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Research Article

# Evaluating Neem's Potential as an Eco-Friendly Insecticide in Brassica Cultivation

**Sajid Ali[1], Adnan Zahid[1*], Ammara Fatima[2], Muhammad Bilal Chattha[1], Muhammad Ali[4], Ahmad Raza[1], Nauman Shafqat[1], Asma Ayub[1,3], Aisha Zahra[1], Munib Ullah Khan[5]**

[1] Department of Agronomy, Faculty of Agricultural Sciences, University of the Punjab, Lahore, Pakistan

[2] Department of Environmental science, Lahore College for Women University, Lahore, Pakistan

[3] Department of Field Crops, Faculty of Agriculture, University of Cukurova, Adana, Turkey

[4] Department of Entomology, Faculty of Agricultural Sciences, University of the Punjab, Lahore, Pakistan.

[5] Department of Plant Pathology, Faculty of Agricultural Sciences, University of the Punjab, Lahore, Pakistan.

*Correspondence: adnan.iags@pu.edu.pk

## ABSTRACT

Brassica crops including cabbage and cauliflower are extremely crucial to food security but are extremely susceptible to bacterial diseases like Xanthomonas campestris pv. campestris (Xcc) and Pseudomonas syringae pv. maculicola (Psm) that drastically lower yield and quality. Even traditional chemical pesticides, popular in use, are too dangerous to human health and the environment because of the accumulation of residues. The laboratory assays indicated that the neem (Azadirachta indica) leaf extract was very effective in preventing the growth of bacteria with mean inhibition zones of 18.9 + 0.6 mm (Xcc) and 15.1 + 0.5 mm (Psm) at 75 percent concentration. Application in the field of the Economic Threshold Level (9% incidence) decreased the disease incidence to 10.5 ± 0.9% compared to 28.4 ± 1.2% and the control plots to 45.6 ± 2.1, respectively. The biodegradability of neem, non-toxicity, and zero residue of the compound render it a potential eco-friendly alternative to using chemical pesticides in the sustainable farmers of Brassica..

**Keywords**: Biopesticide; Plant extracts, Eco-friendly control, Biological Control, Sustainable agriculture.

## INTRODUCTION

Productivity, yield, taste, vigor, nutrition, and texture are parameters to define the status of the food on one hand and the choice of the consumer on the other. Due to continuing challenges and alarming situations, it is estimated that 4 to 5 decades will be questionable for the production of enough food to satisfy the needs of human beings (Prosekov et al., 2018). Brassica includes highly rated agricultural and horticultural crops & vegetables (cabbage, cauliflower, broccoli, canola, turnip & Brussels sprouts). The crucifer members have quite significant health and beneficial nutritional values due to the presence of contents like Beta-carotene, vitamin C, and fiber. Cabbage & cauliflower yielded 74,282 & 227,195 tons in an area of 4,983 & 13,103 ha in Pakistan in 2011, respectively (Huzaifa et al., 2025).

Cabbage and other crucifers are perishables and can only be preserved for as long as 7 days if stored at 4ºC (Demirbas, 2016). Their diseases have considerable importance and can wipe out production, e.g. soft rot & black rot. Black rot is a destructive seed-borne disease, caused by *Xanthomonas campestris*. As it is a bacterial and seed-borne disease as well, the seed treatment with chemicals or hot water seems better than all other control measures. However it is not perfectly effective and it is recommended not to use hot-water-treated seeds for transplant germination since hot-water treatment reduces seed viability (Sanchez et al., 1997). Soto get productive yield, one has to apply a lot of control measures which have sometimes been used more than needed, e.g., chemical control. But for high productivity, more and more chemicals are being added to the natural environment. Continuous evidence has shown the potential risk of pesticides to human health and bad effects on the environment (Kim et al., 2017). Now, pesticide accounts for 258,234 deaths worldwide, annually (Lee et al., 2009).

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Brassica vegetables having enormous-sized stomata increase the chances of taking chemicals from pesticides as a part of their metabolic constituent, so issues of residual effect-related health problems arise. To combat this situation, pathologists are trying to come up with alternate strategies to control diseases. Biocontrol and organic farming are the results of these efforts done by researchers to provide healthy and pure food to the consumer without using chemicals.

The use of neem is found to be very effective in many cases against many pathogens. It belongs to the family Meliaceae and is found to have huge agricultural and medicinal uses. It has a unique chemical, Azadirachtin, which has been proved to be effective against pests & diseases. With various other chemical combinations, neem extract is more effective & safer against pathogens in various cases than chemical applications. Not having a residual effect makes it better than other applications. Its chemicals are biodegradable in the presence of sunlight. Degradation can be performed due to light, temperature, UV effect, and microbial activity (de Oliveira et al., 2014).

