Research Article

Efficacy of Fungicides and Botanical Extracts against *Alternaria brassicicola* Causing Leaf Spot of Cabbage (*Brassica oleracea* var capitata L.)

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ABSTRACT

Background: The leaf spot of cabbage caused by pathogenic fungi, *Alternaria brassicicola*, tends to cause serious diseases in crucifers with considerable economic losses in their yield and storage. Application of chemical fungicides in high doses can have an adverse effect both on the host and the environment, and hence, an alternative eco-friendly approach of plant disease management by means of plant extract is 'the need of the hour'. **Materials and Methods:** Pathogen was isolated from diseased plant part by tissue segmentation followed by screening and maintenance. *In vitro* poisoned food technique was followed to study the comparative effectiveness of commercial fungicides and aqueous botanical extracts of *Azadirachta indica*, *Ricinus communis*, *Aloe vera*, *Oscimum sanctum* and *Cassia alata*, against *A. brassicicola*. **Results:** Hexaconazole showed complete inhibition of mycelial growth of the pathogen at 0.1, 0.15 and 0.2 per cent concentration, whereas the least mycelial inhibition was observed in 0.2 % of Ridomil (67.1%). *In vitro* assays for botanical extracts against mycelial growth of *A. brassicicola* revealed that aqueous extract of *A. indica* showed highest inhibition (upto 97.2%) at 10 and 15% concentration among all the other plant extracts.

Keywords: *Alternaria brassicicola*, Botanical extracts, Fungicides, Poisoned food technique.

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INTRODUCTION

Cabbage (*Brassica oleracea* var capitata L.) belongs to the family Brassicaceae (Cruciferae) and is one of the most popular and widely grown vegetables in the world and has many essential nutrients such as Vitamin C, Vitamin K, Vitamin B6 and folate. It cultivars originated in Western Europe and the northern Mediterranean coast.[1] In 2017, global production of cabbage and other brassicas was 71,451,138 mt, with an area harvested of 2,513,707 ha.[2] In terms of cabbage production, China ranks first (33.42 million mt) followed by India (8.807 million mt). *Alternaria* spot disease is the most destructive disease in cabbage and is caused by *Alternaria brassicicola*. The pathogen causes severe damage to leaf tissues, which affects photosynthesis and, in turn, results in yield loss. The crop reduces its yield due to this pathogen infection ranges from 10-70% in India [3,4] and 32-57% in Nepal.[5,6] Though different chemical fungicides are used for controlling phytofungal disease, their use at higher doses causes adverse effects on the environment and human health, as well as increasing input costs.[7] In view of the high price and adverse effects of chemical fungicides use of plant-based formulation is considered an economical, healthy practice and eco-friendly approach to disease management, without any environmental hazards.[8] Plant extracts have been used in disease management for a long time, as foliar spray is a rarely used approach and requires screening in different crops and under different environmental conditions. As a result, their

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use is considered environmentally friendly and does not cause any environmental pollution. Hence, the present study was carried out to screen the effectiveness of fungicides and botanical extracts against *A. brassicicola* under *in vitro* condition.

MATERIALS AND METHODS

Study Area

The *in vitro* experiment was performed in the Microbiology Laboratory, and the field experiment was carried out in a greenhouse of the Department of Botany, B. P. Chaliha College, Kamrup, Assam.

Isolation of fungal specimen

The fungal pathogen, *Alternaria brassicicola* was isolated from the leaf spot infected leaves of cabbage plant (Figure 1 B) collected from a cabbage farm in Goalpara, Assam, (Figure 1 A) following the tissue segmentation method. The pure culture of the isolated pathogen (Figure 1 C) was maintained on Potato Dextrose Agar slants at 4±1°C.

Preparation of plant extract

Aqueous extracts from fresh leaves of *Azadirachta indica*, *Ricinus communis*, *Aloe vera*, *Oscimum sanctum* and *Cassia alata* were prepared by mechanical grinding followed by cold maceration with sterile distilled water for 24 hr. The homogenate was filtered through Whatman No. 1 filter paper and the extracts were concentrated by air drying and further diluted to prepare a stock of the aqueous extract. The plant extract was then evaluated for its inhibitory effects against *Alternaria brassicicola* under *in vitro* conditions.

Anti-fungal assay

A comparative toxicity assay for the activity of fungicides on the growth of the fungal mycelium under *in vitro* conditions was evaluated following the poisoned food technique.[9] Five fungicides of analytical standard, viz. Hexaconazole, Blitox, Captan, Mancozeb, and Ridomil (Sigma-Aldrich) at different concentrations of 0.1, 0.15, and 0.2%, were used for *in vitro* assay. The fungicides were poured into the sterilized PDA medium and homogenised and to it, a five mm sterile disc of a freshly prepared culture of the test fungus was inoculated with and incubated at 28±1°C for 7 days. The efficacy of fungicides was determined by the radial zone of inhibition and expressed as percent of radial growth over control. The percentage of growth inhibition was calculated by using the method of Vincent (1947).[10]

$$I = \left(\frac{C - T}{C} \right) \times 100$$

Where,

I=Percent inhibition of fungal growth,

C=Radial growth in control,

T=Radial growth in treated petriplates.

