2024-06-06

Pesticidal Plant Treatments Combined with Improved Soil Fertility Can Reduce Damage Caused by Fusarium Wilt (Fusarium oxysporum f.sp. phaseoli) and Bean Fly (Ophiomyia phaseoli) in Common Bean Production (Phaseolus vulgaris L.)

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Pesticidal Plant Treatments Combined with Improved Soil Fertility Can Reduce Damage Caused by *Fusarium* Wilt (*Fusarium oxysporum* f.sp. *phaseoli*) and Bean Fly (*Ophiomyia phaseoli*) in Common Bean Production (*Phaseolus vulgaris* L.)

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Abstract: Common bean production is constrained by a multitude of biotic constraints including bean flies and *Fusarium* wilt in tropical and subtropical farming systems globally. As these pests and diseases attack the crop beneath the soil, excessive applications of synthetic pesticides are frequently used for their control. The use of plant-based pesticides could be a more sustainable management approach; however, few studies have investigated their application for controlling soil-borne pests and diseases. This study aimed to evaluate the efficacy of pesticidal plants and soil fertility management for controlling bean fly (*Ophiomyia* spp.) and *Fusarium* wilt (*Fusarium* spp.) using extracts and pastes of *Azadirachta indica*, *Tephrosia vogelii*, *Tagetes minuta*, *Lippia javanica*, *Cymbopogon citratus* and *Ocimum gratissimum*. To protect against *Fusarium* wilt and bean fly, pesticidal plants were applied as a seed coating and/or foliar spray, and demonstrated that common bean seeds coated with *T. vogelii* resulted in higher yields than other pesticidal plants and the synthetic pesticide control treatment. Treatments to target bean fly damage showed no significant difference between application methods on the oviposition rate of bean fly. An integrated treatment of *T. minuta* with 2 g Diammonium phosphate fertilizer and high compost led to higher yields than other treatments. Our results indicate that key soil-borne pests and pathogens of common bean can be effectively managed without synthetic pesticide inputs, while seed ball pastes of pesticidal plants combined with soil fertility management can increase crop yields using cost-beneficial agroecological farming systems.

Keywords: bean stem maggot; bean fly; *Fusarium* wilt; pesticidal plant; botanical pesticide

Citation: Ngoya, Z.J.; Mkindi, A.G.; Vanek, S.J.; Stevenson, P.C.; Ndakidemi, P.A.; Belmain, S.R. Pesticidal Plant Treatments Combined with Improved Soil Fertility Can Reduce Damage Caused by *Fusarium* Wilt (*Fusarium oxysporum* f.sp. *phaseoli*) and Bean Fly (*Ophiomyia phaseoli*) in Common Bean Production (*Phaseolus vulgaris* L.). *Sustainability* **2024**, *16*, 4866. https://doi.org/10.3390/su16114866

Academic Editor: Chaohe Huangfu

Received: 12 March 2024
Revised: 28 May 2024
Accepted: 4 June 2024
Published: 6 June 2024

1. Introduction

Bean flies and *Fusarium* wilt are known to limit the production of common bean in most bean production systems across tropical and subtropical regions of Asia, Africa, the Americas and Oceania [1,2]. As these pests and diseases attack crops beneath the soil, there is often a high use of synthetic pesticides for their control [3]. Bean stem maggot or bean fly (*Ophiomyia* spp.) is a major pest [4], while *Fusarium* wilt diseases (*Fusarium* spp.) are the most important fungal pathogens globally affecting common bean production [5,6]. The bean fly (*Ophiomyia* spp.) is considered a soil-borne pest as they complete their growth

cycle in or near the soil surface [7]. *Ophiomyia phaseoli* (Tryon) is widely distributed and regarded as a growing pest [8,9]. The range of *O. phaseoli* overlaps with two similar species *O. spencerella* (Greathead) and *O. centrosematis* (DeMeijere) in eastern Africa, where it is often difficult to distinguish the species in the field [10–12]. Plants are attacked by bean flies as the primary leaves begin to unfold [10]. The larval stage of the bean fly can inflict substantial weakening of young plants by attacking the base of the emerging stem, which causes yellowish leaves and stunted growth and can lead to complete yield loss [8,9]. Common bean *Fusarium* wilt, caused by *Fusarium oxysporum.* f.sp. *phaseoli*, is a pathogen adapted to beans physiologically and pathologically in which the pathogen germination, dissemination and survival occur in soil [8]. This fungal species is widely distributed in all bean-producing areas in Africa [13] and East Africa [14–17]. The pathogen affects the plant during the seed germination phase, causing severe impairment in plant growth due to reduced water and nutrient absorption, resulting in pronounced stunting and wilting of the plants [18]. *Fusarium* wilt pathogenesis can cause losses of crop yield between 70% and 100% [5,6,19].

Synthetic pesticide seed treatments have been recommended to control bean flies [13,20] as well as soil treatments and post-emergence foliar sprays [21]. Similarly, seed and soil treatments and fumigation with synthetic fungicides can be used to manage *Fusarium* wilt [13,22]. However, the health and environmental problems associated with synthetic pesticides are well documented, including the development of pesticide resistance among pathogens or insect pests [23,24], as well as their financial burden on resource-poor smallholder farmers and their misuse by farmers with low literacy levels. The accumulation of harmful residues poses a major risk to human health and disrupts pollinators [25], soil-borne natural enemies [26,27] and soil microbes [28].

The use of plant-based pesticides is increasingly promoted for insect pest control, enabling farmers to sell their produce under organic standards while adopting agroecological farming practices [29,30]. Extracts of *Tephrosia vogelii* have broad-spectrum pesticidal properties, which make it effective against many crop pests [31,32]. Neem (*Azadirachta indica*) has been reported to be effective against some soil-borne insects where [33] reported *A. indica* to be effective in controlling bean fly, and [13] reported its efficacy in managing *Fusarium* wilt in common bean.

