Journal of
Agriculture and Biology
ISSN: 3007-1763 (Online), 3007-1755 (Print)

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Research Article

Integrated Management of Alternaria Leaf Spot in Cabbage caused by *Alternaria brassicicola* using Chemicals and Phyto Extracts

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ABSTRACT

Cabbage (*Brassica oleracea* var. *capitata* L.) belongs to *Brassicaceae* family is main vegetable grown for its edible head. Cabbage is susceptible to different bacterial and fungal diseases among which *Alternaria* leaf spot caused by *Alternaria brassicicola* is potential extortion to successful production of cabbage. The aim of study was to manage the disease by using chemicals and plant extracts in in-vitro and in-vivo conditions. In lab experiment, five different fungicides (Nanok, Canbinax, Score, Revus and Defeater) and five different plant extracts (Neem, Aloevera, Moringa, Citrus and Pepper) at different concentrations with three replications of each treatment were evaluated. In field experiment, the in vitro best resulted fungicide and plant extract were applied both in separate and in combination on cabbage plants. The minimum fungal growth was expressed by Nanok (2.015 mm) and Neem (5.878 mm) under in vitro conditions and minimum disease incidence was showed by combination of Nanok + Neem (24.444%) under field conditions. The use of Nanok and Neem separately and in combination is recommended to farmers to overcome Alternaria leaf spot of cabbage.

Keywords: Cabbage, Fungicides, Neem, Nanok, *In vitro*, *In vivo.*

OPEN ACCESS

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Article History
Received: March 05, 2024
Accepted: May 26, 2024
Published: June 30, 2024

INTRODUCTION

Cabbage (*Brassica oleracea* var. *capitata* L.), member of family *Brassicaceae* and genus Brassica is a leafy vegetable important due to its edible head that varies in shape from flat to long (Mwangi 2011; Singh et al. 2006). The word “Cabbage” is derived from French word “Cobbache” which means “head” (Yadav et al. 2014). Cabbage was native to Europe in the mediterranean region (Maggioni et al. 2018). In 2017, its total worldwide production was 71.45 million tons from 2.51 million ha harvested area (FAOSTAT 2019). China is first and India is second largest country with their 33.42 million tons and 8.807 million tons production. In Pakistan, about 4983 ha area was cultivated with 77233 tons production in recent times (GOP 2016). Cabbage has important economical and healing properties (Stefan and Ona, 2020). It has important vitamins such as vitamin A, B, B6, C, E, K and minerals such as iron, calcium and magnesium. It is used as baked, fried, salad, fresh or dried as a spice and as fermented product possessing anti-cancerous, anti- inflammatory,antioxidants, antibacterial, anti-obesity properties (Samec et al. 2018;vLee et al. 2018). Cabbage quality and yield is diminished by different virulent diseases such as downy mildew, black rot, club rot, soft rot, damping off, sclerotinia rot and *Alternaria* leaf spot (Yadav et al. 2014).

Alternaria leaf spot is most prevalent disease caused by *Alternaria brassicicola* that is susceptible to all developmental stages from seedling to maturity (Al-Nadabi et al. 2018). The appropriate conditions for its spore formation are 20-30°C, moist period of 13h and repeated rainfall that causes outburst of disease and 70% yield loss globally. The disease is dispersed through wind, water, humans and agricultural machinery (Choudhary et al. 2018; Kumar et al. 2014). The pathogen spreads mostly through the seed coat, which accounts for 20-50% of crop output losses (Gilardi et al. 2018). The infection cycle of *A. brassicicola* includes formation and attachment of conidia to leaf surface. The fungus directly sticks to the host or invade through stomata or appressorium during germination. Small secondary metabolites, lipases and cell wall degrading enzymes are excreted by fungus to kill the host (Ellis 1971; Singh 1982). Initial symptoms are smaller dark leaf spots around 1cm, that becomes large and round with rings. Yellow halo is produced when lesions flourish on tips and margins of leaf affecting leaf veins and stem (Siciliano et al. 2017; Garibaldi et al. 2011). Several factors promote *A. brassicicola* germination that includes humidity, temperature and conidial concentration (Macioszek et al. 2020).