Yet, various species of *Salmonella*, *Pseudomonas*, *Xanthomonas*, *Escherichia coli*, *Staphylococcus*, and *Streptococcus* spp. have gone through experimentation with neem, producing positive results in a controlled manner. Neem has effective control against X*anthomonas campestris* due to its antimicrobial and medicinal properties (Sharma et al., 2012). In spite of the growing awareness of the environmental risks of synthetic pesticides, there is a lack of field-based research on the application of neem (A. indica) extract in the control of bacterial diseases in Brassica crops under field conditions. Thus, the objective of the study was to assess the effect of neem leaf extract on the antibacterial activity against X. campestris and P. syringae using laboratory and field experiments, and to identify whether it could be applied as an alternative to sustainable management of Brassica disease and be considered an eco-friendly approach. There are signs of fine inhibitory actions by the neem against *Pseudomonas* spp. either in pure or in the form of dilution (Irshad et al., 2011; Jahan et al., 2007).

## MATERIALS AND METHODS

### Preparation of Seedbed

For preparation of seedlings to transplant, seeds of cabbage and cauliflower were taken from Federal Seed Certification and Registration Department (FSC & RD) Pakistan. Nursery was prepared in green house because of frosting in the field at night, which is not suitable for germination of seeds (Murison et al., 2006a). 50% germination rate was supposed and the seeds were sown double than required seedlings in field. According to the size of field, 10g seeds of each vegetable were sown in seedbed as approximate 100 seeds are found in 1g and required no. of transplants for each vegetable was 500. River soil (bhal) was used for seedbed preparation as it is more suitable than field soil for seeds to grow (Cacho, 2013). Seeds were sown when soil moisture content was suitable.

In case of Cabbage, 200 g/acre seed rate is used for seedling production (Murison et al., 2006b). Duration between sowing & transplanting was 2-3 weeks (Agriculture, 2012). Plant spacing was 30-40 cm(Khatiwada, 2011) and row spacing was 45-55 cm (Khatiwada, 2011). Irrigation Crop requirement before transplanting was 2-6 inches and total irrigation requirement was 1-1.5 acre feet (Daugovish et al., 2008). 122kg/Acre Nitrogen, 62.5kg/Acre $P_2O_5$and 62.5kg/Acre $K_2$was used (Onduso, 2011). When transplants were 2-3 weeks old they were able to transfer.

In case of Cauliflower, Seed rate for seedling production was 120 g/ac (Zvalo et al., 2007) and duration between sowing & transplanting was 4-5 weeks (Zvalo et al., 2007). Plant spacing and Row spacing was 25 cm and 45cm respectively (Sanders, 1999). Irrigation was applied immediately after transplanting, thereafter, with a week interval (Agriculture, 2012). Manuring & Fertilizing was used as 80 kg N, 100 kg P and 90 kg/ac K (Center, 2004).

### Preparation of Field

Field was selected where vegetable cultivation was not practiced, because crops residues and debris are good sources of inoculum for these bacteria. 72 plots/blocks were designed in field for 3 crops (cabbage, cauliflower & canola) with 24 plots for each, covering 2 disease inoculums (*X. campestris* & *P. syringae*) 1 treatment and 1 Control with 5 replicates. 2 plots in middle and 2 plots on front and rear, respectively, were designed as boundaries to evaluate disease incidence.

These plots were not inoculated or treated by bacteria and neem extract. According to field size and rows length, 21 seedlings were estimated with 7 plants for each row, out of 3 rows in each plot. Total 1008 plants of both vegetables were required for 48 plots. 12 plots were made for canola cultivation. Canola seeds were sown directly in the field on the rows but couldn't germinate properly because of highly cold weather and excess of water at initial stage, which brought the frost and seeds couldn't germinate. So, these plots were excluded from experimental area. Field was designed on CRD (Completely Randomized Design), the dimensions of field was set according to this layout. Plots were randomly assigned treatments with the use of random number tables to reduce positional bias. Row distance and planting distance was 45cm and 30cm respectively. Net plot size was 1.35 x 3.0 $m^2$, area covered by ridges/boundaries was 14m and total cultivated area was 680. 6m$^2$.Immediately after transplanting, surface irrigation was applied. Approximately 95% plants succeeded to stand.

