

Evaluation of bio pesticides against Major Pests of Cole crops (Broccoli)

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1. Background

Bio pesticides are derivatives of plants, microorganisms and insects. Bio pesticides are potential alternatives to synthetic pesticides. Sources of bio pesticides are readily available, easily biodegradable, exhibit various modes of action, are less expensive and have low toxicity to humans and non-target organisms. Botanical pesticides are derivatives of plant that repel, inhibit growth or kill pests. Most plants produce chemicals that deter pests, often producing a mixture of compounds that repel and stop from feeding.

Plants with pesticidal properties also possess compounds that have effects on plant pathogens such as bacteria, fungi, viruses as well as nematodes. Botanical pesticides are highly biodegradable, have varied modes of action, are less toxic to humans, are non-pollutant and they are readily available in the environment. The use of chemical pesticides for the control of crop pests and diseases is increasingly in question due to their detrimental side effects on humans and environment. There are several alternative measures to conventional methods, one of which is the use of bio-pesticides. The use of bio pesticides and botanical extracts are a key component of IPM together with other crop protection strategies that include host resistance or tolerance, good agricultural practices, use of natural enemies such as predators and parasitoids, microbial pesticides and limited use of safe synthetic pesticides. Under the NOFP, the use of bio pesticides and botanicals are being promoted as an alternative to synthetic pesticides to manage pests in Bhutan.

Broccoli (*Brassica oleracea*) is one such vegetable that is at par with local asparagus and fetches reasonably high prices ranging from Nu.100 to 150 per bundle (200-300 grams). It is known as the "Crown of Jewel Nutrition" because it is rich in vitamins and minerals. It is a good source of vitamin A, potassium, iron, calcium and fibre (Mukherjee & Mishra, 2012).

It grows well in reasonably fertile, well-drained, moist soils with plenty of added organic matter with soil pH ranging between 6.0 and 7.0 for optimum growth and temperature ranging from 18 ° C to 23 ° C. It can be grown anywhere except in the hottest and coldest climates, but does require cool weather to reach maturity (Mukherjee & Mishra, 2012). However, in the lower and mid altitude areas production of broccoli seed is really challenged by pests and diseases

especially aphids, Cabbage white butterfly and Cabbage looper. Keeping in view the importance of broccoli crop and the damage caused by the pest to the crop, the current experiment was designed to compare the effectiveness of a bio pesticide and botanical extract against the pest in broccoli with support from horticulture Program, ARDC, Bajo.

1.1 Problem Statement

Cabbage white butterfly (CWB), cabbage aphids and cabbage looper are the important pests of Cole crops. Cabbage aphid has been reported to cause up to 80 percent loss by leaf distortion, poor growth and by acting as vectors of viral diseases (Munthali, 2009). The larvae of *P. brassicae* eat the leaves of plants and contaminate the marketable heads of crop and have been reported to cause up to 24- 90 percent damage (Rejesus, 2001; Smith and Villet, 2002).

In Bhutan, these insect pests have been reported to cause significant damage in Cole crops. However, till now no information exists on the field efficacy of potential bio-inputs that can be recommended to manage these pests. Moreover, the identification and population dynamics of these pests and their associated natural enemies have not been documented. Therefore, there is need to evaluate the efficacy of available bio pesticides and botanical extracts against these pests and understand the activity of natural enemies to develop an integrated and environmentally friendly field management strategies against these insect pests.

1.2 Objectives:

- ✓ Evaluate the effectiveness of bio-pesticides against major pests (cabbage white butterfly, cabbage aphids).
- ✓ Identify major natural enemies.

2. Materials and Methods

The on-station trial was established at ARDC, Bajo (1,200 masl) in the month of October till December 2025 for the first season trial and for the 2nd season from February end till March 2026. The trial was laid using a randomized complete block design with four treatments (Jholmol 2, plant and herb extract, baking soda, and a control), each replicated three times across twelve 4 m² plots. Broccoli seedlings were transplanted at a spacing of 45 x 45 cm, with each plot

accommodating 20 plants for a total of 240 plants. Treatments were applied at seven-day intervals starting 21 days after crop establishment, using specific dilution rates for Jholmol 2 (1L: 3 L of water), botanical extract (1.5 L:20 L water), and baking soda (1 tbsp: 1 L water) Sandovit sticker was used in all the treatment to prevents the active ingredients from being washed off by light rain or dew.