Less resistance in host and presence of inoculum by infected plant material and seed for attack make this disease difficult to control (Meena et al. 2012). Management through plant extracts with chemical fungicides has been examined to assess the efficiency of their combination (Radhakrishnan et al. 2018). Sidlauskiene (2001) investigated the efficiency of different fungicides (Champion 50 WP, Euparen 50 WP and Amistar 250 SC) in preventing black leaf spot of cabbage due to *Alternaria*. Amistar 250 SC resulted efficient in expressing lowest disease intensity (88.8%) as compare to others. Greater use of chemical fungicides is costly and due to their effects on human health and environment, control by plant extracts is most efficient and helpful approach. Phytoextracts are non-polluting, environment friendly and enhance plant disease resistance to conquer the growth of *Alternaria brassicicola* (Ahmad and Ashraf 2016; Khalse et al. 2017). Therefore, the present study was carried out to evaluate the efficiency of different fungicides (Nanok, Revus, Canbinax, Score and Defeater) and plant extracts (Neem, Aloevera, Citrus, Moringa and Pepper) at different concentrations under in vitro and in vivo conditions against *A. brassicicola* causing black leaf spot of cabbage.

MATERIALS AND METHODS

Isolation, Identification and Purification of *A. brassicicola*

Different fields were surveyed in Faisalabad and Cabbage leaves showed typical black spots symptoms were collected and brought to laboratory. Diseased leaves were primarily washed with tap water and cut into small pieces of 3mm size having diseased and healthy portion and surface sterilized in ethanol for 30 seconds, after that washed with distilled water twice, to remove ethanol. Potato dextrose agar (PDA) media was used to isolate the pathogen. PDA was poured into sterilized petri plates and kept for solidification, then samples were placed onto media (3 samples per petri plate). The plates were properly wrapped and incubated in an incubator at 25°C. Initially isolated pathogen was further processed for purification using single tip hyphal technique (Hansen 1926). For identification, temporary slides were prepared using pure fungal culture and observed under microscope, the characters of mycelia and spores including shape and size were then compared with available literature for confirmation.

Pathogenicity Test

Cabbage was grown at field area of Department of Plant Pathology, UAF. Spore suspension was prepared by adding 5-10 mL of distilled water in pure culture plates of *A. brassicicola* and scratched the upper mycelia, then it was sieved using muslin cloth and further distilled water was added. Spore suspension was sprayed on fresh leaves of cabbage using hand sprayer at morning time. After a few days, blackish spots were observed on leaves of cabbage, infected leaves were collected and brought to laboratory for re-isolation procedure. The morphological characters and spores were noted following microscopy and compared with the parent culture for confirmation of the pathogen for fulfilling Koch's postulates (Khan et al. 2010).

In-vitro Evaluation of Fungicides Against *A. brassicicola*

Five chemical fungicides (Score, Revus, Defeater, Canbinax and Nanok) were evaluated against *Alternaria brassicicola* using poisoned food technique. Three different concentrations (250, 500 and 750 ppm) of each fungicide were prepared from stock solutions of each fungicide. Required amount of every treatment was added to 100ml of PDA media and poured into petri plates. 5mm discs of 7-days old Alternaria culture were taken with the help of cork-borer and placed onto the middle of each petri plate. Plates without any fungicide were considered as control. All plates were incubated in the incubator at 25°C for 7 days. The colony's growth was measured with scale after every 24 hours for

three days. Completely Randomized Design (CRD) was used with three replications of each treatment (Kachelo et al. 2022).

In-vitro* Evaluation of Plant Extracts Against *A. brassicicola

Five natural plant extracts (Neem, Moringa, Aloevera, Pepper and Citrus) were used against *Alternaria brassicicola in vitro* using poisoned food technique. Fresh plant leaves were collected and brought to laboratory. Plant materials were washed and ground to make fine powder. The powder of each plant material was dissolved separately in flasks containing sterile distilled water and the then it was filtered using muslin cloth. The extracts were kept for 24 hours at room temperature. Three different concentrations (10, 15 and 20%) of each plant extract were prepared by adding 10, 15 and 20 mL of aqueous extract into 100 mL of media (Atiq et al. 2022). After pouring, Petri plates were inoculated with *Alternaria* culture (5 mm) disc with help of sterile needle (Khalse et al. 2017). Control plates contained un-amended PDA media. The plates were placed in the incubator at 25°C for 3 days. Mycelial growth was noted for three consecutive days with 24 h or interval.