**Preparation of Inoculum**

Samples infected with *Xanthomonas campestris* pv. *Campestris* and *pseudomonas syringae* pv. *Maculicola* were collected from the infected field of cauliflower& cabbage. Isolation was done from leaves on basis of symptoms. Usually, series of biochemical experiments is conducted for confirmation of bacterial path over from diseased sample. Isolation from field was done to provide alternate source of inoculum, while pure and confirmed cultures were taken from the inventory of FCBP (Fungal culture bank of Pakistan), accession numbers of these pathovars were found, in cultural inventory of bacterial cultures collection, as 007 for *Xanthomonas* and 097 for *Pseudomonas*. These cultures were immediately multiplied by growing them on culture media. King's medium B (20g agar, 20g proteose peptone no.3, 1.5g $K_2HPO_4$ anhydrous, 1.5g $MgSO_4.7H_2O$and 15ml glycerol) was used to cultivate *Pseudomonas* and Wilbrinck agar (20g sucrose, 12g agar, 5g peptone, 0.5g $K_2HPO_4$ and 0.25g $MgSO_4.7H_2O$) for *Xanthomonas* spp. (Atlas, 2005). After purification, cultures were stored at freezing temperature, and re-cultured repeatedly after 15 days interval for maintaining viability of cultures. Isolations from leaves were first cultured on LBA (Bertani, 1951) and King's medium B and then purified but these cultures were stored and never used.

**Treatment**

**Plant Material**

Bacterial cultures were tested with different concentrations of Neem (*Azadirachta indica*) extract. Leaves were taken from the graveyard, situated in front of IAGS.

**Data Collection**

**Laboratory Trial**

Leaves were cleaned, chopped, mixed and grinded to make paste. The crude extract obtained was filtered through muslin cloth then through Whatman No.1 filter paper and serial dilutions of 25, 50, 75 and 100 (v/v) of the solvent (ethyl alcohol) was prepared. Spectrophotometric analysis was performed at 600 nm to verify that the extracts had equal concentrations. Standard extract was made of 1g/ml dilution. Final standard extract was further diluted in ethyl alcohol to make 25%, 50%, 75% concentrations. 100% concentration used as pure extract & 0% as pure diluent (Sarmiento et al., 2011).

These dilutions were made to evaluate their comparative effect in controlling bacterial infection. For these concentrations, Gel diffusion method was used (Reddy et al., 2012) to show inhibition zones against *Xanthomonas campestris* pv. *campestris* and *Pseudomonas syringae* pv. *Maculicola* by different concentrations. Concentration which showed best inhibition ability in controlled condition was taken to field. Before evaluation, bacterial colonies were re-cultured on agar containing medium from LB broth in the form of suspension. 5mm suspension was inoculated through pipette and swirled on King's B for and Wilbrinck agar. Plates were incubated at 27$^0$C for 24 hours. After bacterial growth, treatments were applied through gel diffusion method. Zones of inhibition were estimated that showed which treatment has better effect on controlling bacterial growth. These zones of inhibition were compared with control having 0% concentration, a pure diluent (Sarmiento et al., 2011).

**Field Trial**

In the field, treatment was applied when the symptoms appeared on leaves and heads and disease severity level reached up to Economic Threshold level (ETL). It took 1 to 2 weeks for bacteria to establish their inoculum and to show symptoms. Bacterial infestation was realized by rate of lesion development and lesion size through disease rating scale, on leaves and other parts, while treatment was applied to control bacterial infection. After 2 weeks when the inoculum was just about to cross damage boundary in field, first treatment was applied. Economic damage usually begins when the detriment is higher than the cost of control and economic damage starts just after the economic threshold level. Disease severity and disease incidence was calculated by using these formulas (Masood et al., 2010)

$$Disease\ severity = \frac{Infected\ tissue\ area}{Total\ tissue\ area} \times 100$$

$$Disease\ incidence = \frac{Number\ of\ infected\ plants}{Total\ no.of\ plants\ assessed} \times 100$$

A point must come where we have to apply control measures because after that point economic losses due to control treatment will happen. That point is economic threshold level and can be calculated by formula (IRRI, 2009);

$$Economic\ threshold\ level = \left[\frac{C}{YP}\left(\frac{K}{100}\right)\right] \times 100$$

Where,

C= cost of treatment

Y=expected yield

P= expected price per production unit

K=expected success of the treatment

Data was obtained by measuring disease severity, and for calculating disease severity, random selection of leaves of different plants was done from different replications in both experiments of bacterial inoculation, before treatment. From 24 plots of each vegetable, 3 plants were randomly selected to check the disease incidence each day, for 28 days (14 after inoculation and 14 after treatment). When treatment was applied by considering ETL level, disease incidence was ceased. Next spray was applied at the time when vegetables were producing heads and flowers, because these parts are sensitive to these bacterial diseases and infection can develop in newly developed plant parts.