Data were collected from 12 tagged plants per plot by visually scanning leaves dorsally and ventrally to record live larvae, eggs, adults, and pupae, along with the presence of beneficial insects (such as coccinellids, syrphids, spiders, and parasitoids). Observations were recorded one day before each application (pre-spray) and six days after (post-spray) to evaluate treatment efficacy.

2.1 Growth and Yield Assessment

Among the tagged plant, five plants were randomly selected from each plot and measured to determine the effect of treatments on the growth and yield.

Growth parameter	Yield parameter
Plant height- measured from ground level to the tip of the inner most leaf using measuring scale.	Curd weight- weigh using weighing balance
Number of leaves-counted manually	Curd diameter-measure using measuring scale/vernier caliper

The yield per plot was calculated using the following formula:

Area occupied by one plant (msq) = Total area of plot/Number of plants per plot

Yield per plot = Average yield per plant (kg) x number of plants per plot (3m²)

Yield per acre (kg) =Total yield per plot/Area of net plot x Area of acre.

3. Data Analysis

Data were entered and organized in Microsoft Excel and subsequently analysed using R statistical software (R Core Team, 2025). Prior to analysis, data were checked for completeness and appropriately coded, with treatment and replication treated as categorical variables.

Insect count data, including aphids, cabbage white butterfly larvae, and beneficial insects, were analysed using generalized linear mixed models (GLMM) with a negative binomial distribution to account for count data structure and overdispersion. Treatment was considered as a fixed effect, while replication (block) was included as a random effect to account for variability among experimental units.

Plant height, head diameter, and yield data were analysed using linear mixed models (LMM), with treatment as a fixed effect and replication as a random effect. Where applicable, model diagnostics were performed to ensure that assumptions of normality and homogeneity of variance were reasonably met.

Treatment means were separated using Tukey's Honest Significant Difference (HSD) test at a 5% level of significance ($p \leq 0.05$). Graphical presentations of temporal trends in insect populations were prepared using line graphs, while treatment effects on growth and yield parameters were presented using bar plots with standard error bars and significance letters.

4. Result and discussion

4.1 Effect of biopesticides on Aphid population

The negative binomial generalized linear mixed model (GLMM), revealed that treatment effects were not statistically significant ($p > 0.05$), indicating that aphid populations did not differ significantly among the tested biopesticide treatments. However, replication effects were significant, with Replication 2 ($p = 0.002$) and Replication 3 ($p < 0.001$) exhibiting higher pest counts than Replication 1. This suggests that variation among field blocks had a greater influence on aphid abundance than treatment application.

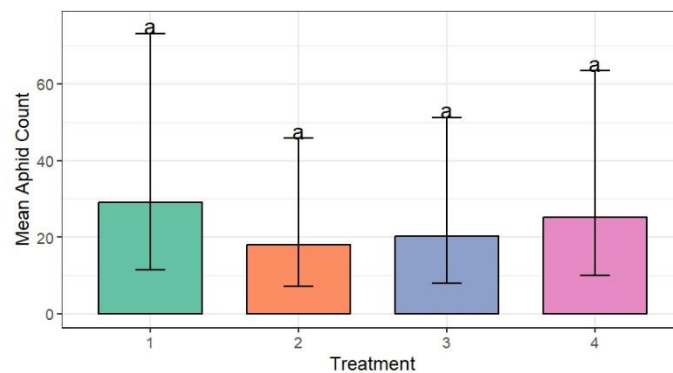


Figure 1. Mean aphid count ($\pm$ 95% confidence interval) across treatments. Treatment effects were evaluated using a negative binomial generalized linear mixed model (GLMM). Treatments sharing the same letter are not significantly different at the 5% significance level.

The present study found no significant differences in aphid abundance among the tested biopesticide treatments. Similar findings have been reported in previous studies where biopesticides exhibited inconsistent performance under field conditions despite showing potential under controlled environments. For example, Prince and Chandler (2020) reported that applications of the fungal biopesticide *Beauveria bassiana* significantly reduced populations of some aphid species but had no significant effect on *Myzus persicae* under field conditions.