The role of soil fertility has also been shown to be important for reducing soil-borne insect pests and diseases [34]. Integrating inorganic and organic sources of soil fertility can improve nitrogen (N) and phosphorus (P) recovery, and improve bean productivity [35,36]. Soil organic matter increases plant vigor, enabling plants to resist insect herbivory [37]. High amounts of phosphorus can result in decreased population densities of all species of bean fly [38] and soil-borne diseases [39]. Some research suggests integrating soil fertility management with other cultural practices to control bean fly [40] or soil-borne pathogens [41]. However, research on the integrated use of inorganic fertilizer with high phosphorus, the use of compost and the application of pesticidal plants for the management of soil-borne insect pests and diseases has not been adequately explored. In this study, it was hypothesized that the use of organic and inorganic fertilizer inputs and pesticidal plants could make bean plants more resistant to soil-borne diseases and insect pests. Therefore, this study used a set of experiments to investigate the use of pesticidal plants in combination with organic and inorganic soil inputs to determine the best combinations for managing bean fly and *Fusarium* wilt damage to common beans, improving soil health and increasing common bean productivity the major constraints of common bean production to smallholder farmers in sub-Saharan Africa.

2. Materials and Methods

Four experiments tested the efficacy of pesticidal plant leaf extracts in controlling common bean *Fusarium* wilt and bean fly (*Ophiomyia phaseoli*) when applied by seed coating, seed coating + foliar spray and foliar spray, followed by testing best-performing pesticidal plant in combination with organic and inorganic soil inputs.

2.1. Preparation of Fusarium Inoculum for Use in the Screenhouse Trials

To be able to use the pathogen in the screenhouse, the isolation and culturing of fungal strains were carried out in the laboratory, followed by inoculation into an autoclaved finger millet, which acts as a fungal carrier to the experimental soil. The fungal species (*Fusarium oxysporum.* f.sp. *phaseoli*) was isolated from common bean root samples collected from smallholder farmer's fields located at an elevation of 1213 m above sea level within the Narumu Ward, Hai District, Kilimanjaro, Tanzania (3°15′12″ S and 37°14′34″ E), with monthly average temperature ranging from 13.6° to mean annual rainfall of 1200 mm. Sampling was conducted on one-month-old common bean plants showing yellowing, wilt or weak growth. Ten plants were sampled in a field and placed in sterile plastic bags. The samples were transported to the Plant Pathology Research Laboratory at the Nelson Mandela African Institution of Science and Technology University in a cooler box within 12 h. Sampled common bean roots were washed with tap water to remove soil debris, then immersed in 1% sodium hypochlorite for 1–2 min, and then transferred to sterile distilled water for 2–3 min, followed by drying on a sterile filter paper. Under sterile conditions in a laminar flow cabinet, the sample root was cut into 2–3 mm pieces and placed on 2.5% potato dextrose agar (PDA) medium. The plates were kept at room temperature in the laboratory for 7 days to allow fungal growth. Fungal growth on each plate was subcultured to a new plate, and the plates were kept in an incubator at 27–29 °C [42]. When the fungi had fully grown, culture purification was carried out by cutting a small piece of the media with mycelia from the edge of a colony and then subculturing it onto new growth media. Pure isolates were transferred to PDA slants, and after 14 days of growth, fungal cultures were stored at 4 °C [43]. The fungus species of *Fusarium oxysporum.* f. sp. *phaseoli* was confirmed by morphological and molecular identification methods by DNA extraction using the Chelex extraction method and sequencing of ITS (Internal Transcribed Spacer) gene region [44], matching to records found for an accession reported to be pathogenic (*F. oxysporum* accession KM268692.1) [45]. Inoculum of the fungal strains was prepared by washing 1.5 kg of finger millet grain in tap water followed by drying. Dry finger millet grain was autoclaved for 60 min at 121 °C. Once cooled, fungal culture from a potato dextrose agar culture was inoculated and cultures were incubated in complete darkness for 3 days at 25 °C [46].

2.2. Preparation of Pesticidal Plant Materials

Plants used included leaves of *Azadirachta indica*, *Tephrosia vogelii*, *Ocimum gratissimum*, *Tagetes minuta*, *Lippia javanica* and *Cymbopogon citratus*. The first three plant species were obtained from Same and Narumu districts, Kilimanjaro Region (3°14′8″–4°16′0″ S and 37°14′47″–38°0′0″ E), and the last three plant species were collected in Meru District, Arusha Region (3°22′26″–3°22′13″ S and 36°47′13″–36°42′23″ E). Plants were chosen due to local availability and known efficacy [47,48] and their known safety [25,49,50]. *L. javanica*, *C. citratus* and *O. gratissimum* are used as flavors in beverages and the food industry ([48] and shown to have low mammalian toxicity [51]. Leaves of each species were used due to their known phytochemical contents [47,52–54]. Fresh leaves of each plant were collected and dried under shade to prevent UV degradation. The dried leaves were ground to fine powder and stored in dark dry conditions until use.

Treatments were applied in three different application methods: (1) seed coating, (2) foliar spraying and (3) combined seed coating and foliar spraying. For bean seed coating, 15 g of *O. gratissimum*, *A. indica*, *T. minuta* and *L. javanica* was mixed with 30 mL of water to make (0.5 g/mL) a pesticidal plant paste for each seed. Amounts required to make a paste using *T. vogelii* and *C. citratus* involved mixing 7.5 g of ground plant material with 45 mL of water (0.25 g/mL). These different rates were required to make a paste-like substance, as some plant species leaf powders were less dense. This was purposefully carried out to make a paste that would hold shape whilst coating seeds. Each bean seed was covered with the pesticidal plant paste to create a small ball of paste around each seed with a bean seed inside. Seed balls were left to dry overnight and then planted the next

day. Pesticidal plants for foliar spraying as a liquid extract were prepared by mixing 100 g of plant powder into 1 L water adding 0.1% liquid soap. The solution was left to extract for 24 h, filtered to produce an extract of 10% (*w*/*v*) and sprayed immediately [53]. For the negative control, seeds were soaked in water for 15 min left to dry overnight and then planted the next day. The positive control seeds were soaked in commercial fungicide (Ridomil Gold manufactured by Syngenta Crop Protection AG) prepared according to the manufacturer's guide (50 g/20 L of water) for 15 min left to dry overnight and then planted the next day.

2.3. Evaluating Pesticidal Plant Efficacy to Control Common Bean Fusarium Wilt

A completely randomized design in a controlled pest-free screenhouse at the Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania, was used for the trial. '*Soya gololi*', a local variety of common bean (*Phaseolus vulgaris* L.) commonly grown in the study area, was planted with 3 seeds per pot with 10 pots (replicates) for each treatment. Treatment of bean seeds with pesticidal plant paste was carried out before planting while plants were sprayed at emergence which occurred 7 days after planting.

