bokashi, Penergetic and boiled manure 7.5% (Figure 1C).

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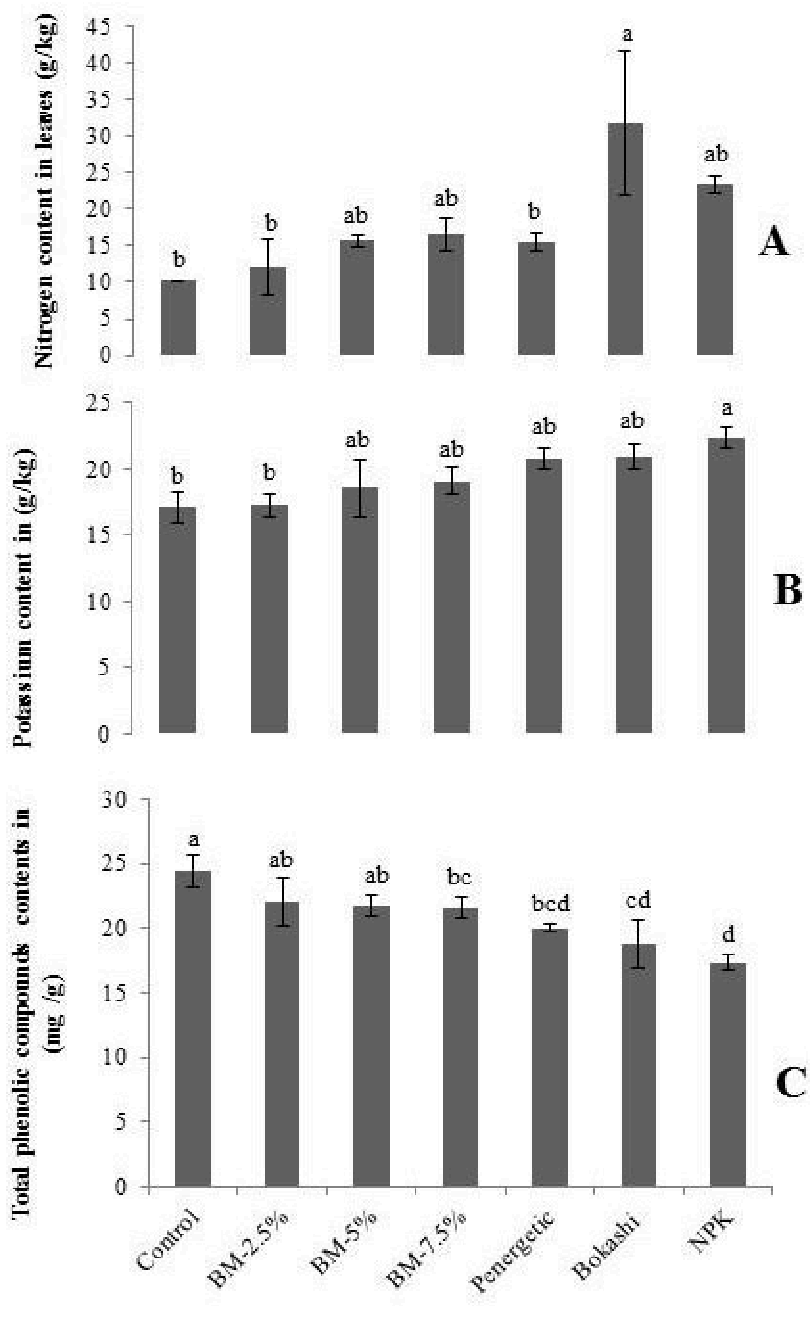
Figure 1. Leaf content of nitrogen (A); potassium (B) and total phenolic compounds (C), present in collards greens leaves fertilized with different doses and sources of organic fertilizers. Different letters on the columns indicate significant difference by the Tukey test (α= 5%). Bars in the columns indicate the standard deviation. Londrina, PR, Brazil, from February to June 2017.