**RESULTS**

**Field Results**

Bacterial inoculum was applied when plants matured. The symptoms appeared on them after two weeks. Neem extract of a concentration of 75% was used as it showed promising results in laboratory conditions. To avoid toxicity in plants 100% concentration was not used. The treatment time for cabbage and cauliflower was determined using the following ETL formula:

$$ETL = \left(\frac{C}{Y \times P}\right) \times k \times 100$$

For instance, assuming the cost of control (C) = PKR 5000, expected yield (Y) = 1300 kg, price (P) = PKR 25 $kg^{-1}$, and expected control success (k) = 60% (0.60), then:

$$Y \times P = 1300 \times 25 = 32{,}500 \qquad \frac{C}{Y \times P} = \frac{5000}{32{,}500} = 0.1538$$

$$ETL = 0.1538 \times 0.60 \times 100 = 9.23\%$$

Thus, the Economic Threshold Level (ETL) was calculated as **9.23%**. This indicates that the control should be applied before 9% disease occurrence or it will cause yield loss. Disease incidence was measured, daily, by taking random readings for both vegetables, from all plots (replicates) of the experiment, and calculating the no. of diseased plants. Those plants which showed severe disease symptoms on the disease rating scale were considered as diseased. This scale was developed based on %age disease severity, which was calculated by formula (Infected tissue area / total tissue x 100). Those plants that showed 0% infection were classified as healthy plants; with 0 on the rating scale. Other plants having 20%, 40%, 60%, 80%, and 100% were rated as mildly infected, moderately infected, highly infected, severely infected and very severely infected respectively. When 9% of plants were found severely infected, Neem extract was applied. In the field, post-inoculation and post-treatment trends are shown in Fig 1, 2 and 3. The trend of increase in infection of both diseases was rapid but when it became near to ETL level, Neem extract as a control treatment was applied, which successfully inhibited disease incidence in both diseases. For the first 14 days, all plots/ blocks were equally inoculated and average disease incidence was calculated. The trend of disease incidence, when reached to ETL level, was changed due to the application of neem extract. After 14 days, plots that were treated with extract showed inhibition in disease incidence for both cabbage and cauliflower, while non-treated plants showed a rapid increase in disease occurrence, ultimately leading to the development of severe infections in all untreated plants. Cabbage showed quite a bit more sensitivity than cauliflower for diseases and led to the early development of symptoms.