Plastic pots (16 × 15 cm) were filled with 1800 g of forest soil obtained from within the Tanzania Agricultural Research Institute (3°23′10″ S and 36°48′17″ E). The soil organic matter of the soil was 4.4%, soil available phosphorus (P) was 19.11 mg P/kg, pH was 7.1 and soil texture was sand loamy. The soil was sterilized to eliminate soil pathogens including soil nematodes, fungi and bacteria by heating the soil for 5 h when the soil reached a temperature of 70 °C. Although this process may have eliminated some beneficial microorganisms [55], the main aim was to reduce competition from unwanted pathogens and, hence, favor the growth of pathogens of interest in the rhizosphere [56]. Sterile soil was then inoculated by placing 15 g of fungal-infested millet grain distributed as a layer in each pot filled with sterile soil (1800 g) and then covered with a 2 cm layer of sterile soil. Thereafter, three common bean seeds were planted in each pot and covered with a further 2 cm of soil [46]. The untreated control involved planting common bean seeds on sterilized soil with no fungal-contaminated millet seed. Untreated and no pathogen control treatments were included to assess plants with no influence of pathogen or pesticidal plants. All pots were watered equally each day as required.

The proportion of emerged plants was observed after 7 days, while disease severity using external symptoms was recorded after three weeks. To assess severity, five plants from each treatment were uprooted and washed with tap water to remove soil debris and observed for external symptoms of the pathogen developing on roots, hypocotyl and stem. At four weeks, disease severity was assessed by checking internal symptoms by cutting the lower part of stem (pith) below the first node to see if plants showed discoloration of the vascular tissues (xylem and phloem) using a 1 to 4 scale developed by [57] as follows: 1 = no or minor discoloration of vascular tissues or pithy stem; 2 = light discoloration of pithy stem; 3 = severe discoloration of pithy stem; and 4 = very severe discoloration of pithy stem. At the maturity stage, plants from each treatment were harvested and yield (g/plant) was determined to assess the effect of the disease pathogen on the yield of common bean.

2.4. Establishment of Laboratory Bean Fly (Ophiomyia phaseoli) Colony

Wild bean fly adults were collected from farmer fields in Mnadani Ward, Hai District, Kilimanjaro, Tanzania (3°21′57″ S and 37°16′53″ E). Male and female adult bean flies were distinguished by size where females were slightly bigger than males and had a bluntly pointed abdominal tip. Adult flies were taken to the laboratory where the collected insects were released in a cage with fine mesh (50 × 50 × 50 cm) containing 11-day-old clean bean plants to facilitate oviposition. The colony was maintained at 27 ± 2 °C with a photoperiod of 12L:12D and relative humidity of approximately 40% [58]. Adults were provided with a 10% honey/water solution in cotton balls placed at the top of the cage. After 25 days, adult insects emerged and could be used to maintain the colony using new bean plants, as well as for use in experimental trials.

2.5. Preparation of Pesticidal Plants for Bean Fly (Ophiomyia phaseoli) Experiment

The pesticidal plants evaluated on bean fly were *O. gratissimum*, *T. vogelii*, *A. indica*, *C. citratus*, *L. javanica* and *T. minuta*. Plant powder and water were mixed together to make a paste, as previously described. Each bean seed was coated with the pesticidal plant paste and then molded to create a small ball of paste around each seed with a single seed inside. Seed balls were left to dry overnight and then planted the next day. The positive control used was the insecticide Selecron 720 EC (Syngenta Crop Protection AG), which was prepared according to the manufacturer's guidelines (50 g/100 mL water), with bean seeds soaked in the solution for 15 min, left to dry overnight and planted the next day. Pesticidal plant water extracts for foliar spraying of bean plants were prepared by mixing 100 g of fine powder into 1 L of water with 0.1% liquid soap. The solution was left to stand in the dark for 24 h and filtered to produce an extract of 10% (*w*/*v*), which was used immediately [53]. For the negative control, seeds were soaked in water for 15 min left to dry overnight, and then planted the next day.

2.6. Evaluating Pesticidal Plant Efficacy to Control Bean Fly (Ophiomyia phaseoli) Damage to Common Bean

As with fungal trials described previously, three different treatments were applied. (1) Seed coating: bean seeds were coated in pesticidal plant paste and then planted in pots following previously described protocols; (2) seed coating + foliar spray: bean seeds were coated with pesticidal plant pastes, and thereafter, seedlings were sprayed with extracts of pesticidal plants; and (3) foliar spray only: bean seeds were planted in pots and extracts of pesticidal plants were sprayed once seeds had germinated. Then, 1- to 3-day-old adult flies (15 in total of mixed male and female) were released into a cage containing 6 pots each (18 plants per treatment) of 11-day-old bean seedlings. Flies were left for three days in cages to copulate and for female flies to oviposit. Cages were maintained at 26 ± 1 °C, 50–70% RH and 12 h photoperiod. Three days post-exposure, adult flies were removed to avoid excessive oviposition and feeding damage. Where treatment application involved spraying plant extracts, bean seedlings were sprayed one week after planting followed by spraying once a week until pupal formation. Spraying was carried out using a small hand sprayer with equal amounts across replicates.

Bean fly oviposition punctures on leaves and larval damage were evaluated on bean plants in all three treatments. Oviposition punctures on plants were examined 7 days after exposure to ensure sufficient time to account for bean fly copulation and oviposition. Two primary (unifoliate) leaves and other trifoliate leaves were observed in each treatment and the number of oviposition punctures was counted and recorded. The number of ovipunctures was used to detect the oviposition activity of bean flies and the oviposition deterrent effect for each treatment [59]. At maturity, one plant per pot was selected for yield evaluation, counting the number of pods per plant and the yield of beans produced (g/plant).

2.7. Evaluation of Pesticidal Plant Treatments on Beans Planted in Two Soil Types on Bean Fly Damage

The same protocols were used as previously described in terms of applying pesticidal plant pastes made from six pesticidal plant species, positive control (Selecron) and untreated (water only) control treatments to seeds and planting in pots. Forest soil was obtained from the Tanzania Agricultural Research Institute (TARI) natural forest. Soil organic matter, phosphorus, pH, and texture were like that described in the first experiment. The other soil used for planting was collected from farmer fields where bean fly insects were collected to establish the laboratory colony. The soil organic matter was 0.80%, the soil available phosphorus was 11.83 mg P/kg, pH was 5.82 and the soil texture was a clay loam. The farmer's soil was mixed with fine sand at a ratio of (3:1) to improve the drainage of soil for use in plant pots. As described for other trials, 3 common bean seeds were planted in pots with 1800 g of soil with 6 replicates/pots (18 plants per treatment) together in a mesh-enclosed cage. Seeds were allowed to germinate and emerge as young seedlings.

