# Efficacy of Neemraj and Beauvitech on Abundance and Distribution of Arthropod Pests in Soil and Hydroponically Grown Strawberry

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**Abstract**

Strawberry is a commercially important crop which has gained global significance in terms of production and consumption. The strawberry farmers have been faced with economic losses due to attack from arthropod and phytophagus pests. To overcome this challenge, management of pests is important with reduced impact on beneficial organisms and the environment. Use of non-chemical approaches as an alternative to insecticide driven management is highly gaining popularity. Identification of the non-chemical production for management of strawberry pest such as entomopathogenic fungi and botanicals is critical. The need for compatibility of entomopathogenic fungi in crop production techniques with other insecticides to enhance efficacy is required. The interaction between these control agents could be synergistic, additive or even antagonistic. Synergistic interactions usually lead to enhanced effectiveness of the microbial control agents while reducing the adverse effects of the pesticides in combination. In this review a description of the compatibility of entomopathogenic fungus *Beauvaria bassiana* and botanical neem in strawberry pest management is given.

The experiment involved five treatments: Neemraj, Beauvitech, Neemraj and Beauvitech, Ampligo and control. These treatments were allocated in a completely Randomized design in two separate greenhouse setups one with soil and other with pumice. The treatments were replicated four times. The data was subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure of the statistical analysis system (SAS) program, version 9.1. Tukey's (HSD) test at $P \leq 0.05$ was used to separate significant means. The combination of Neemraj and Beauvitech was most effective in the management of pests in both hydroponics and soil grown media. Pest populations were reduced significantly as follows; thrips, *Frankliniella* spp 2.1, red spider mites *Tetranychus species* 11.5, tarnished plant bug *Lygus* species 3.0, there was 0% cricket *Gryllus* species damage and 0 number strawberry weevil in hydroponics cultures while in soil culture pest mean populations were: thrips 5.6, spider mites *Tetranychus* species 18.2, straw-

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berry weevil (*Otiorhynchus* species) 7.0, tarnished plant bug 3.0 and there was 13.8% cricket *Gryllus* species damage on strawberry plants.

**Introduction**

Strawberry *Fragaria ananassa* (Weston) family Rosaceae Duchesene ex Rozier is an important fruit, grown throughout the world (Manganaris *et al.,* 2014). In highland regions of Indonesia farmers make strawberry products for agroindustry and agribusiness (Arystia *et al*., 2019). The fruits are bright red color, juicy texture, aromatic and sweet (Massetani *et al*., 2017). Large quantities of strawberry fruits are majorly included in making of pies, chocolates or in foods juice (Massetani *et al*., 2017). The fresh form the fruits are used as botanical extracts for dietary supplements or can even be processed food products (Khatun *et al*., 2019). The juice extract has significant amounts of antioxidants that targets hydroxyl radicals, superoxide radicals, singlet oxygen free radicals as well as hydrogen peroxide (Khatun *et al*., 2019)

Commercial production of strawberry has increased by 42% in the last decade (Simpson, 2018; FAO, 2018). Florida and California are the leading states in production of strawberry in the United States with the latter producing over 91% of total strawberry (Guthman and Soto, 2021). In most areas, strawberries are grown in soil but in temperate regions, their production is inside greenhouses under soilless media [1]. Engagement in worthwhile adaptation studies is necessary to access application of modern strawberry production techniques such as greenhouse, irrigation, mulching, drippage and use of different cultivation approaches and use of improved species from breeding programs (Khatun *et al.,* 2019). Greenhouses has been preferred for crop production such as strawberry since it ensures early crop maturity, long shelf life, high yields and reduced chances of fungal diseases such as botrytis and also provides low humidity on the fruits and leaves (Oguz *et al*., 2021).