***In-vivo* Management of *Alternaria* Leaf Spot of Cabbage**

For *in vivo* trial, most effective phytoextract and fungicide under lab conditions were evaluated solely and in combination against leaf spot of cabbage. Cabbage was grown at field area of Department of Plant Pathology, UAF. At leafy stage of crop, it was artificially inoculated using spore suspension through hand sprayer. The most effective concentration of phytoextract and fungicide were sprayed thoroughly, where control plants were treated with distilled water. Experiment was designed under Randomized Complete Block Design (RCBD) with three replications of each treatment. Data regarding disease incidence was recorded for next 21 days with one week of interval.

Statistical Analysis

The experimentally collected data was analyzed using Completely Randomized Design (CRD) and Randomized Complete Block Design (RCBD) for *in vitro* and *in vivo* trials. The statistical analysis was performed using “Statistics 8.1” software. To analyze the difference between treatments least significant difference (LSD) was used at level of 0.05% probability.

RESULTS

Symptomology and Disease Development

Leaf spots are characteristics symptoms after the fungal invasion on plant. The pathogen causes small black or brown spots on older leaves that transforms into large, round spots with rings declaring target point of pathogen. Dark lesions with yellow halo are usually visible on leaf with crack in the middle (Figure 1).

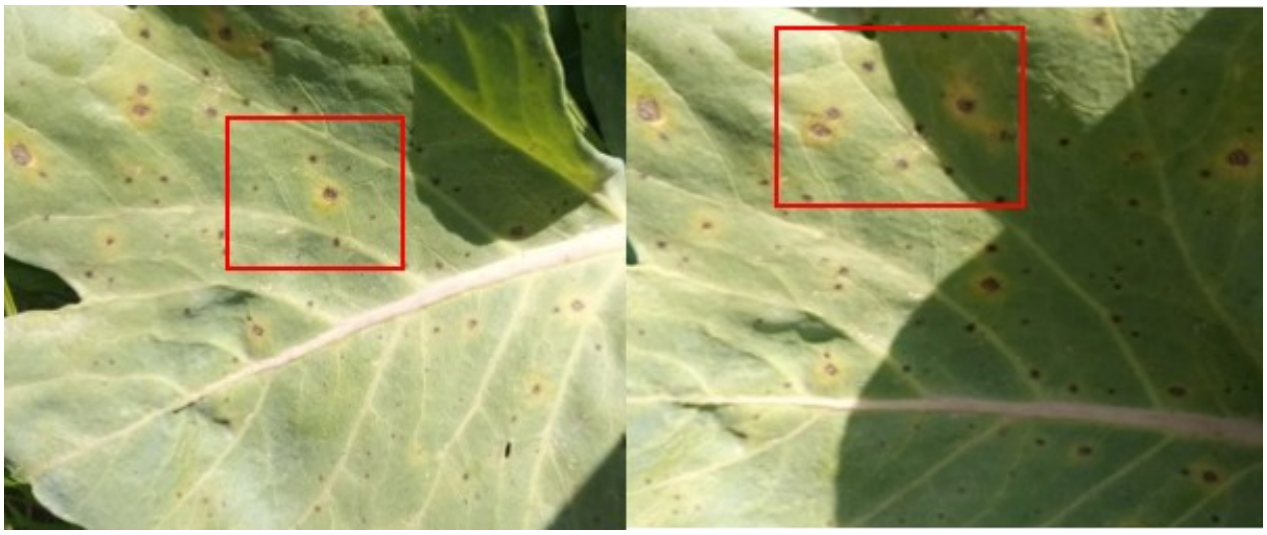

Figure 1. Symptoms of black leaf spot of cabbage.