After 10 days, 15 adult bean flies were released in each cage to mate and for females to oviposit. Data collection on yield was carried out as previously described.

2.8. Assessment of Pest and Soil Fertility Management on Bean Fly (Ophiomyia phaseoli) Damage

Based on results from the above trials, *T. minuta* was selected as the most effective pesticidal plant to evaluate as a botanical seed coat treatment in combination with soil amelioration using organic inputs (compost made from livestock beddings) and/or DAP synthetic fertilizer (23 N: 46 P) additions for control of bean fly damage in a typical farmer managed soil to assess some best selected practices in a more real setting. Six pots each with 1800 g of farmer's soil were mixed with compost or synthetic fertilizer. Treatments included (1) *T. minuta* seed treatment; (2) *T. minuta* seed treatment + 30 g compost; (3) *T. minuta* seed treatment + 120 g compost; (4) *T. minuta* seed treatment + 2 g DAP; (5) *T. minuta* seed treatment + 30 g compost + 2 g DAP; and (6) *T. minuta* seed treatment + 120 g compost + 2 g DAP. The insecticide Selecron was used as a positive control and untreated seeds as the negative control. For each treatment, there were six replications, and data were collected as per previous trials.

2.9. Data Analysis

The effects of the treatments observed were subjected to Analysis of Variance (ANOVA). The means of treatments and interactions were compared using the least significant difference (Fisher LSD) test at a significant level of $p \leq 0.05$. For some inferences regarding treatments, post hoc statistical contrasts were used to compare groups of treatments that allowed exploration of treatment factors within the more complex treatment structure, such as the effects of spraying versus seed treatment or of groups of plant treatments. The normality of data was assessed, and, where required, data were transformed using square root or log transformations to meet requirements for homogeneity of variance across treatments. Most analyses were conducted using XLSTAT statistical package version 2022 (Addinsoft, New York, NY, USA), while statistical contrasts were performed within ANOVA analyses comparing treatment means in JMP 16.0 statistical software (JMP, Version 16.0. SAS Institute Inc., Cary, NC, USA, 1989–2023). Contrasts for differences between groups of means were calculated and evaluated for significance using *t*-tests.

3. Results

3.1. Evaluating Pesticidal Plant Efficacy to Control Common Bean Fusarium Wilt

None of the six pesticidal plant species tested had a significant effect on the germination rate, which ranged from 92% to 100% across pesticidal plant species. Comparing the treatment's main effect, disease severity was significantly different at four weeks (Figure 1). Disease severity was significantly less on bean plants treated with *L. javanica* (5%) and *T. vogelii* (15%) treatments. There was no significant difference observed among treatments, although *T. vogelii* had a higher yield than all other treatments and all plant species treatments were still better than the untreated pathogen-infected control (Figure 1).

Further evidence of efficacy in yield was assessed by using statistical contrasts of ANOVA means. Within plant extract treatments, there were no significant interactions between plant species and application method, allowing these to be treated separately in their effects. Post hoc contrasts of means showed a significant difference between the six pesticidal plants as a group and the untreated control ($p = 0.002$, Supplementary Table S1), as well as differences between *T. vogelii* and other plant treatments ($p = 0.025$) and seed coating versus the seed coating + spray and spray-only treatments ($p < 0.0001$). However, plant extracts as a group were not different from the fungicide control ($p = 0.065$), and spray vs. seed coating and seed coating + spray as a group were not significantly different from each other ($p = 0.11$).

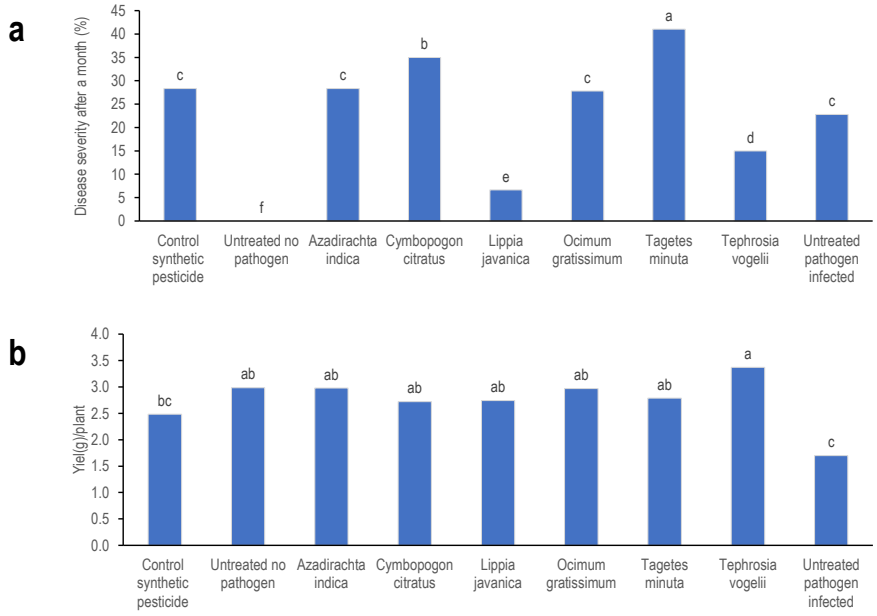

Figure 1. Impact of *Fusarium* wilt: (**a**) percent disease severity after one month and (**b**) bean yield (g/plant) when common bean was treated with different pesticidal plants in a screenhouse study. Internal symptoms were evaluated by splitting open the base stems followed by visual rating discoloration of vascular tissues. Analysis of Variance followed by Fisher's LSD test where treatments with different letters are significantly different at the level $\alpha = 0.05$. The displayed values represent mean values.