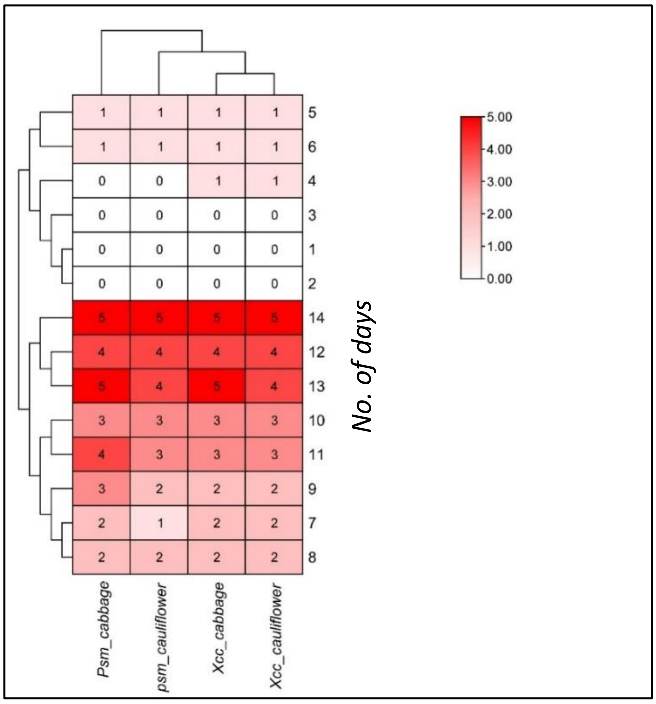


**Figure 1.** Post-inoculation trends of disease incidence in field

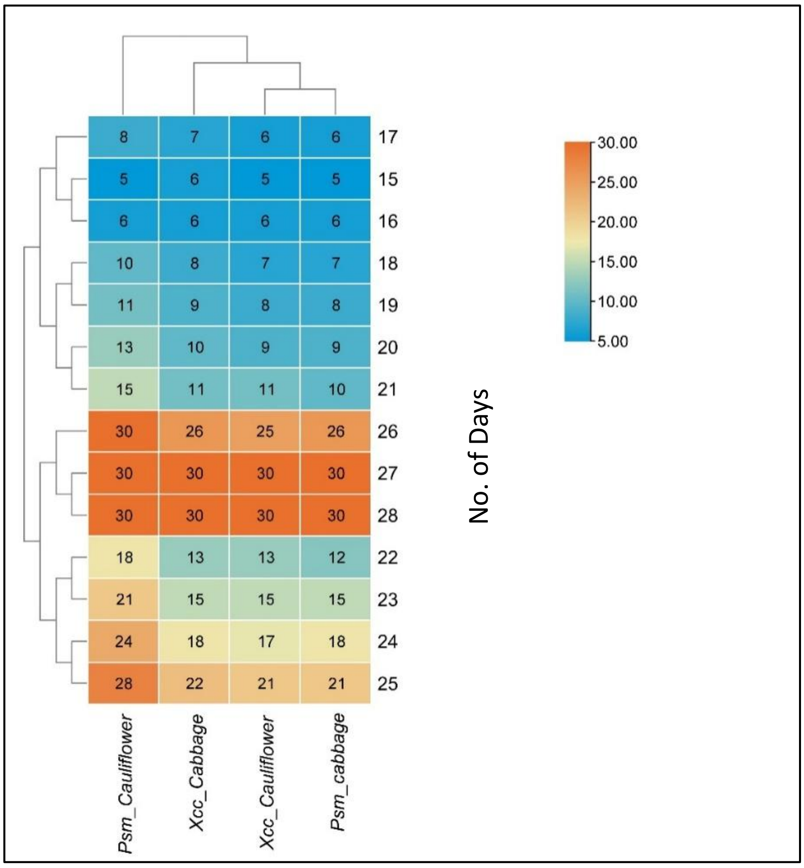


**Figure 2.** Control, where inoculum was applied but not the treatment

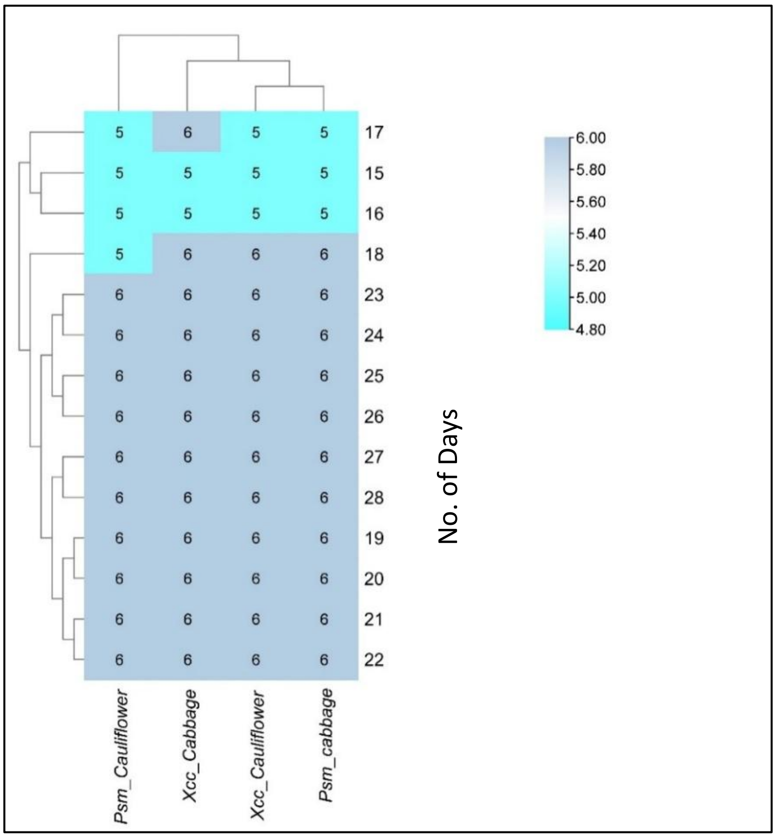


**Figure 3.** Post-treatment trends of disease incidence in field

The average disease incidence in neem-treated plots decreased from 28.4 ± 1.2% to 10.5 ± 0.9%, while control plots showed an increase to 45.6 ± 2.1%. Cabbage exhibited higher susceptibility to bacterial infection than cauliflower, developing symptoms earlier. The application of neem extract effectively suppressed further spread once the ETL threshold was reached.