Fungal Isolation and Identification

After isolation of pathogen associated with leaf spot of cabbage, it was identified as *Alternaria brassicicola*. Dark grayish or black colored mycelial growth of pathogen was observed on purified culture plates (Figure 2a). Morphological identification of fungi was done by preparing slides and observed under the microscope, where spore’s characters were cylindrical to oblong shaped, smooth walled, dark brown conidia were identified (Figure 2b).

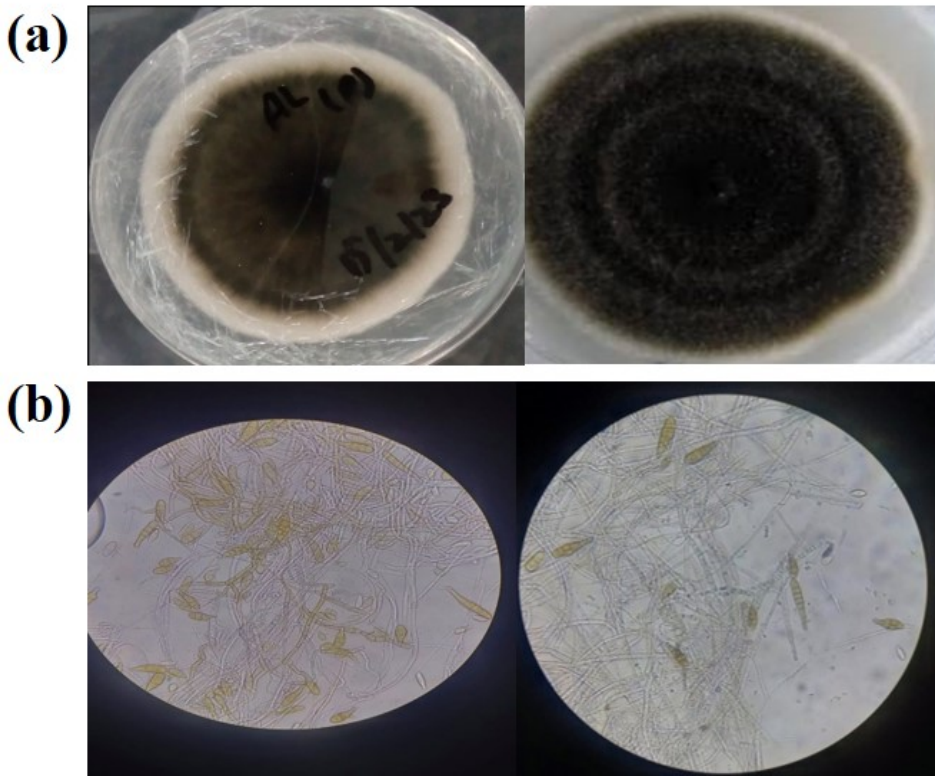

Figure 2. (a) Colonial growth of fungus on PDA media (b) Morphology of spores under microscope.

***In-vitro* Evaluation of Different Fungicides Against *Alternaria brassicicola* Causing Black Leaf Spot of Cabbage**

Five different fungicides were evaluated at different concentrations against *Alternaria brassicicola* causing *Alternaria* leaf spot. Among all treatments, the greatest reduction in fungal growth was shown by Nanok (2.01 mm) followed by Score (3.17 mm), Canbinax (4.66 mm), Revus (7.03 mm) and Defeater (8.18 mm) as compared to control (20.77 mm) (Figure 3a). The impact of treatment and concentration (T×C) showed that as compared to control, Nanok was best in controlling fungal growth at concentrations of 250 ppm (2.37 mm), 500 ppm (2.21 mm) and 750 ppm (1.45 mm). The second effective fungicide in inhibiting fungal growth was Score followed by Canbinax, Revus and Defeater at concentration of 250 ppm (3.68, 5.5, 8.51 and 9.93 mm), at 500 ppm (3.15, 4.72, 7.17 and 8.13 mm) and at 750 ppm (2.66, 3.76, 5.41 and 6.47 mm) (Figure 3b). The interaction between treatment and days (T×D) revealed that Nanok expressed maximum fungal growth inhibition at day 1 (1.01 mm), day 2 (2.05 mm) and day 3 (2.97 mm) followed by Score, Canbinax, Revus and Defeater at day 1 (1.34, 2.21, 3.15 and 4.31 mm), day 2 (3.30, 4.56, 6.94 and 8.41 mm) and day 3 (4.86, 7.21, 11 and 11.82 mm) (Figure 3c). The relation between treatment, concentration and days (T×C×D) as also observed (Figure 3d).