Positive correlations between *B. tabaci* population and *M. persicae* (r^2= 0.92; p< 0.01); *B. tabaci* and potassium content in leaves (r^2= 0.84; p< 0.01) were found (Figure 2). A negative correlation

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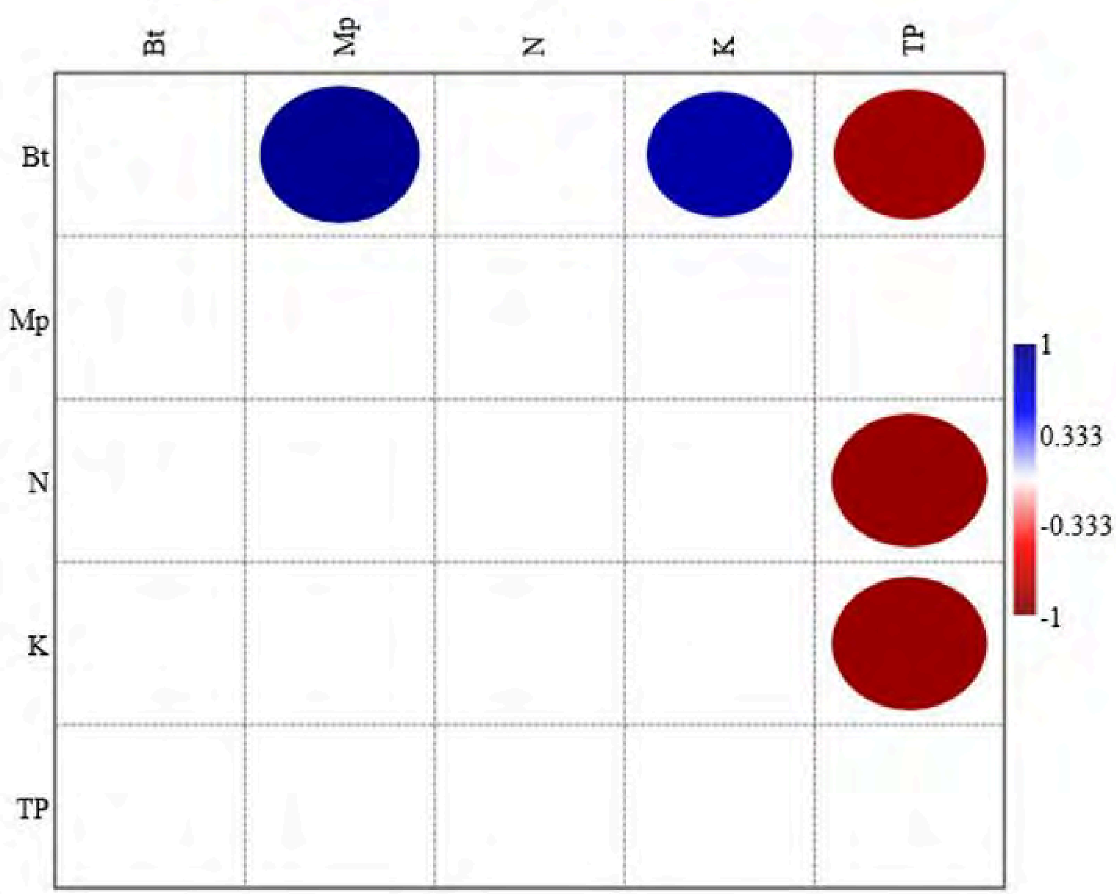
was observed between total phenolics and *B. tabaci* (r^2= -0.87; *p*< 0.01), nitrogen (r^2= -0.9; *p*< 0.01) and potassium (r^2=- 0.9; *p*< 0.01) (Figure 2).

Figure 2. Pearson correlation matrix between variables evaluated in the experiment. Bt= *Bemisia tabaci*; Mp= *Myzus persicae*; N= nitrogen; K= potassium; TP= total phenolics. Blue color represents the '+1' correlation coefficient and red color represents the '-1' correlation coefficient. Only significant (*p*< 0.05) correlations were shown). Londrina, PR, Brazil, from February to June 2017.



Discussion

Treatments affected the contents of the two nutrients studied (Figure 1). The nitrogen content in the current study was generally lower than that found in collard plants fertilized with pig slurry, poultry manure, and urea which varied from 29 to 35.7 g kg^{-1} N in collards leaves (Steiner *et al*., 2019). The potassium contents for control and boiled manure treatments were similar to the previous cited study, in which, varied from 17.1 to 18.7 g kg^{-1} K (Steiner *et al*., 2019).

Nitrogen is one of the most important nutrients for plant development but also impacting on arthropods plant susceptibility mostly to phloem sucking insects (Bala and Tahur, 2018). However, in the present study, no correlation was observed between nitrogen and the insects assessed. Similarly, no relation between nitrogen and *Delia radicum* L. or *B. brassicae* on rapeseed (*Brassica napus* L. ssp. *oleifera* Metzg) was reported (Szwarc *et al*., 2021).