Despite the fact that strawberry is a high potential fruit, farmers are faced with abiotic and biotic constraints such as lack of proper training for production, lack of capital, arthropod pests and diseases (Khatun *et al*., 2019). Among the most serious arthropod pests are the two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae), greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) Zalom *et al*. (2018), and Scirtothrips *Frankilienella* species. Other economic pests include the root boring pests, seed bug *Neopamera bilobata* (Say) (Hemiptera: Rhyparochromidae) and the tarnished plant bug *Lygus Hesperus* (Knight) (Hemiptera: Miridae) which causes damage to both leaves and fruits (Hata *et al*., 2020; Talton *et al.,* 2020).

The management of strawberry pests has been majorly reliant on application of chemical pesticides which over time leads to resistance development causing inefficiency and retention of residues (Varma and Saran, 2019). Natural extracts such as neem based biopesticides and entomopathogenic fungal formulations e.g *Beauvaria bassiana* are effective, safe and have no residual effect. They are also biodegradable and exhibit stimulating effect on plant metabolism.

**Materials and Methods**

*Study site*

The study was conducted between August 2019 to March 2020 at Silanga farm, Olkalou, Nyandarua County, Kenya. The farm lies at 0°13'59.99"S and 36°22'0.01"E at 2,700 meters above sea level (asl). The area is reasonably cool and the temperatures range between 21°C and 26°C with the average annual temperatures of 23°C. Njenga *et al*. (2014) reported that the area has deep alluvial volcanic loam soils suitable for crop growing and that it receives an average rainfall of 750-1500mm annually which increases from west to east and the rainfall pattern is bimodal with long rains starting in March to May and short rains from September to December.

*Land preparation and treatment application*

Two separate greenhouses each measuring 248m$^2$ were used to set up the experiment. One greenhouse had the soil grown strawberry and the other had pumice grown strawberry.

Treatments included Beauvitech *(Beauvaria bassiana)*, Neemraj (Azadirachtin 0.03%), combined Beauvitech and Neemraj, Ampligo and untreated control. Each treatment included 20 experimental plots each measuring 5m by 1 m and a total of 60 strawberry plants in 4 rows. The treatments were replicated four times and arranged in a completely Randomized Design. The treatments were applied on weekly basis for 3 months between August and October 2020 using a knapsack sprayer. Pest populations of strawberry weevil, thrips, tarnished plant bug, spider mites and crickets were monitored on 20 inner plants randomly selected 3 and 4 days after each spray application. The data on the type of pests and the numbers observed were recorded and then subjected to general linear model of ANOVA and Tukey's HSD means of separation test.

*Effects of selected treatments on thrips population*

Assessment of thrips population on flowering strawberry plants was conducted in the morning hours between 7:00-9:00 am as described by Shaffie and Abdelraheem (2012). This was done on a weekly basis from 4$^{th}$ to 12$^{th}$ weeks after transplanting (WAT) by randomly selecting 20 well developed flowers from 20 strawberry plants in the two inner rows of each experimental plot. The flowers were then cut and placed in a glass bottles (35ml) containing 70% ethanol and taken to the laboratory. The flowers were placed in petri dishes, dissected and washed with 70% ethanol to ensure maximum collection of the thrips. The thrips were identified using taxonomic keys as described by Kumar and Omkar 2021.Counting was then done using a tally counter under the dissecting microscope (NTB-3A) at x10 magnification, and numbers recorded. The specimens were taken to Integrated Crop Management Laboratory in Nakuru for confirmation.

*Effects of treatments on spider mites population on strawberry*

The number of spider mites was collected in the early morning hours between 7-9am. This was done weekly from 4$^{th}$ to 10$^{th}$ WAT for five times by randomly selecting 20 well developed leaves from 20 strawberry plants in each experimental plot. The plucked leaves were placed on a white paper and hand lens was used to observe the mites from the underside of the leaves after which counting of the adult spider mites was done. The samples of the thrips collected were placed in containers and taken to the Integrated Crop Management laboratory for identification and confirmation. The mean numbers of the mites per plot were recorded and analyzed.