In-vitro* Evaluation of Different Plant Extracts against *Alternaria brassicicola

Among all treatments, the greatest reduction in fungal growth was shown by Neem (5.87 mm) followed by Moringa (7.47 mm), Aloevera (8.19 mm), Citrus (10.12 mm) and Pepper (11.20 mm) as compared to control (22.58 mm) (Figure 4a). The impact of treatment and concentration (T×C) showed that as compared to control, Neem was best in controlling fungal growth at concentrations of 10% (7.22 mm), 15% (5.77 mm) and 20% (4.63 mm). The second effective plant extract in inhibiting fungal growth was Moringa followed by Aloevera, Citrus and Pepper at concentration of 10% (8.44, 9.3, 11.73 and 12.98 mm), at 15% (7.41, 8.21, 10.24 and 11.03 mm) and at 20% (6.55, 7.07, 8.38 and 9.57 mm) (Figure 4b). The interaction between treatment and days (T×D) revealed that Neem expressed maximum fungal growth inhibition at day 1 (3.47 mm), day 2 (5.61 mm) and day 3 (8.54 mm) followed by Moringa, Aloevera, Citrus and Pepper at day 1 (4.66, 5.26, 6.17 and 7.27 mm), day 2 (7.42, 8.11, 10.06 and 11.54 mm) and day 3 (10.32, 11.21, 14.12 and 14.77 mm) (Figure 4c). The relation between treatment, concentration and days (T×C×D) was also observed (Figure 4d).