3.2. Evaluating Pesticidal Plant Efficacy to Control Bean Fly (Ophiomyia phaseoli) Damage to Common Bean

Results show that there was a significant difference between the three application modes of pesticidal plants (seed treatment, foliar spray, seed treatment + foliar spray) on the oviposition rate of bean fly (Table 1), as well as a significant interaction between the treatment and mode of application ($p = 0.04$). However, when the analysis was repeated with only the pesticidal plant treatments without the synthetic control, neither the mode of application nor the interaction was significant, indicating that differences in mode of action with treatment were mainly due to the difference between synthetic pesticide and plant performance. Although the effect of mode was not significant within plant treatments ($p = 0.19$), the ordering of means was the same as shown in Table 1 for the analysis, including the synthetic control, suggesting that earlier pesticidal application to seed was able to produce lower oviposition, as well as higher yields (Table 1, right side). Meanwhile, in the case of yield, there was no significant interaction between plant species and application mode on the oviposition rate of bean fly ($p = 0.815$, Supplementary Table S2), indicating that the effects of treatment and mode could be separately examined.

Table 1. Impact of pesticidal plant application method on rate of bean fly (*Ophiomyia phaseoli*) oviposition and bean seed yield per plant. Analysis of Variance (n = 6) followed by Fisher's LSD test where treatments with different letters are significantly different at the level $\alpha = 0.05$. The displayed values represent mean values.

Application of Pesticidal Plants	Oviposition Rate Plant^{-1}	Seed Yield Plant^{-1} (g)
Seed	4.9 b	1.5 b
Spray only	16.0 a	1.3 a
Seed and spray	4.5 b	1.5 b
Pr > F (Model)	0.001	0.001
Significant	Yes	Yes

3.3. Evaluation of Pesticidal Plant Treatments on Beans Planted in Two Soil Types on Bean Fly (Ophiomyia phaseoli) Damage

The yield of common bean was significantly higher in bean plants planted in forest soil compared to farmer's soil regardless of pesticidal plant species treatment. All treatments including positive and negative control treatments were not significantly different across the forest soil treatments (Figure 2). However, farmer's soil showed different effects, where *T. minuta* had the highest yield. The lowest yield observed in farmer's soil was with *L. javanica* and the positive synthetic control treatments, which were more than 50% lower than the best treatment yields. These results were used to select *T. minuta* for the next trial, whereby only farmer's soil was used to evaluate the use of *T. minuta* integrated with soil fertility management treatments.

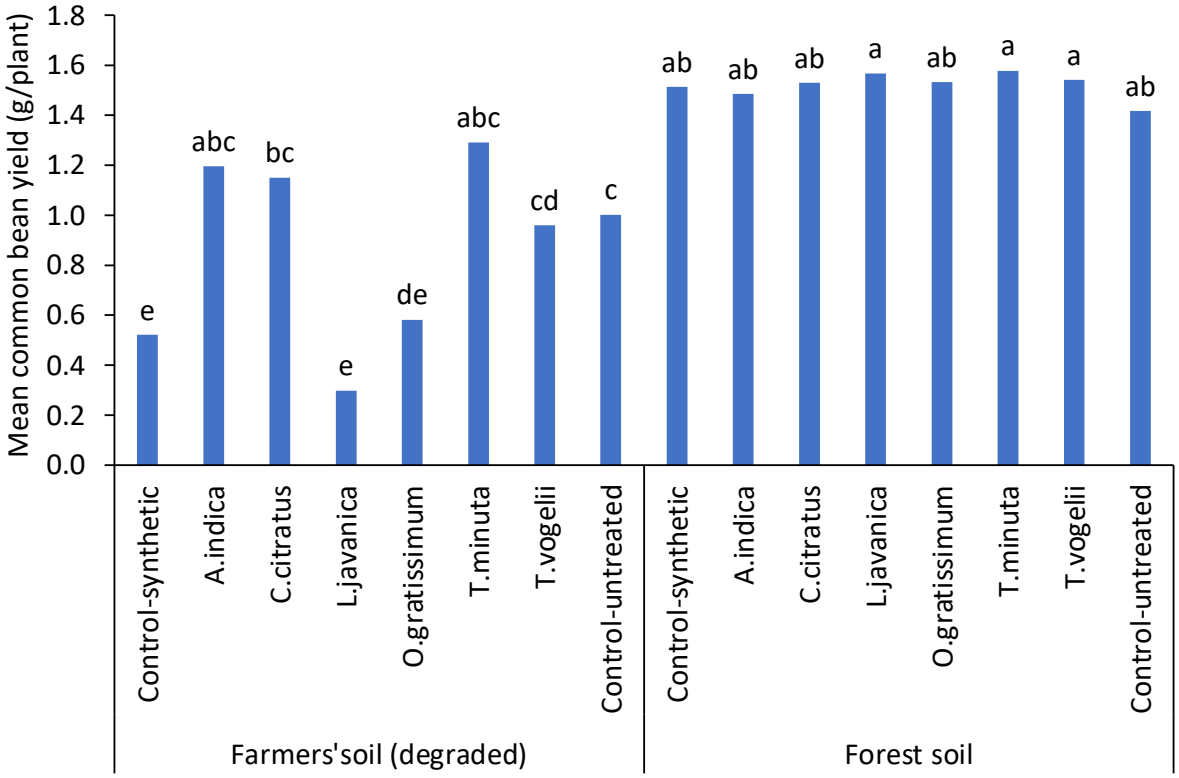

Figure 2. Effects of pesticidal plants coated in seeds tested on forest and farmer's soil to observe impact of soil fertility differences on bean yield following bean fly infestation under screenhouse conditions. Analysis of Variance followed by Fisher's LSD test where treatments with different letters are significantly different at the level $\alpha = 0.05$. The displayed values represent mean values.

3.4. Assessment of Pest and Soil Fertility Management on Bean Fly (Ophiomyia phaseoli) Damage

In measuring the number of ovipunctures per pot, there were some strong differences between the point estimates of means (Figure 3). Treatments with *Tagetes minuta* and *Tagetes minuta* + 2 g DAP + 120 g compost had the lowest number of ovipunctures compared to other treatments with *Tagetes minuta* combined with improved soil fertility.

The yield of common beans was affected positively using *Tagetes minuta* and improved farmer's soil with DAP fertilizer and compost treatments (Figure 4). Most of the treatments were statistically the same as the synthetic control with the exception of *Tagetes minuta* + 2 g DAP + 30 g compost and the untreated control, which had lower yields. All treatments significantly improved the bean yield compared to the untreated control. Statistical contrasts of ANOVA means showed that treatments with 120 g compost added were significantly different by having lower oviposition and higher yields than the negative control, with 30 g compost + DAP alone and 30 g compost (Supplementary Table S3).