Likewise, Dunan et al. (2021) observed that botanical biopesticides based on essential oils demonstrated promising insecticidal activity under laboratory conditions, but their effectiveness declined substantially when evaluated under greenhouse conditions. Such findings

suggest that environmental factors, pest immigration, product persistence, and variability in pest susceptibility can influence the field performance of biopesticides.

In the present study, replication effects were highly significant, indicating substantial spatial variability within the experimental area. Higher aphid densities observed in Replications 2 and 3 suggest that local environmental conditions, such as microclimate, crop vigor, or initial infestation levels, exerted a greater influence on aphid populations than the treatments themselves. Therefore, the absence of significant treatment effects does not necessarily imply that the biopesticides were ineffective, but rather that their effects were not sufficiently large or consistent to overcome the inherent variability present under field conditions.

Similar challenges in achieving consistent field control with entomopathogenic fungi have been reported in previous studies, where treatments that performed well under laboratory conditions failed to provide significant suppression under field environments (Prince & Chandler, 2020).

4.2 Effect of biopesticides on cabbage white butterfly

The negative binomial generalized linear mixed model (GLMM), indicated significant treatment effects on cabbage white butterfly larva abundance. Relative to Treatment4, Treatment1 (Estimate = 1.644, $p = 0.048$), Treatment2 (Estimate = 2.659, $p = 0.001$), and Treatment3 (Estimate = 2.070, $p = 0.013$) exhibited significantly higher counts. Treatment2 recorded the greatest increase in abundance. Replication effects were also observed, with Replication3 showing significantly higher counts than Replication1 ($p = 0.006$), while Replication2 was marginally significant ($p = 0.067$). These findings indicate that the tested biopesticides did not provide effective suppression of cabbage white butterfly larvae under the conditions of the present study.

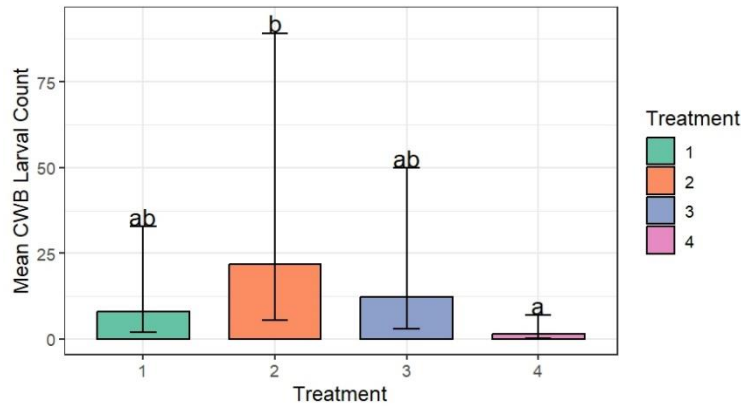


Figure 2. Mean CWB larva count ($\pm$ 95% confidence interval) across treatments. Treatment effects were evaluated using a negative binomial generalized linear mixed model (GLMM). Treatments sharing the different letters are significantly different at the 5% significance level.

Several factors may explain the lack of efficacy observed. Biopesticides are often influenced by environmental conditions such as temperature, rainfall, and ultraviolet radiation, which can reduce the persistence and activity of biological agents in the field (Dunan et al., 2021). In addition, application timing may not have coincided with the most susceptible larval stages, thereby limiting treatment effectiveness. Previous studies have reported that the efficacy of microbial and botanical biopesticides can vary considerably under field conditions due to environmental influences and pest pressure, resulting in inconsistent levels of pest suppression (Prince & Chandler, 2020). Variation in initial pest infestation levels among plots may also have contributed to the observed differences.

Significant replication effects further suggest that spatial variability within the experimental field influenced cabbage white butterfly populations. Environmental heterogeneity, including differences in microclimate, plant vigor, and pest colonization patterns, may have affected larval abundance independently of treatment application. Similar observations have been reported in field evaluations of biological control products, where local environmental conditions significantly influenced pest populations and treatment responses (Atwa et al., 2009).

Consequently, while treatment effects were statistically significant, the higher larval counts observed in treated plots indicate that the tested biopesticides did not achieve the desired level of control under field conditions. Further studies involving multiple application timings, larger plot

sizes, and repeated seasonal evaluations are recommended to better assess their efficacy under Bhutanese conditions.