*Effects of selected treatments on tarnished plant bug on strawberry*

The tarnished plant bugs were collected by placing a white plain paper below the plant canopy and shaking gently the strawberry flower clusters just before flower open as described by Ernest (2020). This was done from 4$^{th}$ -12$^{th}$ WAT by randomly selecting 20 flower clusters in each plot. The numbers of tarnished plant bug observed were recorded. Samples of the tarnished plant bug were put in containers and taken to the laboratory for identification and confirmation.

*Effects of selected treatments on strawberry vine weevil on strawberry plants*

Strawberry vine weevils were monitored according to Bennison *et al*. (2017) by observation of 4 grooved boards placed under the canopy of mature strawberry plants on weekly basis between 4$^{th}$ -10$^{th}$ WAT in each experimental plot. Regular inspection for the presence of weevils in the grooves during the day was done and counting was done. Collected strawberry weevils were put in containers then taken to the laboratory for identification and confirmation.

*Effects of selected treatments on cricket damage on strawberry*

To evaluate the damage of crickets on the strawberry plants, leaves were observed on weekly basis between 4th to 10th WAT and given a rating depending on percentage area of individual leaf damaged as described (table 1) by Kahuthia-Gathu (2000). The percentage ratings (table 1) were recorded. The destructive cricket species were also collected and put in containers and take to the laboratory for identification and confirmation.

**Results**

*Effect of different treatments on the number of Frankliniella sp. on hydroponically and soil grown strawberry*

There were significant differences (F=5.5, df=4,19 and $p \leq 0.006$) on the number of *Frankliniella* sp., observed at on plots treated with Ampiligo, Beauvitech, Neemraj, a combination of Beauvitech and Neemraj and the control on hydroponically and soil grown strawberry plants, respectively (Table 2). In both cases, the mean number of *Frankliniella* sp., in soil were highest in the control at 41.9 and 11.7 for hydroponics while the lowest numbers of thrips were observed in the treatment containing a combination of Beauvitech and Neemraj at 5.6 and 2.1, respectively. There was no significant difference on the number of *Frankliniella* sp., observed upon treatments with Beauvitech 6.4 and Neemraj 2.6 alone. Use of Ampligo 5.2 and a combination of Neemraj and Beauvitech 2.1 had significant impact on *Frankliniella* sp., on strawberry plants in hydroponics setup as compared to where Neemraj 2.6, Beauvitech 6.4 and control 11.7.

Table 1. Visual rating score for leaf damage

| Visual rating scale | Leaf area damaged extent (%) |
|---|---|
| 1 | 0-25 |
| 2 | 26-50 |
| 3 | 51-75 |
| 4 | >75 |

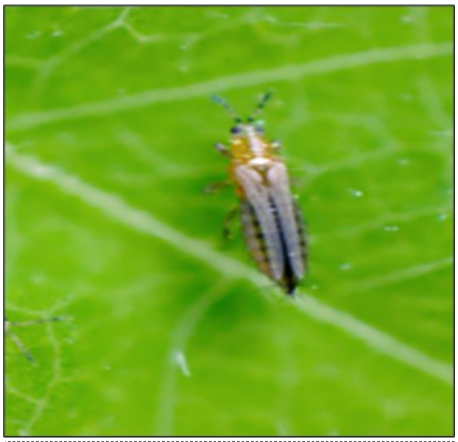

Figure 1. Frankillienlla sp recovered from strawberry flowers (Photo by Gladys Mbau)

*Effect of different treatments on the number of Tetranychus urticae on hydroponically and soil grown strawberry*

Application of selected treatments Neemraj, Beauvitech, Ampligo, combined Neemraj and Beauvitech and control on strawberry plants had no significant influence (Table 2) at F value 9.68, df=4,19, and p≤0.0004 on the number of *T. urticae* observed during the strawberry growing period (Table 2). In both instances the numbers of the *T. urticae* were highest in control plots with soil 46.3 and hydroponics at 41.5. Plots treated with Ampligo and Neemraj indicated no difference between them but indicated a significant difference compared to plots treated with a combination of Neemraj and Beauvitech 11.5 for hydroponics, 18.2 for soil and those treated with Beauvitech alone 33.4 and 28.8 for soil and hydroponics, respectively.