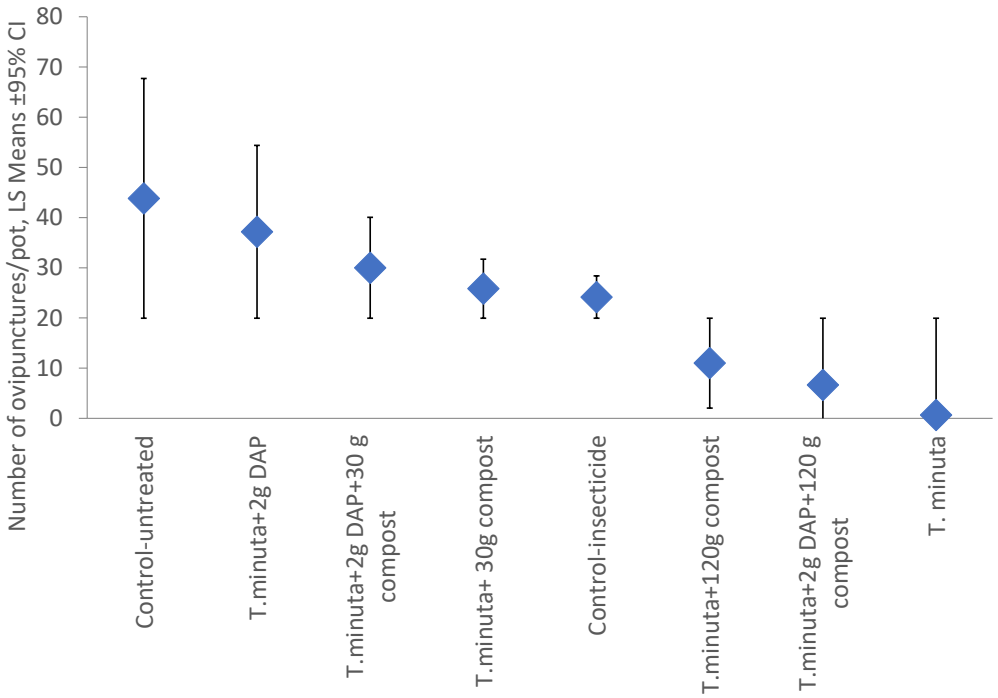

Figure 3. Mean oviposition rate of bean fly on bean plants treated with *T. minuta* combined with different amounts of compost (30 or 120 g) and synthetic fertilizer (2 g DAP) in pots in a screenhouse. Error bars indicate standard error (SE) on mean.

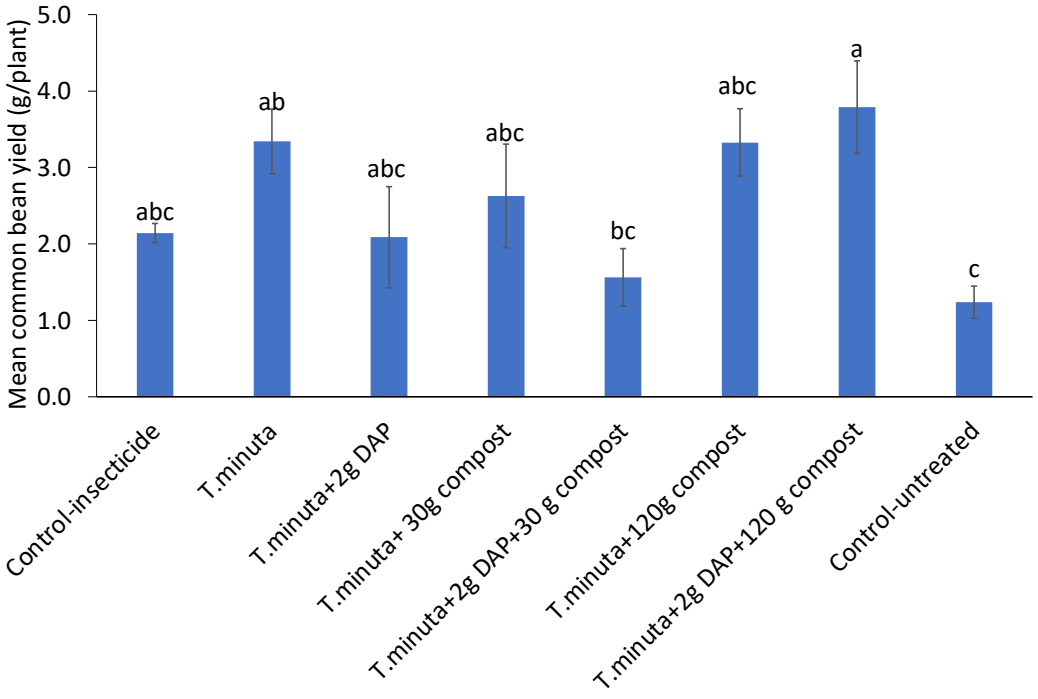

Figure 4. Effect on bean yield of common bean seed treated with *T. minuta* planted in farmer's soil with different amounts of compost (30 or 120 g) and synthetic fertilizer (2 g DAP) in response to bean fly infestation. Analysis of Variance followed by Fisher's LSD test where treatments with different letters are significantly different at the level $\alpha = 0.05$. Error bars indicate standard error (SE) of the mean.

Also, the correlation result showed that there was a negative correlation of yield to bean fly ovipunctures, so the highest yield had the lowest oviposition (Figure 5). This strongly suggests that at least one explanatory factor is bean fly damage from female bean flies during oviposition. Although there was less difference in ovipunctures among different pesticidal plants used, the significant trend across all treatments shows that their effect on yield may be pronounced.