4.3 Effect of biopesticides on beneficial insects

The generalized linear model showed no significant treatment effects on beneficial insect abundance ($p > 0.05$). Although Treatment1 tended to support lower beneficial insect populations than the reference treatment (Estimate = -1.121 , $p = 0.066$), the difference was only marginally significant. Treatments 2 and 3 did not differ significantly from the reference treatment. Replication effects were also non-significant, indicating that beneficial insect abundance was relatively consistent across blocks. Overall, the treatments did not substantially affect beneficial insect populations.

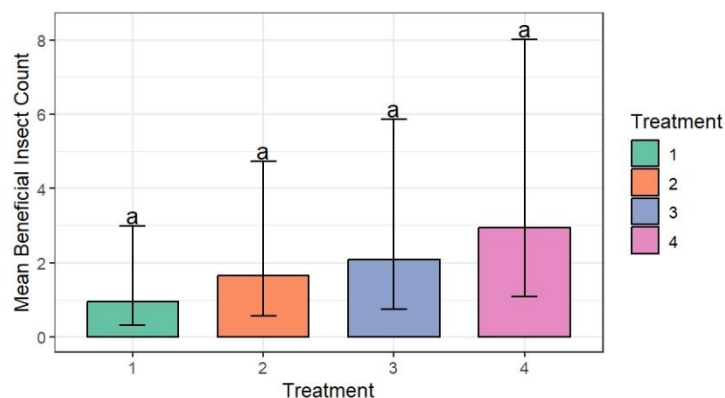


Figure 3. Mean beneficial insects count ($\pm$ 95% confidence interval) across treatments. Treatment effects were evaluated using a negative binomial generalized linear mixed model (GLMM). Treatments sharing the same letter are not significantly different at the 5% significance level.

No significant differences were observed in the abundance of natural enemies among treatments. This suggests that the tested biopesticides were relatively compatible with beneficial arthropods and did not adversely affect naturally occurring predator populations. Similar observations have been reported for microbial biopesticides such as *Bacillus thuringiensis* (Bt), which are generally regarded as selective insecticides with minimal impact on non-target organisms and natural enemies. Helson (1960) reported that Bt applications against cabbage pests had no observable

effects on syrphid larvae and hymenopteran parasitoids while effectively targeting lepidopteran larvae.

The conservation of natural enemies observed in the present study is important from an integrated pest management perspective, as biological control agents contribute to the natural suppression of pest populations. Therefore, although the treatments did not significantly reduce pest abundance, their apparent compatibility with beneficial insects supports their potential role within sustainable pest management programs.

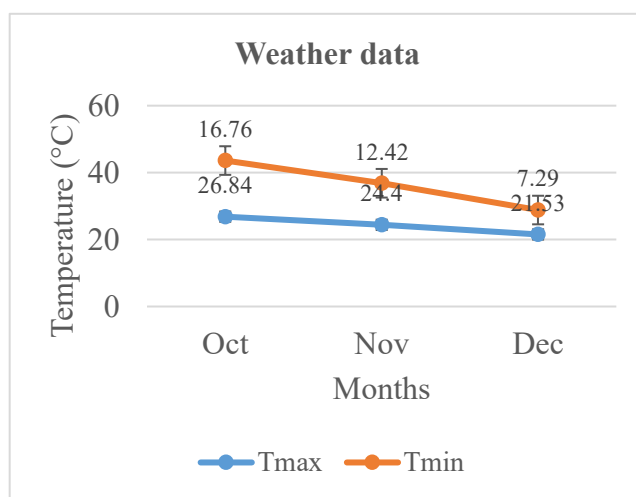


Figure 4. Monthly maximum (Tmax) and minimum (Tmin) temperatures recorded during the broccoli experimental period at Bajo, Wangdue Phodrang. Temperatures declined progressively from October to December.

4.4 Effect of biopesticides on population dynamics of aphids

Aphid populations exhibited a declining trend throughout the experimental period across all treatments (Figure 5). At the initial assessment, aphid abundance was relatively high in all plots, after which populations progressively decreased toward the final observation date. Although numerical differences among treatments were observed, statistical analysis revealed no significant treatment effects ($p > 0.05$). Similar population trajectories across treated and untreated plots indicate that the tested biopesticides did not significantly influence aphid abundance under the conditions of the present study.