*Effect of different treatments on the number of strawberry vine weevil (Otiorhynchus ovatus) on strawberry plants*

The number of strawberry vine weevils on soil grown strawberry upon treatments with Ampiligo, Beauvitech, Neemraj, Neemraj and Beauvitech combined and in control treatments was significantly different (Table 2) at F=5.6, df=4,19, P≤0.0059. In hydroponics no observations of strawberry weevils were made while in soil culture the control treatment had highest observations of 26.5 and least number of strawberry weevils on plots treated with a combination of Neemraj and Beauvitech treatment at 6.95 which did not have a significant difference with plots treated with Ampligo 7.80. Independent use of Neemraj and Beauvitech did not have significant influence between the two treatments but was significantly different from control plots.

Figure 2. Adult *Tetranychus urticae* (photo by Gladys)

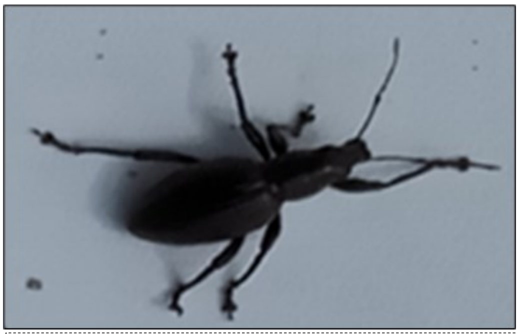

Figure 3. Photo of strawberry vine weevil (By Gladys)

*Effect of different treatments on the number of tarnished plant bug on strawberry*

There were significant effects (f values 6.07, df=4,19, p≤0.004) on the number of tarnished plant bug in both hydroponic and soil cultures. The observations were highest in soil media with 10.4 which was control treatment and on hydroponic culture (Table 2) at 7.2. Lowest mean observations were made on Ampiligo treated plots in both soil and hydroponic cultures in which a 2.9 tarnished plant bugs and 2.4 tarnished plant bugs were recorded respectively. There was no significant difference in observations made on plots treated with Ampligo and Neemraj and Beauvitech treatment combination in soil grown strawberry.

*Effect of different treatments on percentage cricket damage on strawberry*

From the results obtained cricket damage on strawberry plants (Table 2) at f values 10.4, df=4,19 and p≤0.0003 on treatment with Ampligo, Neemraj, Beauvitech, Neemraj and Beauvitech and in control was affected significantly. However, damage was only observed on strawberry planted in soil culture plots and not hydroponic setup. In control plots, cricket damage on the leaves was recorded at 31.3% whereas combination of Beauvitech and Neemraj recorded at 13.8% which was not significantly different as where Neemraj 17.5% was used independently. In Ampiligo treated plots there was least damage recorded at 6.3%.

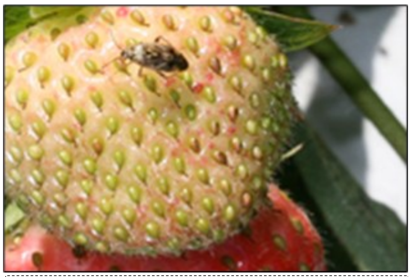

Figure 4. Tarnished plant bug on strawberry fruit (photo by Gladys)