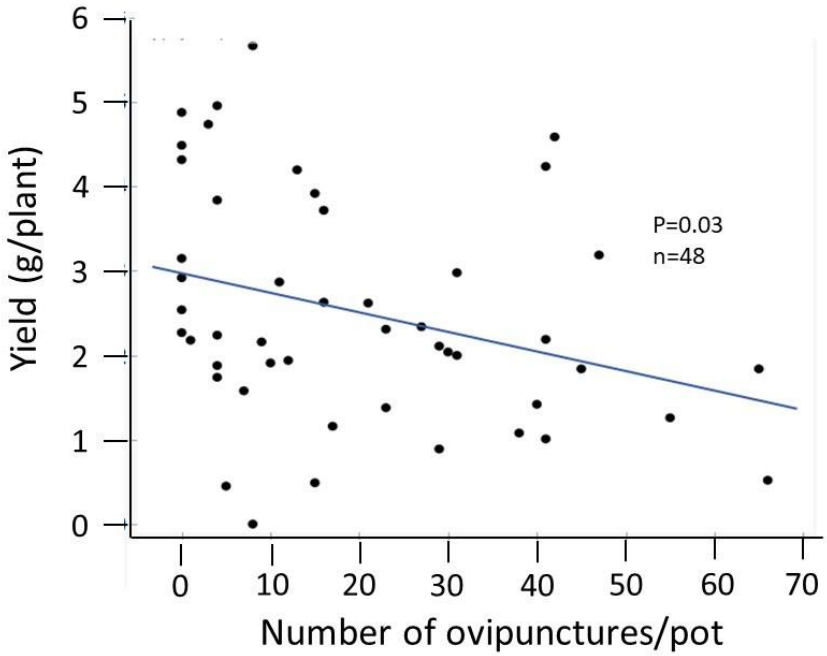

Figure 5. Regression analysis plotting leverage residuals indicating correlation between common bean yield and bean fly oviposition rate showing a significant trend across treatments in common bean seed treated with *T. minuta* planted in farmer's soil with different amounts of compost (30 or 120 g) and synthetic fertilizer (2 g DAP).

4. Discussion

4.1. Pesticidal Plant Efficacy on Fusarium Wilt Disease

The results from the first trial evaluating pesticidal plant treatments to control *Fusarium* wilt did not indicate any significant effect on bean seed germination rates. A small yet insignificant difference was recorded when seeds were coated with pesticidal plants compared to when pesticidal plants were applied as a foliar spray. A small reduction in germination rate was observed in just a few of the pesticidal plants, including, notably, *O. gratissimum*, *L. javanica* and *A. indica*. The leaf extracts of *A. indica* have been reported to inhibit germination in some cereals and legumes [60,61]. The inhibitory effects on germination of *O. gratissimum* have been reported among medicinal plants traditionally used in Ghana [62]. However, storing common beans with *L. javanica* powder is reported to promote seed germination of bean seeds when stored for a month [63]. Variability in germination rate could be the normal variability expected across a large number of pots, pesticidal plants or other potential causes. Overall, we would argue that if there are relatively small reductions in germination caused by treatment with pesticidal plants, these are likely to be offset by higher yields achieved in germinated bean seeds, if pesticidal plants are effective, as long as germination rates remain relatively high.

Comparing overall pesticidal plant treatments, *L. javanica* and *T. vogelii* reduced common bean *Fusarium* wilt compared to other plant species. The reduced disease severity was more pronounced when these pesticidal plants were applied on seeds than the combination of seed and foliar application, which suggests that spraying seemed to negate the effect of seed application. The highest suppression of disease was by *L. javanica* and *T. vogelii*,

which could be due to the efficacy of known antifungal bioactive constituents, linked to the way that *T. vogelii* has been used in Africa for controlling insect pests for decades [64]. Phytochemical analysis shows that *T. vogelii* has rotenoids (isoflavonoids) as the most active ingredient highly present in leaves especially dried ones [65]. Leaf analysis of *T. vogelii* shows rotenone and deguelin are the main constituents of rotenoids [66,67] with deguelin having the highest insecticidal properties [64]. This has made the plant highly useful in controlling field and storage insects [67]. Although fungicidal usage of *T. vogelii* has been less reported for field diseases [68], its broad-spectrum nature paves the way for further research on its antimicrobial properties [69]. Leaf extract of *L. javanica* has verbascoside oil a major compound in leaf extract [51]. Verbascoside oil in leaf extract has been reported to have antifungal activities and insecticidal activities [50,70]. In addition, the suppressed disease severity from either of these apparently promising species could have come about from the fertility impacts of pesticidal plant pastes applied to bean seeds [71]. When these plants are applied as biomass they break down and release N, a growth-promoting mineral. Studies have found that the incorporation of both *T. vogelii* and *L. javanica* biomass can lead to increases in plant-available N in soils [72,73]. The N supplied by plant extracts promotes the vegetative growth of the host plant, enabling it to escape pathogen parasitism [74].

4.2. Effects of Plant Extract Delivery Mode on Fusarium Wilt Disease

A comparison of yield among three modes of treatment application of six pesticidal plant species shows that seeds coated and later on sprayed with pesticidal plant had lower yield than seeds only coated with pesticidal plant paste or seedlings sprayed with pesticidal plant extract. Combining seed coating and foliar spray with pesticidal plants may have caused an alteration of morphological, physiological and yield parameters in the bean plants [71]. In this way, our results disagree with studies testing the efficacy of the combined effect of seed coating with foliar spray of pesticidal plants in common bean, which have shown high yield [75]. Therefore, our results suggest that further research on parameters affecting bean yield when comparing different modes of treatment applications needs to be carried out. Common bean plants coated with *T. vogelii* had a high yield compared to other treatments and negative control. Seed coating is a cost-effective method of providing nutrients that improve plant growth [71]. Our study recommends further research on the effect of studied pesticidal plants on physiological parameters, as well as the way that fertility impacts of seed-applied plant products interact with their biocidal and physiological effects on crops.

4.3. Response of Bean Fly to Plant Extracts

Our trial evaluating different pesticidal plant species treatments on beans planted in farmer's soil or forest soil for the control of bean fly suggested the most important factor was the broad differences in soil fertility and related soil quality between a higher organic matter forest soil and a cultivated forest soil. When comparing the impact of the pesticidal plant treatments between soil types, there was generally high bean yield across all treatments planted in forest soil, with no significant differences among pesticidal plant treatments. However, the fact that in farmer's soil, a synthetic pesticide control did not alleviate yield constraints, suggests that the issue was fertility and did not affect the pest or performance of pesticidal plant treatments. The impact of soil fertility on the proliferation of bean fly appears to be important. In one study, P deficiency in soil resulted in an increase in population densities of all species of bean fly while higher levels of total soil N were associated with increased populations of *O. spencerella* [38]. A plant that has access to more soil P may also be able to grow out of the effects of insect herbivory or disease damage [68]. It has been argued that an excess of nitrogen can cause an excess production of amino acids [68], which favor insects' herbivory [2]. A low level of N fertilizer reduces oviposition preference and feeding by bean fly, as well as the survival of bean fly larvae inside the plant [76]. Bean plant damage by bean fly larvae can be observed particularly at the third instar feeding on the first pair of leaves and beneath the soil on the stem epidermis [10],

which ultimately results in yellowish, wilted bean plants. High plant mortality is observed when the bean flies are pupating due to a concentration of puparia in the stem tissue, which leads to swelling, stem split, and rotting of the stem base [8].