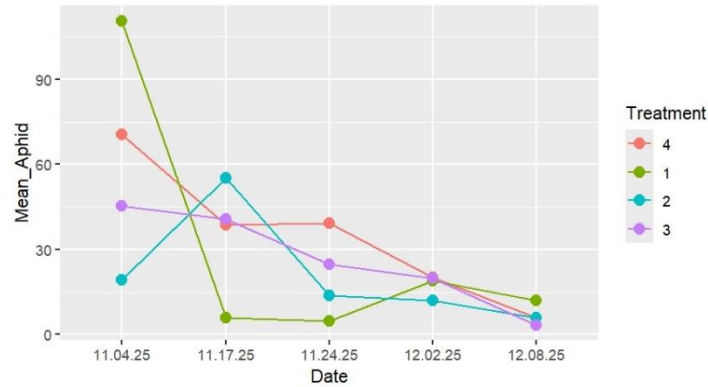


Figure 5. Showing population dynamics of aphid over time

4.5 Effect of biopesticides on population dynamics of cabbage white butterfly larvae

Cabbage white butterfly larval populations varied throughout the sampling period, with peak abundance observed during the early stages of crop growth, followed by a marked decline toward the end of the experiment (Figure 6). All treatments exhibited similar temporal patterns, and no significant differences among treatments were detected ($p > 0.05$). The decline in larval abundance coincided with decreasing temperature during the later stages of the trial.

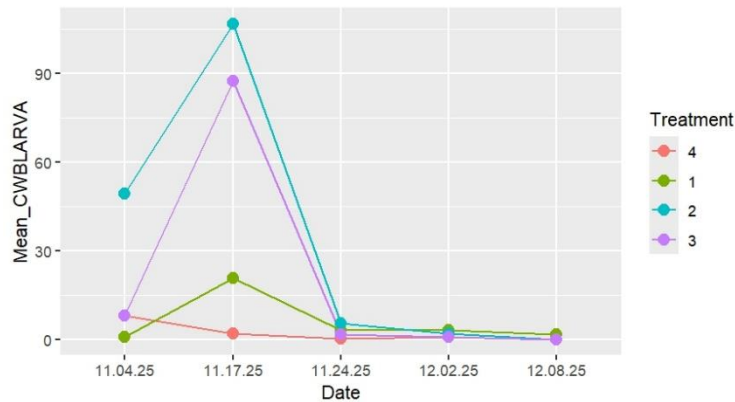


Figure 6. Showing population dynamics of Cabbage white Butterfly over time

4.6 Effect of biopesticides on population dynamics of beneficial insects

Beneficial insect abundance remained relatively low during the initial observations, increased during the middle of the cropping period, and subsequently declined toward the final assessment (Figure Z). Despite slight numerical variation among treatments, no statistically significant treatment effects were observed ($p > 0.05$). The temporal fluctuations in beneficial insect

populations appeared to correspond with changes in prey availability and prevailing environmental conditions.

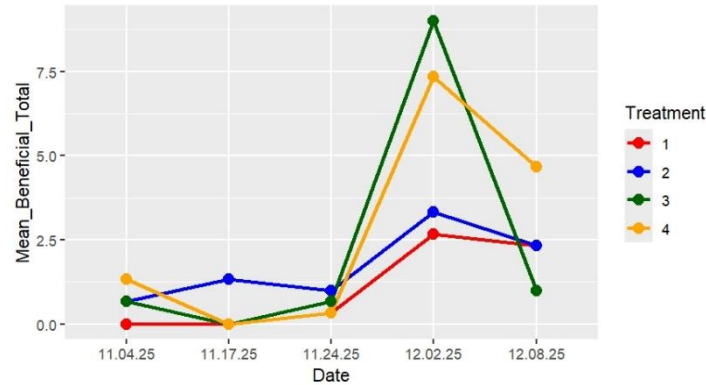


Figure 7. Showing population dynamics of beneficial insects over time

The absence of significant differences among treatments suggests that the tested biopesticides were not sufficiently effective to suppress aphid and cabbage white butterfly populations under field conditions. Similar population trends across all treatments, including the untreated control, indicate that environmental factors may have exerted a stronger influence on insect abundance than treatment applications.