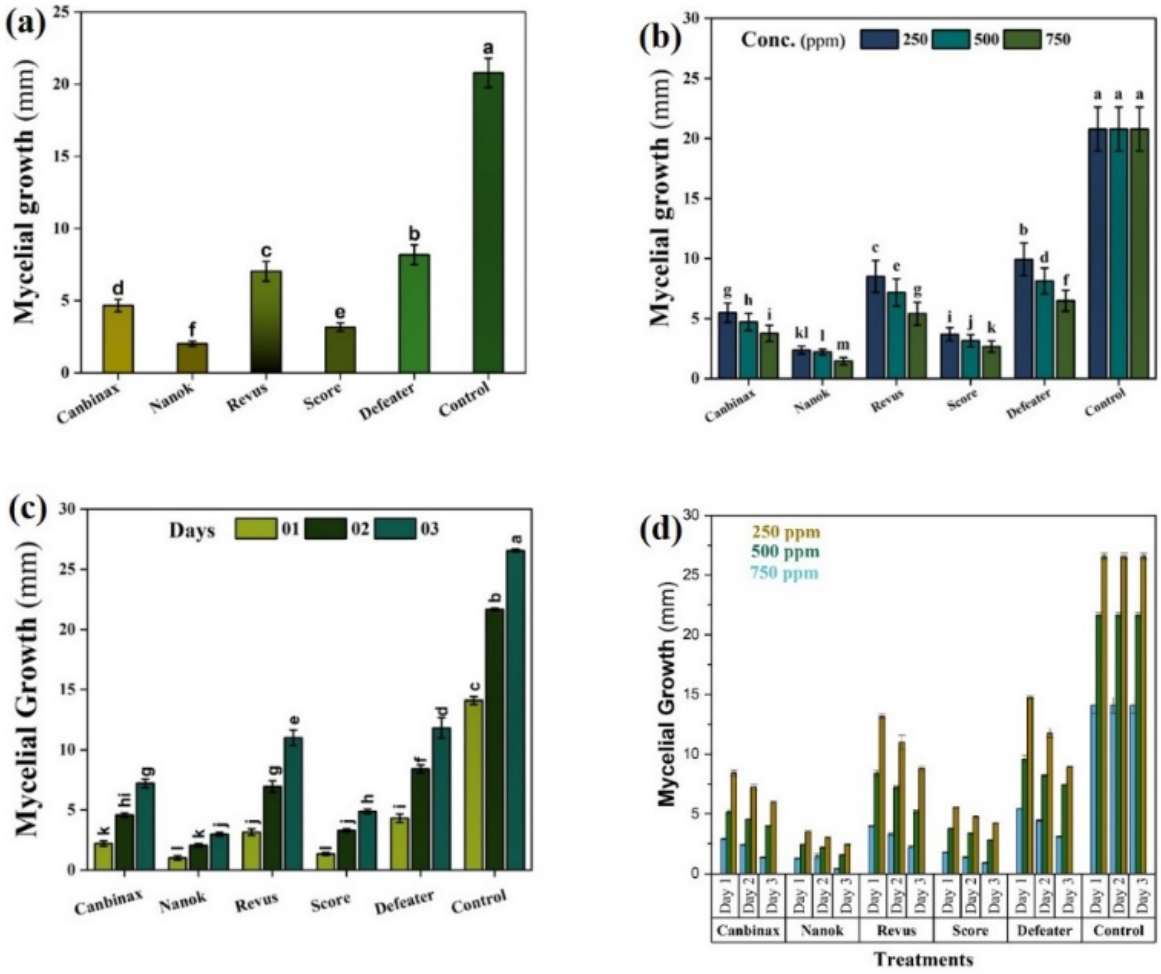

Figure 3. (a) *In-vitro* evaluation of different fungicides against *A. brassicicola* (b) Evaluation of different fungicides at different concentrations against *A. brassicicola* (c) Interaction of treatment and days against *A. brassicicola* under *in vitro* conditions, (d) Interaction of treatment, concentration and days against *A. brassicicola* under *in vitro* conditions.

Evaluation of Fungicides and Plant Extract against *Alternaria brassicicola* under Field Conditions

Among all treatments, Nanok + Neem showed minimum disease incidence (24.44%) followed by Nanok (30%) and Neem (42.22%) as compared to control (53.33%) (Figure 5a). The interaction between treatment and days (T×D) showed that minimum disease incidence was expressed by Nanok + Neem at 7 days (13.33%), 14 days (23.33%) and 21 days (36.66%) followed by Nanok and Neem at 7 days (20% and 26.66%), 14 days (30% and 40%) and 21 days (40% and 60%) (Figure 5b).