As *T. minuta* was one of the more effective plant species to protect beans planted in farmer's soil from bean fly, based on a higher yield compared to other treatments, this plant species treatment was used to investigate potential interactions between pesticidal plants and adding organic or inorganic fertilizers. The outcomes on bean crop yield suggest that the best methodology is to combine both DAP fertilizer and compost with the pesticidal plant treatment. However, using either DAP, compost or *T. minuta* on their own still has a higher yield than the untreated control. Leaf extracts of *T. minuta* have been reported to be effective in reducing aphid abundance [77]. The plant is known to contain a number of bioactive constituents that will be responsible for efficacy in our trials [78]. In the context of bean fly damage prevention, the provision of adequate soil nutrients for plant growth and vigor is reported to be important in reducing the damage caused by bean flies [40]. Ref. [36] report that the use of organic and inorganic soil amendments creates a synergistic effect of increasing leaf area and leaf area index. Our results show that applying a high amount of compost and a half rate of DAP produced the highest grain yield, which arguably is helping bean plants resist damage caused by bean fly larvae. However, the combination of pesticidal plant with DAP and 30 g of compost resulted in low yield compared to their single treatment, which could be due to antagonistic effect [79] or to a one-season experiment where it was difficult to discern systematic variation in the treatments from the different inputs, due to high levels of variability [80].

4.4. Interactions of Plant Extracts with Fertility Inputs for Bean Fly

Our results show that either DAP or compost had some effects on improving yield in the presence of soil-borne insect pests. DAP fertilizer applied in our trials aimed at increasing the availability of P, as phosphorus deficiency is widespread in many common bean-producing regions [81]. P deficiency decreases the plant's ability to fix nitrogen [82]. Our results show that either DAP fertilizer or compost had a higher yield and overcame some constraints from the pests. This is because improved fertility can make crops more vigorous and, hence, less vulnerable to insect attack, and overcome insect damage [4,13,68,83]. Applying about twice the amount of P to that of N results in a high grain yield of common bean [84]. Other studies have shown that adding compost to the soil is associated with changes in populations of the antagonistic resident soil bacteria and fungi species, allowing improved root health that could improve access to soil nutrients and improve tolerance to bean fly attacks [85]. Adding a quarter rate (30 g) of compost other than just *T. minuta* may have made the plant more attractive to bean flies, due to a higher ratio of soluble to less labile forms of N within the inputs, compared to the high rate of 120 g compost per pot, which may have overwhelmed the effect of the DAP and bean fly attack resulting from the soluble N and low compost application. Using *T. minuta* will aid in controlling bean fly oviposition, which adds to a growing body of research on pesticidal plant bioactive agents [86]. In addition, combining with more compost will not only allow better effects of the plant pesticides but also have a longer-term impact on soil health. Our study suggests that relatively high application rates may be needed for composts to achieve suppression of bean fly pressure (as ascertained by the overall link between oviposition and bean yield) and/or positive nutritional impacts on beans. These effects from high rates of compost could be associated with crop vigor, which outcompetes bean fly herbivory, and, hence, low oviposition [40]. Regression analysis showed that the lowest oviposition rate was observed in plants with high amounts of compost and was associated with the highest yield. The highest oviposition rate affects plants in later stages of growth causing yellowing and wilting symptoms [8], which can significantly affect plant yield [20,87]. However, our study was unable to completely clarify whether using organic or inorganic soil inputs was beneficial on its own in terms of insect pest control and improving bean yield.

5. Conclusions

Coating seeds with pesticidal plant material could be the best application method for managing soil-borne *Fusarium* wilt. Coating seeds with *T. vogelii* can suppress disease severity, as well as improve the yield of common beans. Soil-borne pathogens and insects can be controlled based on these new analyses of pesticidal plant effectiveness, especially by combining plant inputs with soil fertility management practices. Soil fertility management using both organic and inorganic fertilizers seems to be important, particularly in the case of bean flies. Therefore, improvement of soil fertility in smallholder common bean producers is important for sustainable production, especially using easily available organic and rational use of synthetic fertilizers, which is important for sustainable management of soil-borne pathogens and insects. Our results suggest further research to analyze important plant chemical characteristics of *T. minuta*, which may have contributed to a higher common bean yield.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su16114866/s1.

Author Contributions: Conceptualization, Z.J.N., A.G.M., S.J.V., P.C.S., P.A.N. and S.R.B.; methodology, Z.J.N., A.G.M. and S.R.B.; software, Z.J.N., A.G.M., S.J.V. and S.R.B.; validation, Z.J.N., A.G.M., P.A.N., P.C.S., S.J.V. and S.R.B.; formal analysis, Z.J.N., A.G.M., S.J.V. and S.R.B.; investigation, P.S and S.V; resources, P.A.N., S.J.V., S.R.B., P.C.S. and A.G.M.; data curation, A.G.M. and S.J.V.; writing—original draft preparation, Z.J.N., A.G.M., P.A.N. and S.R.B.; writing—review and editing, Z.J.N., A.G.M., P.A.N., S.J.V., P.C.S. and S.R.B.; visualization, S.R.B., S.J.V. and P.C.S.; supervision, A.G.M., P.A.N., S.J.V., P.C.S. and S.R.B.; project administration, S.R.B.; funding acquisition, A.G.M., P.A.N., P.C.S. and S.R.B. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by the McKnight Foundation- Global Collaboration for Resilient Food Systems (grant number 20-034).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data oresented in this study are available on request from the corresponding author.

Acknowledgments: We would like to acknowledge the technical support from the microbiology laboratory at the Nelson Mandela African Institution of Science and Technology where laboratory experiments were conducted. Thank you to the technical staff from Tanzania Agricultural Research Institute (TARI) Tengeru for the technical assistance during the bean fly rearing experiment.

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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