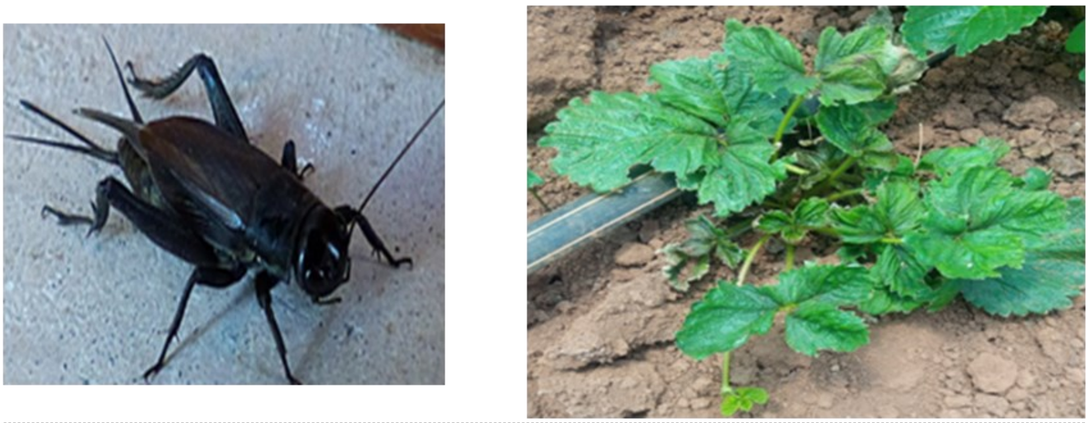

Figure 5. (a) Cricket (b) Cricket damage on strawberry leaves

Table 2. Means of pest incidences from selected integrated pest control treatments on strawberry

| | ***Franklienella spp*** | | ***Tetranychus urticae*** | | ***Otiorhynchus ovatus*** | | ***Lygus lineolaris*** | | Cricket damage Different species | |
|---|---|---|---|---|---|---|---|---|---|---|
| **TREAT-MENTS** | Hydroponic | Soil | Hydro-ponic | Soil | Hydro-ponic | Soil | Hydropon-ic | Soil | Hydro-ponic | Soil |
| Control | 11.7ᵃ | 41.9ᵃ | 41.5ᵃ | 46.3ᵃ | 0 | 26.5ᵃ | 7.2ᵃᵇ | 10.4ᵃ | 0 | 31.3ᵃ |
| Beauvitech | 6.4ᵃᵇ | 31.0ᵃᵇ | 28.8ᵃᵇ | 33.4ᵃᵇ | 0 | 17.5ᵃᵇ | 6.9ᵃ | 7.7ᵃᵇ | 0 | 22.5ᵃᵇ |
| Ampligo | 5.2ᵇ | 15.4ᵇᶜ | 20.7ᶜ | 24.4ᵇ | 0 | 7.0ᵇ | 2.4ᶜ | 2.9ᵇ | 0 | 17.5ᵇᶜ |
| Neemraj | 2.2ᵃᵇ | 8.1 ᶜ | 14.6ᵇᶜ | 18.9ᵇ | 0 | 13.6ᵃᵇ | 3.8ᵃᵇᶜ | 5.6ᵃᵇ | 0 | 13.8ᵇᶜ |
| Neemraj &Beauvitech | 2.1ᵇ | 5.6ᶜ | 11.5ᵇᶜ | 18.2ᵇ | 0 | 7.8ᵇ | 3.3ᵇᶜ | 3.0ᵇ | 0 | 6.3ᶜ |
| Msd | 7.1 | 24.2 | 17.0 | 21.8 | 0 | 14.7 | 3.89 | 5.27 | 0 | 12.69 |
| P value | 0.006 | 0.001 | 0.0004 | 0.0057 | 0 | 0.0059 | 0.004 | 0.002 | 0 | 0.0003 |
| F value (4.19) | 5.5 | 8.0 | 9.68 | 9.67 | 0 | 5.6 | 6.07 | 7.0 | 0 | 10.43 |

Means (±SE) followed by the same letter along the column are not significantly different according to Tukeys' (HSD) test at p≤0.05.

**Discussion**

*Effects of Neemraj and Beauvitech on pest incidences on soil and hydroponically grown strawberry*

From this research a total of six different pest species were observed. These included Thrips *Franklienella occidentalis*, spidermites *Tetranychus spp*, tarnished plant bug *Lygus lineoraris*, strawberry weevil *Ortiorhynchus spp*, crickets and predatory spiders. The study reveals that pest abundance and diversity was observed in soil based grown strawberry than in hydroponic grown media.