**Lab Results**

These outcomes were attained in a controlled setting. Different doses of Neem extract displayed distinct patterns in the lab during screening tests against bacterial pathovars, indicating the superior control treatment for assessment. For each treatment, average values were obtained for duplicates. Zones of inhibition and concentrations were measured to determine the results, which were then used to compare the effects of inhibition on Xcc and Psm after three days. Neem extract had a greater controlling effect on Xcc than Psm in the initial observation.

Neem extract exhibited dose-dependent antibacterial activity against *Xanthomonas campestris* pv. *campestris* (Xcc) and *Pseudomonas syringae* pv. *maculicola* (Psm). The mean inhibition zones (mm ± SD) for Xcc were 8.3 ± 0.5, 13.7 ± 0.8, 18.9 ± 0.6, and 19.2 ± 0.7 for 25%, 50%, 75%, and 100% neem concentrations, respectively. Corresponding values for Psm were 6.9 ± 0.4, 10.8 ± 0.6, 15.1 ± 0.5, and 16.0 ± 0.5.

Inhibition zones expanded progressively up to seven days, after which no further increase was observed. The 75% neem extract demonstrated comparable performance to the 100% extract, validating its selection for field trials. Neem was particularly effective against Xcc, exhibiting broader inhibition zones than for Psm.

Following three days, inhibition zones increased consistently until the point at which the rate of increase in the inhibition zone stopped.

Inhibition zones were expanded in the area after 7 days, indicating the degree of concentrations when handling bacterial isolates.

The final values were obtained after ten days since the third computation was completed after that time and no additional rise in inhibition zones was estimated.

Lab results showed that neem extract was effective for seven days. During this time, doses of 75% and 100% shown impressive effects in limiting the growth of bacterial colonies. In laboratory experiments, Xcc colonies were more effectively restrained by neem extract than Psm colonies.