Aphid populations declined progressively throughout the experimental period. This decline coincided with decreasing maximum and minimum temperatures from November to December. Temperature is one of the most important abiotic factors influencing aphid development, survival, reproduction, and dispersal. Reduced temperatures generally slow population growth and may limit colony establishment, resulting in lower aphid densities later in the season (Dixon, 1998). The observed decline across all treatments therefore suggests that seasonal weather conditions contributed substantially to aphid suppression.

Similarly, cabbage white butterfly larval abundance decreased during the latter stages of the experiment. Larval development and adult activity of *Pieris* species are strongly influenced by temperature, with cooler conditions reducing feeding activity, developmental rates, and reproductive success (Courtney, 1986). The reduction in larval populations observed during

December may therefore be partially attributable to declining temperatures rather than treatment effects alone.

Beneficial insect populations increased during the middle of the season before declining toward the end of the experiment. Such fluctuations are common in agroecosystems and are often associated with prey availability and environmental conditions. As aphid populations declined, food resources available to predators and parasitoids may also have decreased, resulting in lower beneficial insect abundance during the final observation period (Landis et al., 2000). Furthermore, declining temperatures may have reduced the activity and foraging efficiency of natural enemies.

Weather conditions during the study period further support this interpretation. Mean maximum temperature declined from 24.40°C in November to 21.53°C in December, while minimum temperature decreased from 12.42°C to 7.29°C. Rainfall also declined from 17 mm in November to zero in December. These environmental changes likely affected both pest and beneficial insect populations and may have masked potential treatment effects. Similar findings have been reported in field studies where seasonal climatic variation exerted a greater influence on insect population dynamics than pest management treatments (Pedigo & Rice, 2014).

Overall, the results indicate that seasonal environmental conditions, particularly declining temperature, played an important role in regulating aphid, cabbage white butterfly, and beneficial insect populations during the experimental period. Consequently, the tested biopesticides did not produce statistically detectable effects under the prevailing field conditions.

4.7 Effect of biopesticides on plant height

Plant height did not differ significantly among treatments ($p > 0.05$) (Figure 8). Although slight numerical variations were observed, all treatments produced plants of comparable height. The absence of significant differences indicates that the application of Jhol Mol, Plants and Herbs extract, and Baking Powder did not substantially influence vegetative growth of broccoli under the conditions of the present study.

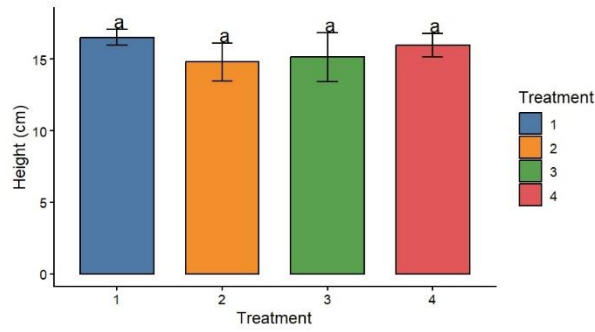


Figure 8. Effect of biopesticide treatments on plant height. Bars represent mean $\pm$ SE. Means followed by the same letter are not significantly different according to Tukey's HSD test at $p \leq 0.05$.

4.8 Effect of biopesticides on head diameter

Similarly, head diameter was not significantly affected by treatment application ($p > 0.05$) (Figure 9). The recorded head diameters were comparable across all treatments, including the untreated control. These findings suggest that the tested biopesticides neither enhanced nor suppressed curd development and marketable head formation.

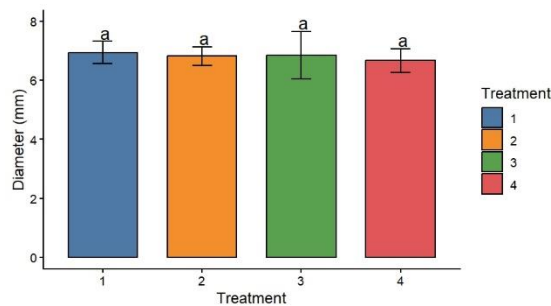


Figure 9. Effect of biopesticide treatments on head diameter of broccoli. Bars represent mean $\pm$ SE. Means followed by the same letter are not significantly different according to Tukey's HSD test at $p \leq 0.05$.