Previously, *B. tabaci* attraction on tomato plants under NPK fertilization, raised from 29.4 (recommended dose) to 37.2 (2× recommended fertilization) (Idriss *et al*., 2015). Excessive use of nitrogen fertilizers was identified as promoters of imbalances in plant metabolism due to protein

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degradation releasing soluble amino acids, which are easily assimilated by insects favoring pest infestation (Chaboussou, 1987). Previous studies also indicated that higher amounts of nitrogen fertilizer change the volatile profile attracting more *B. tabaci* adults in olfactometer assays (Islam *et al*., 2017).

A positive correlation between potassium and *B. tabaci* was observed (Figure 2), what was also previously observed when evaluating *Tetranychus urticae* Koch (Acari: Tetranychidae) populations on strawberry leaves (Hata *et al*., 2019). However, in general, the potassium content in leaves is negatively correlated with arthropods infestations due to the improvement of secondary compounds and reduction of carbohydrate accumulation (Bala *et al*., 2018).

Decreasing populations of *Hydrellia philippina* Ferino (Diptera: Ephydridae), *Cnaphalocrocis medinalis* Guenee (Lepidoptera: Crambidae), *Scirpophaga incertulas* Walker (Lepidoptera: Crambidae) on rice (*Oryza sativa* L.) were observed after foliar sprays of potassium dihydrogen phosphate (Chatterjee *et al*., 2021). In the present study, the population of *B. tabaci* and *M. persicae* were higher on NPK-fertilized plants than organically-fertilized or no-fertilized plants. Similarly, on chemical-fertilized collards plants, higher *B. brassicae* and *M. persicae* populations were observed than on organic-fertilized plants (Cividanes *et al*., 2020).

In cabbage, reduction of populations of *M. persicae* was obtained by fertilizing plants using organic amendments (Staley *et al*., 2010). Lower aphids' populations were also observed in maize grown using organic fertilization than those using formulated synthetic fertilizers (Morales *et al*., 2001). Although the N levels were higher in Bokashi treatment and similar to NPK treatment, Bokashi did not trigger growth of *B. tabaci* and *M. persicae* insect populations per plant.

Organic amendments and fertilizers release nitrogen and other nutrients slowly and improve the microbial community (Rowen *et al*., 2019). By using organic fertilizers, increasing abundance of predatory insects has been reported (Peñalver-Cruz *et al*., 2019). Furthermore, the number of natural enemies was even higher than pests (*B. brassicae*, *M. persicae* and *B. tabaci*) on organically-fertilized collard plants than on chemical fertilized-plants (Cividanes *et al.*, 2020).

Then, organic manure may activate indirect defenses by increasing defensive chemical production, modifying herbivore-induced plant volatiles (Rowen *et al.*, 2019), which should attract or 'call' more natural enemies for pest control. Organic fertilization and amendments, besides increasing N levels, may also increase secondary compounds. For Brassicaceae plant species increases of up to three-fold in glycosinolates (sulfur-rich compounds) have been reported (Staley, 2010).

Organic fertilizers can provide a number of nutrients, including sulfur, which are included in the composition of glucosinolates. Under these conditions, generalist insects such as *B. tabaci* and *M. persicae* would be underprivileged. The phenolic compounds may also impair the biological parameters or reduce of pest-arthropods populations (Hata *et al*., 2019; Sousa *et al*., 2021).

This was observed through a negative correlation between total phenolics and *B. tabaci* (Figure 2). In previous controlled study, landing and oviposition preferences of B. tabaci on cabbage were negatively correlated to total phenolic contents (Yang *et al*., 2020). Phenolic compounds may also exhibit toxic and antifeedant effects on insects (Singh *et al*., 2021).

The results reported here may be relevant to understand the dynamic of insects mostly on organic cultivation to develop suitable management strategies. Due to lower pest development by using organic fertilizers, adoption of a sustainable approach, avoiding pesticides may be facilitated. By using amendments that disfavor insect pest occurrence, complementary measures such as botanicals extracts, biological control, repellent plants etc. may work better.

Conclusions

In summary, major number of *B. tabaci* adults and *M. persicae* were found in NPK-fertilized plants. Plants fertilized with Bokashi compound showed higher N contents than those fertilized with boiled manure 2.5%, control and Penergetic. Overall, NPK-fertilized plants showed higher K contents than control and boiled manure 2.5% while the remaining treatments were intermediate. Positive

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correlations between *B. tabaci* population and *M. persicae*, *B. tabaci* and potassium content in leaves were found. Otherwise, negative correlation was observed between total phenolics and *B. tabaci*, nitrogen and potassium.

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