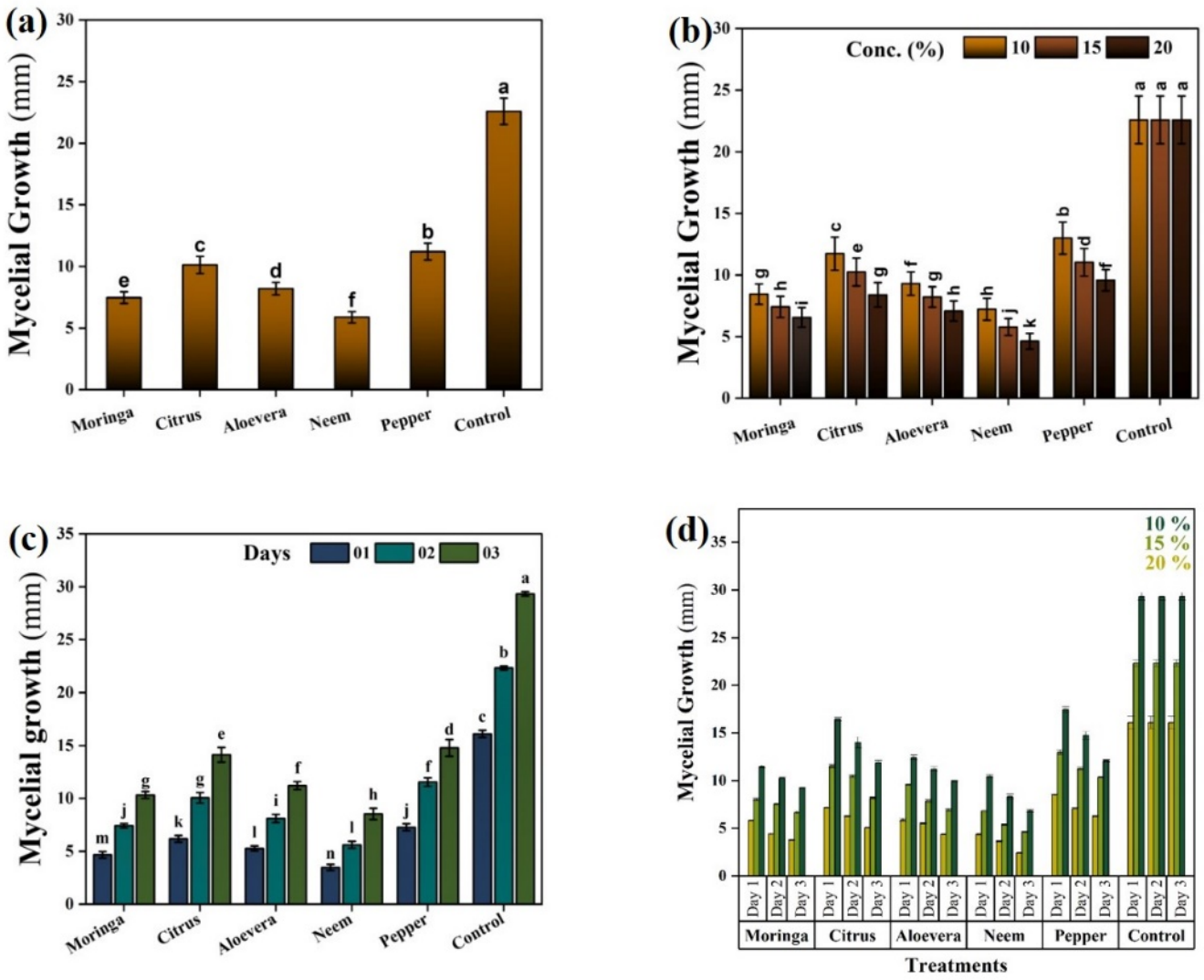

Figure 4. (a) *In-vitro* evaluation of plant extracts against *A. brassicicola*, **(b)** Impact of interaction between phytoextracts with concentrations against *A. brassicicola* **(c)** Impact of interaction between treatment and days against *A. brassicicola,* **(d)** Impact of interaction between treatment, concentration and days against *A. brassicicola* under lab conditions.

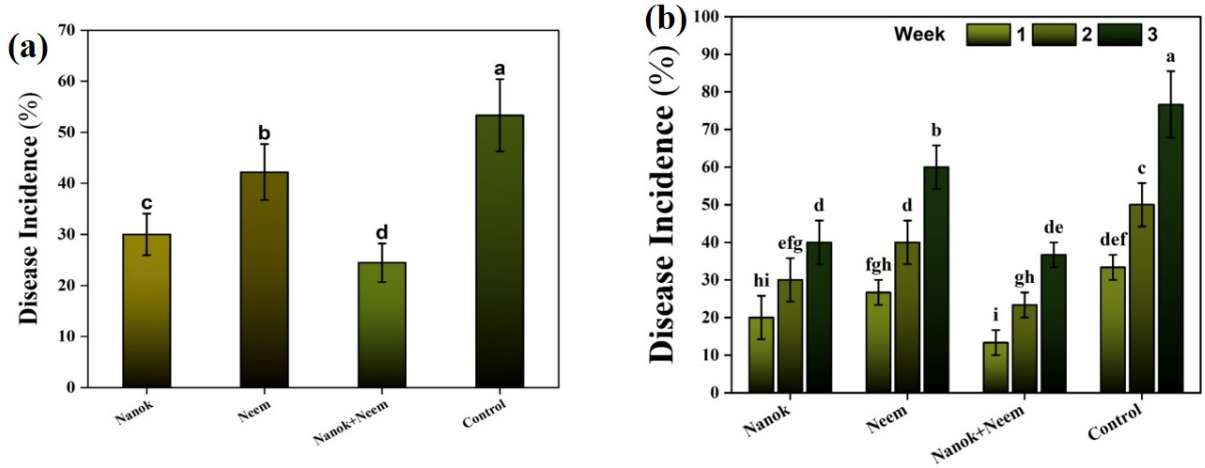

Figure 5. (a) Appraisal of most effective fungicide and plant extract against leaf spot of cabbage under open field conditions (b) In Vivo evaluation of treatments with respect to weeks against leaf spot of cabbage.