Based on this research, management of various pests such as spidermites, thrips, strawberry weevil and tarnished plant bug using Neemraj and Beauvitech was found to have significant differences in both soil and pumice grown strawberry. Additional research by Pascoli *et al*. (2019) oil extracted from the Neem plant has higher potential as a pesticide because of fungicidal, nematicidal, insecticidal and bactericidal activities.

The potential of using Neem and *Beauvaria bassiana* for pest control is supported by Islam and Omar (2012) reported that combining the two products had synergistic effect that giving a significant deterrence index in management of white flies whereby reduction in oviposition rates as well as number of adults in egg plants as compared to cases where each treatment was used alone.

Guruligappa *et al*. (2010) reported that inoculation of plants with *Beauvaria bassiana* and other entomopathogens have a wider ecology as well as more influence on herbivorous insects performance. In relation to this study, Dara (2016) observed that use of entomopathogenic fungi is able to manage all life stages of insect pests but nymphal stages that molt earlier before infection occurs, have shown minimal susceptibility and therefore combination of *Beauvaria bassiana* or *Metarrhizium brunneum* with Azadiractin has more potential to target young and mature stages of different pests.

Further support on the findings have been given by Mantzoukas and Eliopoulos (2020) by indicating that growth medium, species of the entomopathogen used, age and plant species, conidial density as well as method of inoculation involved are major factors that may also affect colonization. Further explanation is by Vidal and Jaber (2015) stating that ability of *B. bassiana* to produce metabolites such as bassianin, beauvaricin, oosporein, oxalic acid, tenellin, several bassinolides as well as other unknown metabolites into the plant system triggers defense mechanism resulting to management of insect pests attacking plants which have been colonized with endophytic EPF.

There were no observations made on cricket damage in pumice media. This can be explained by the fact that crickets prefer warm loamy soils to hide in and later emerge at night to feed. However damage was recorded in soil grown strawberry whereby young leaves were eaten and irregular and rugged markings were seen on the leaves. As the plants matured the cricket damage was not noticeable and the crop survived well.

In the current study, there were significant differences observed on the pest populations of different pests observed to have affected the soil and hydroponically grown strawberry crop. Combined use of Neemraj and Beauvitech has additive effects on the pest populations as compared to using each treatment individually. This is supported by Konecka *et al*., (2020) who reported that combination use of microbial and botanical pesticides have synergistic effects and majorly targets the immune system of the insects.

Schintzer (2007) mentions that the use of soilless growing cultures does not wholly guarantee complete absence of pests and diseases but provides ways to reduce infestations and as such hydroponics use in the greenhouse provides ways for protecting plants with ability to easily control external factors as compared to crop growing in the soil media.

In a study on pest and disease management in soilless cultures Schitnzer (2007) noted that similar pests and diseases can attack crops grown in both soil and soilless cultures but extents of severity and frequency may differ. This concept has been confirmed in this study through observations that there were lesser pests in hydroponic (pumice) set up as compared to soil grown strawberry

In the present study combined use of *Beauvaria bassiana* and Neemraj in management of thrips in both hydroponic and soil grown strawberry indicated significant effect on the number of thrips in strawberry plants.

The possibility of combining various neem products with various strains of *Beauvaria bassiana* was investigated and found worth by several researchers [2]. The potential to give synergistic effects by combining entomopathogenic fungi (EPF) and neem product against various pests was reported against *Epilachna deodecastigma* [3], *Spodoptera litura* (Mohan *et al*., 2007), *Bemisia tabaci* [4] and *Anacridum melanorhadon* [5] among others.

Similar reports by *Islam et al.,* (2010a) indicated that use of *Beauvaria bassiana* and neem as a combined foliar application gave the highest mortality incidence (97.2%) of *Bemisia tabaci* while individual applications of the treatments gave lower mortalities with *Beauvaria bassiana* (107conia/ml) at 70.4% neem (0.5%) at 77.3% respectively. From a research on eggplant (Islam *et al*., 2010b) found that there was toxicity increase on nymphs of *Bemisia tabaci* when *Beauvaria bassiana* was used as a foliar application and that combining neem and *B.bassiana* as a treatment showed whitefly nymphs reduced significantly on eggplants.