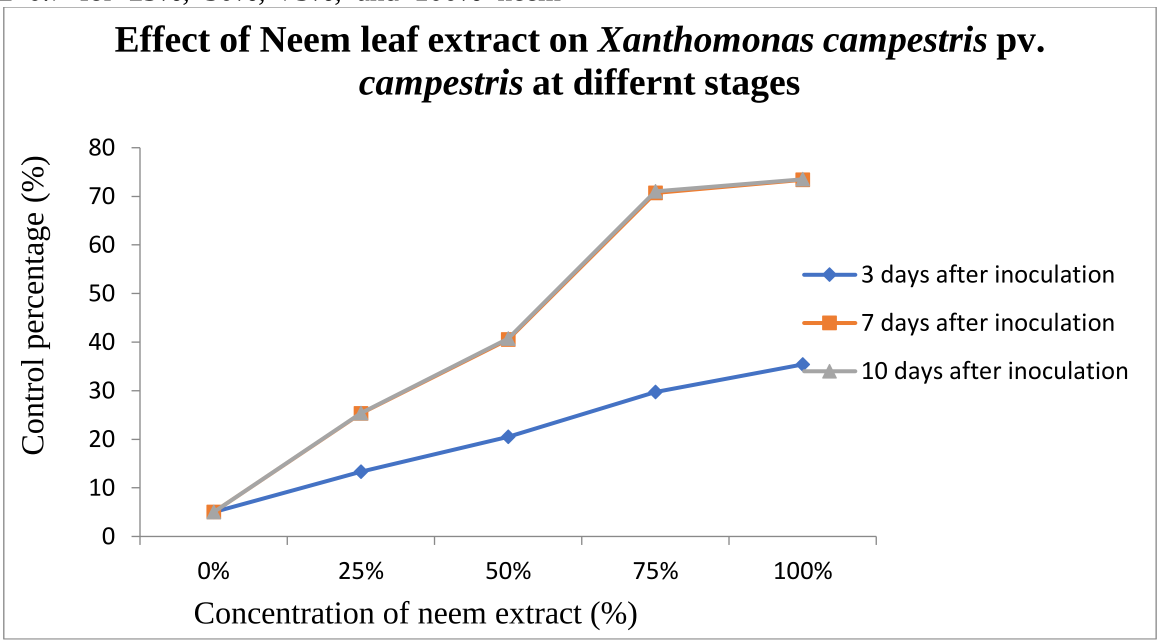


**Figure 4.** General inhibition trends of *A. indica* against *Xcc*

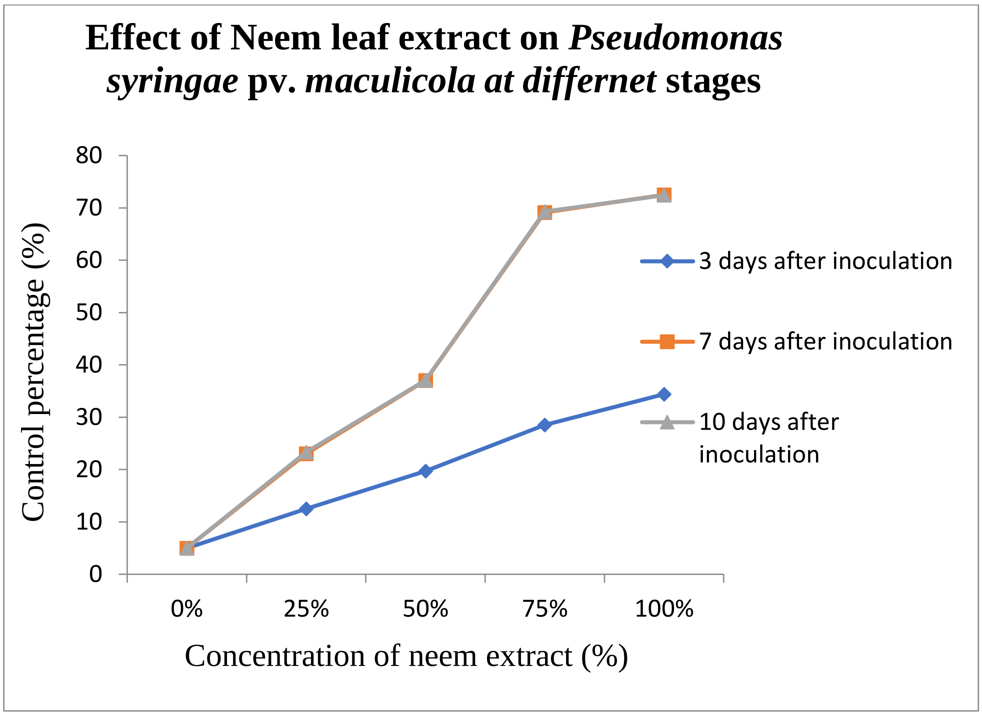


**Figure 5.** General inhibition trends of *A. indica* against *Psm*

**DISCUSSION**

In light of the evolving challenges faced by global agriculture, the pursuit of sustainable and eco-friendly solutions has become imperative (Balusamy et al., 2023). The intricate interplay between agricultural productivity, environmental impact, and human health necessitates a paradigm shift in disease management strategies. The study at hand not only investigates the efficacy of neem extract but also situates its potential within the broader context of global food security and the need for resilient agricultural practices.

The growing demand for food production in the face of climatic uncertainties and resource constraints emphasizes the urgency of adopting sustainable agricultural practices (Tubiello et al., 2008). The introduction of neem as an insecticide aligns with these principles, offering an eco-friendly alternative to traditional chemical treatments. Neem's biodegradability and absence of residual effects underscore its potential to address not only immediate disease concerns but also long-term environmental sustainability (Nkansah-Dwamena, 2023). Neem, with its non-toxic nature and biocompatibility, emerges as a beacon of hope in mitigating the risks associated with chemical residues in food crops (Prakashkumar et al., 2021). This aligns with the global trend towards organic farming and biocontrol mechanisms, championing the production of uncontaminated and nutritionally rich food.

This current research highlights the issue of neem (Azadirachta indica) being an ecologically friendly alternative to bacterial disease management of Brassica crops. Since there is growing demand in the world to seek sustainable agricultural remedies, neem-based formulations are in consonance with the reduced chemical dependency and environmental conservation principles. Neem showed great antibacterial properties against Xanthomonas campestris pv. campestris (Xcc) and Pseudomonas syringae pv. maculicola (Psm) which is in consonance with the previous research (Irshad et al., 2011; Jahan et al., 2007). These findings are closely related to findings by Abalaka et al. (2012), who found a strong inhibition of gram-negative bacteria by neem extract. Nevertheless, the current results demonstrate successful inhibition at moderate levels (75 percent), which demonstrates better efficiency that could be caused by new preparation of fresh aqueous extract and the best pH conditions during use. Neem is rich in more than 140 bioactive substances including azadirachtin, nimbin, and nimbidin that produce its broad-spectrum antibacterial and insecticidal properties (Biswas et al., 2002). Azadirachtin disrupts the operation of bacterial enzymes and cell wall construction, whereas nimbin and nimbidin change the membrane permeability and induce oxidative stress that kill bacteria cells (Balusamy et al., 2023). These biochemical pathways clarify the inhibition pattern observed especially that Xcc is sensitive to neem extract. The fact that the disease incidence decreases after the treatment is also an additional factor proving the potential of neem in field applications. The synergistic nature of neem-based treatment at the Economic Threshold Level (ETL), will have the benefit of ensuring that diseases are controlled at the desired cost and that the environmental hazards are kept to a minimum. These applications are an example of the Integrated Pest Management (IPM) principles that include prompt interventions and environmental safety. With a disease incidence of nearly 9, farmers will be able to avoid massive losses in yield without the use of synthetic bactericides which are harmful to the environment.