4.9 Effect of biopesticides on broccoli yield

Broccoli yield did not differ significantly among treatments ($p > 0.05$) (Figure 10). Although numerical differences in yield were observed, the variability among replications was sufficiently large that treatment effects were not statistically detectable. The results indicate that application of the tested biopesticides did not significantly influence final yield under field conditions.

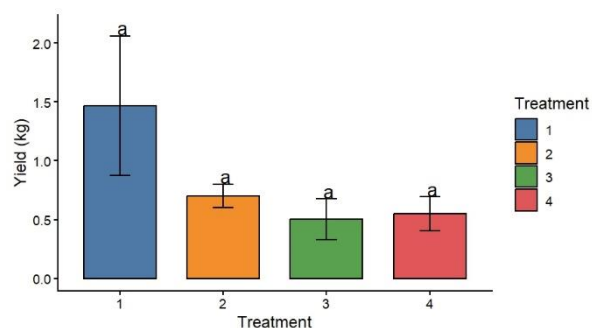


Figure 10. Effect of biopesticide treatments on broccoli yield. Bars represent mean $\pm$ SE. Means followed by the same letter are not significantly different according to Tukey's HSD test at $p \leq 0.05$.

The absence of significant differences in plant height, head diameter, and yield among treatments suggests that the tested biopesticides had limited influence on broccoli growth and productivity. Similar growth performance across treated and untreated plots indicates that the products neither promoted plant growth nor caused phytotoxic effects.

One possible explanation is that pest populations may not have reached levels sufficient to cause substantial crop damage. Although aphids and cabbage white butterfly larvae were present during the growing season, their populations declined over time across all treatments, including the untreated control. Consequently, the level of pest injury may have been insufficient to reduce vegetative growth or yield formation. Under such conditions, differences in pest suppression among treatments are less likely to translate into measurable agronomic benefits (Pedigo & Rice, 2014).

Environmental conditions may also have contributed to the lack of treatment effects. During the experimental period, maximum and minimum temperatures declined progressively from November to December, while rainfall decreased substantially. These environmental changes likely influenced both pest populations and plant growth. Favorable growing conditions can often compensate for moderate pest damage, allowing crops to maintain growth and yield despite pest infestation (Dent, 2000).

The lack of yield response despite numerical differences among treatments is consistent with findings from many pest management studies, where reductions in pest abundance do not necessarily result in significant increases in crop productivity. Yield is a complex trait influenced by multiple interacting factors, including environmental conditions, nutrient availability, plant genetics, and pest pressure (Nault & Kennedy, 2003). Therefore, even when treatments provide some degree of pest suppression, corresponding improvements in yield may not always be observed.

Overall, the results indicate that the tested biopesticides did not significantly affect broccoli growth and yield under the prevailing field conditions. The similarity of plant height, head diameter, and yield across treatments suggests that environmental factors and relatively low pest pressure may have played a greater role in determining crop performance than the applied treatments.

Conclusion

This study evaluated the effectiveness of three biopesticide treatments, namely Jhol Mol, Plants and Herbs extract, and Baking Powder, for the management of aphids and cabbage white butterfly in broccoli under field conditions. The results showed that none of the tested biopesticides significantly affected aphid populations, cabbage white butterfly larval abundance, beneficial insect populations, plant height, head diameter, or yield compared with the untreated control.

Although numerical differences among treatments were observed, pest populations generally followed similar temporal trends across all treatments. Aphid and cabbage white butterfly populations declined progressively during the cropping period, while beneficial insect abundance fluctuated throughout the season. These changes coincided with decreasing temperature and rainfall from November to December, suggesting that seasonal environmental conditions may have played a greater role in regulating insect populations than the applied treatments.

Likewise, the absence of significant differences in plant height, head diameter, and yield indicates that the tested biopesticides neither enhanced nor adversely affected broccoli growth and productivity. The relatively low pest pressure and favorable growing conditions may have limited the ability of treatment effects to translate into measurable agronomic benefits.

Overall, the findings suggest that the tested biopesticides provided limited pest management benefits under the conditions of the present study. Environmental factors, particularly seasonal changes in temperature and rainfall, appeared to exert a stronger influence on pest and beneficial insect dynamics than treatment application. Further research across multiple growing seasons, locations, and pest pressure levels is recommended to better assess the potential of these biopesticides as components of sustainable broccoli pest management programs.

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