DISCUSSION

Alternaria is a deadliest fungal genus, comprising a list of species (Ali et al. 2024). *Alternaria brassicicola* is also one of them, causing leaf spot of cabbage, a potential threat to cabbage production, which infects at all the developmental stages of the crop and results a vast damage (Meah et al. 2002). No doubt, use of resistant cultivar and cultural practices are too much desired management strategies, but in the absence of resistant varieties and sudden outbreak of disease, the only possible way to control disease is use of synthetic fungicides due to their quicker action and easier availability (Moosa et al. 2016). In contemporary study, five different fungicides (Nanok, Revus, Score, Canbinax and Defeater) with three concentrations were evaluated under laboratory conditions against *A. brassicicola*. Results expressed that Nanok exhibited significant control against mycelial growth of the fungus respectively at all three concentrations. Results of our study are in line with Vasilescu et al. (2004) and Iacomi et al. (2004) who examined the sensitivity of different fungicides against *A. brassicicola*, where Iprodione was shown to be more efficient in lowering the growth of *A. brassicicola*. Similarly, Wang et al. (2016) reported the outstanding inhibitory effect of Novice against *A. brassicicola*, when appraised under lab conditions. A bunch of fungicides against *A. brassicicola* were investigated by Sailaja et al. (2017) and Rai et al. (2017) and found that Propiconazole was highly effective against pathogen. Additionally, hexaconazole and flusilazole showed 100% growth inhibition against *A. brassicicola* (Pun et al. 2020; Kumari and Chandra 2022).

Due to detrimental consequences of chemicals to human health and environment, phytoextracts are preferred over chemicals (Seydou et al. 2024). In present study, five different plant extracts (Neem, Aloevera, Moringa, Pepper and Citrus) were used to check their efficacy against *Alternaria brassicicola*. Among all, Neem showed significant growth inhibition followed by Moringa and Aloevera. Neem (*Azadirachta indica*) has antifungal compound like Nimbidin and nimbolide that cause lysis of fungal cell wall. The results are in line with the work of Singh et al. (2015) where Neem exhibited maximum inhibition of the pathogen. Similarly, Gaine et al. (2013) assessed the efficiency of different phytoextracts and declared that *O. sanctum* was found highly prominent in inhibiting the fungal growth. Results of our study are supported by Aboomer et al. (2019) who reported the antifungal efficacy of neem against *Alternaria* species. In contemporary study, open field trial showed that combination of Neem+Nanok exhibited minimum disease incidence followed by solo application of Nanok and Neem. The current study is indorsed by the work of Kushare et al. (2017), Biswas and Ghosh (2018) and Valvi et al. (2019), who determined the effectiveness of different fungicides against leaf spot of cabbage caused by *A. brassicicola* in field conditions. Whereas, the efficacy of neem in controlling leaf spot of cabbage was studied by Rai et al. (2017) and Gupta et al. (2019), where 5% of neem extract indicated maximum decrease in disease incidence percentage.

CONCLUSION

Alternaria leaf spot caused by *Alternaria brassicicola* is potential threat to cabbage responsible for low production, degradation of both quality and quantity of the plant. The present study is based on the results that fungicide Nanok and plant extract Neem were found highly efficient against *Alternaria* leaf spot of cabbage. Nanok and Neem expressed least fungal growth under *in vitro* conditions and combination of Nanok and Neem showed minimum disease incidence percent under field conditions. Therefore, this investigation could be helpful for the selection of suitable fungicide and plant extract to overcome *Alternaria* leaf spot of cabbage.

ACKNOWLEDGEMENTS

Authors are thankful to the Plant-Pathogen Interaction Laboratory, Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan for providing the space for conducting experiments.

AUTHOR CONTRIBUTIONS

All authors contributed equally to this research.

COMPETING OF INTEREST

The authors declare no competing interests.

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