*A. indica* has demonstrated remarkable control effects against Xanthomonas campestris pv. campestris (Xcc) and Pseudomonas syringae pv. maculicola (Psm) across various concentrations. Notably, the plant contains a rich array of 140 bio-chemicals, sparking scientific interest in its diverse properties (Hazmi et al., 2013). The trends observed in graphs depict a proportional increase in control effectiveness with rising concentrations of Neem extract. The initial readings align with subsequent data, confirming that pure extract exhibits the most potent control over bacterial growth, as evidenced by well-defined inhibition zones in both tests. Graphs further illustrate the nuanced impact of different concentrations on restricting bacterial colonies. Points of inhibition are evident, revealing a sudden rise after reaching 50% concentration in 75% and 100% extracts. Interestingly, the 75% extract demonstrates potency and rapid effects comparable to the 100% extract. This deliberate choice aims to minimize the impact on the living environment while ensuring substantial control over bacterial diseases. The experiment builds on existing literature that underscores the antimicrobial prowess of A. indica, showcasing its effectiveness under controlled conditions before transitioning to field application. Considering the challenges posed by pathogens like Xcc and Psm, which are notoriously sensitive even to trace amounts of inoculum, A. indica emerges as a promising solution. The plant's unique compounds, including phenols, sterols, flavonoids, and resins, exhibit robust antibacterial activities (Biswas et al., 2002). Chemical combinations within Neem contribute to a broad spectrum of antimicrobial activities. Additionally, studies highlight A. indica's role as a repellent to various insects, providing a natural defense mechanism and protecting crops from secondary invaders (Abalaka et al., 2012).

In the context of disease management, understanding key thresholds becomes essential. Control measures, ideally applied at the Economic Threshold Level (ETL), must be preceded by an awareness of economic injury levels (EIL) and damage boundaries. ETL proves more economically viable, as damage beyond this point results in economic losses. Pest scouting and sampling methods, akin to those employed in this study, help assess damage levels. In plant diseases, severity is quantified by the number of lesions or the damaged tissue area per plant. In the case of insects, severity is determined by the presence of pests or the extent of damage inflicted by them.

The findings emphasize the need for a strategic approach to disease control, incorporating the benefits of *A. indica* at optimized concentrations. Moreover, the study's meticulous exploration of Neem's bio-chemical constituents contributes to the broader understanding of its multifaceted properties. This research not only substantiates the effectiveness of Neem extract against bacterial pathogens in controlled conditions but also underscores its potential for real-world applications in sustainable agriculture. Further integration of Neem-based solutions into pest and disease management strategies promises to contribute to the development of eco-friendly and economically viable agricultural practices.

## CONCLUSION

The current research established that application of neem (azadirachta indica) extract would be very useful and more environmentally friendly than the use of chemical pesticides to combat bacteria diseases of Brassica plants. This laboratory experiment also determined that Xanthomonas campestris pv. campestris Xcc and Pseudomonas syringae pv. maculicola Psm were both highly inhibited by neem extract at 75 percent and that Xcc was more effectively inhibited by the same extract. The same results were confirmed by field tests and application of neem at Economic Threshold Level (9% incidence) was very efficient in reducing the severity of the disease and thereby preventing loss of high yield. These findings also predisposed cabbage to infection more than cauliflower, but they both reacted to neem treatment. The future studies should be aimed at massively validating neem-based therapies in different agro-climatic settings and standardization of extract preparations to provide uniformity to the concentration and efficacy of active constituents. Also, by investigating nano-formulations, or combining other bio-based agents, the stability, shelf-life, and field-efficacy of neem-derived biopesticides might be improved. These developments will play an important role in commercial integration of neem as an alternative to conventional pesticides in the treatment of Brassica diseases in a more sustainable manner. Neem, among others, can be one of the most realistic Biopesticides within the Brassica crops category, which can help to stabilize the yields and protect the environment and human health. Its usage and optimization can also be improved to support the need of its application in sustainable crop protection systems.

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