The findings have also been supported by studies conducted by Islam *et al*., (2010b) and indicated that combined use of neem and *B. bassiana* in management of *Bemisia tabaci* in eggplants gave 27.6 and 20.5% more nymphal mortality within 7 days after application as compared to applying each treatment individually.

The potential of neem in pest control has been strongly affirmed from by Kumari *et al*. (2020) research work that showed effective management of *Helicoverpa amigera*, *Spodoptera frugipeda*, *Anopheles stephensi, Cnaphalocrocis medinalis* and *Diaphorina citri* among others.

Neem (azadirachtin) does not directly kill pests but rather alters life processes of the pests such as feeding, reproduction, metamorphosis. This has been associated with the presence of and organic molecule tetranortriterpenoids which has a similar structure to insect hormones referred to as ecdysones. Neem is known to possess antibacterial and germicidal properties which are important for protecting plants from different kinds of pests [6].Uses of *B. bassiana* in thrips control have been supported by Bennison *et al*. (2017) indicating that different types of entomopathogenic fungi have been used in management of thrips.

In the present study combined treatment of Neemraj and Beauvitech had significant results in management of thrips in both hydroponic and soil grown strawberry as compared to using each treatment alone and thrips populations were highest in control plots Beauvitech 6.4 and Neemraj 2.6 and Neemraj &Beauvitech 2.1 and control 11.7 for hydroponic media. For soil media thrips populations were as follows; Beauvitech 31.0, Neemraj 8.1, Neemraj and Beauvitech 5.6 and control 41.9.

For the tarnished plant bug, there was notable difference in the numbers observed in both soil and hydroponic media upon treatment with Neemraj and Beauvitech but best management was in plots treated with combined Neemraj and Beauvitech 3.3, followed by Neemraj 3.8, Beauvitech 7.2.

*Beauvaria bassiana* has been found to have effective impact in the management of adults major strawberry pests such as *Otiorhynchus ovatus*, tarnished plant bug *Lygus lineolaris* and bud weevil clipper *Anthonomus signatus* in Quebec , Canada [6] with a concentration of 1X $10^7$ causing a mortality rates of between 23.3 and 100%.

Application of neem(800pm) and combining with a *B. bassiana* trap on palm tree trunks was very efficient in managing egg laying by red palm weevils in wounded palm tree trunks [7]. Similarly a combination of neem and *B. bassiana* in this study has been effective in managing the thrips, spidermites, lygus bugs and strawberry weevils.

The potential of Neem as a pesticide has also been supported by Minista *et al*. ( 2017) in Cameroon where research indicated that use of Neem extract on sweet potato crop *Ipomoea batatas* L., caused mortality of sweet potato weevil *Cylas puncticollis* (Boheman) which is a major pest causing approximately 97% damage. Saleem *et al.,* (2019) reported that use of azadiractin had a moderate efficacy in controlling pests in hydroponically grown cucumber andanaged *Tetranychus urticae* (69%) and Cotton aphid *Aphis gossypii* Glover (Hemiptera: Aphididae)(56%).

In comparison between hydroponically and soil grown strawberry studies, it was observed that there was higher strawberry plants survival (80%) than in soil grown system (<50%) which is largely associated with pest incidences and severe damage on soil grown crop than in hydroponics and that despite providing similar pest management practices to both soil and hydroponic growing cultures, soil grown plants had severe pest damage as aphids and spider mites thrived best in soil than in hydroponics [8].

## Conclusion

This study has demonstrated that using a combination of Azadiractin and *Beauveria bassiana* as pesticides in integrated pest management in strawberry greatly reduced various pest populations. However more trials on other crops and possible natural combinations can be conducted to encourage safer and friendly pest management options.

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