The anti-fungal properties of botanical extracts were assayed under *in vitro* conditions following the Poisoned Food Technique[11] The working media was prepared from the stock solution, by combining different concentrations of aqueous extracts, *viz*., 5 mL, 10 mL and 15 mL to 95 mL, 90 mL and 85 mL of PDA media, respectively to attain and uniform composition of 5%, 10% and 15% concentrations from which 20 mL media were poured into sterile Petri plates. A sterile cork borer was used to cut out five mm discs of mycelium from the periphery of an actively growing culture, and one of these discs was placed in the centre of each agar plate. A control was maintained containing only fungal discs on PDA media. The percent inhibition of plant extract against mycelial growth over control was calculated.

RESULTS

Effectiveness of fungicides

In vitro culturing of *Alternaria brassicicola* in PDA supplemented with the fungicides revealed that all were significantly superior in reducing the mycelial growth when compared with the control (Figure 4). While

Figure 1: (A) Cabbage field; (B) Infected Cabbage leaf; (C) Microscopic image of the isolated *A. Brassicicola.*

Table 1: *In vitro* effect of different concentrations of fungicides on mycelial growth of *Alternaria brassicicola*.

Sl. No.	Fungicides	Percent inhibition of mycelial growth		
		Concentration (%)		
		0.1	0.15	0.2
1.	Hexaconazole	100±0.00	100±0.00	100±0.00
2.	Mancozeb	83.4±0.56	85±1.21	85.2±1.42
3.	Blitox	81.1±0.85	82±0.68	84±1.12
4.	Captan	60.3±3.19	69.3±2.51	70.4±2.63
5.	Ridomil	53.4±3.96	59.4±4.31	67.1±2.85

Values shown are the mean±SE of 3 replicates.

Table 2: *In vitro* effect of different concentrations of fungicides on mycelial growth of *Alternaria brassicicola*.

Sl. No.	Leaf extracts	Percent inhibition of mycelial growth		
		Concentration (%)		
		5	10	15
1	*Azadirachta indica*	60.2±0.70	85.4±0.98	97.2±0.82
2	*Ricinus communis*	60±0.92	72.5±0.70	83.3±1.31
3	*Aloe vera*	42.6±1.08	48.7±1.66	56.9±1.21
4	*Oscimum sanctum*	34.4±1.85	45.2±1.41	54.1±1.39
5	*Cassia alata*	41.8±2.35	43.1±1.59	46.2±2.05

Values shown are the mean±SE of 3 replicates.

considering 0.1% of fungicide in the media (Table 1), Hexaconazole (100% inhibition) was found superior over the other treatment, followed by Mancozeb (83.4% inhibition) and Blitox (81.1% inhibition). Whereas Captan and Ridomil were found least effective with 60.3 and 53.4% inhibition respectively. Hexaconazole showed complete (100%) inhibition against the fungal mycelium at all three concentrations (0.1, 0.15 and 0.2%). Considering the effect of 0.2% fungicide against the fungal mycelium, the best effective treatment other than Hexaconazole was observed in Mancozeb (85.2% inhibition), followed by Blitox (84% inhibition) while Captan and Ridomil were least effective *i.e.* 70.4% and 67.1% inhibition of mycelial growth (Figure 2).

Effectiveness of plant extract

The effects of aqueous botanical extracts on *Alternaria brassicicola* (Table 2) indicated significant variations in percentage inhibition for radial growth among the different treatments. Among the botanical extracts evaluated against the pathogen (Figure 3), *Azadirachta indica* extract (5, 10 and 15%) was found most effective with percentage inhibition of 60.2±0.70%, 85.4±0.98% and 97.2±0.82%, followed by *Ricinus communis* 60±0.92%, 72.5±0.70% and 83.3±1.31% percentage inhibition. The extracts of *Aloe vera, Oscimum sanctum* and *Cassia alata* were observed to have percentage inhibition of 42.6±1.08%, 34.4±1.85% and 41.8±2% at 5% concentration and 56.9±1.21%, 54.1±1.39% and 46.2±2.05 at 15 % respectively.

DISCUSSION

Alternaria brassicae, A. brassicicola and *A. raphani* have been earlier reported to cause alternaria blight in members of the Cruciferae family, with a huge loss of yield due to a reduction in photosynthetic area, growth and seed yield.[3] *In vitro* assays for the antifungal activity against

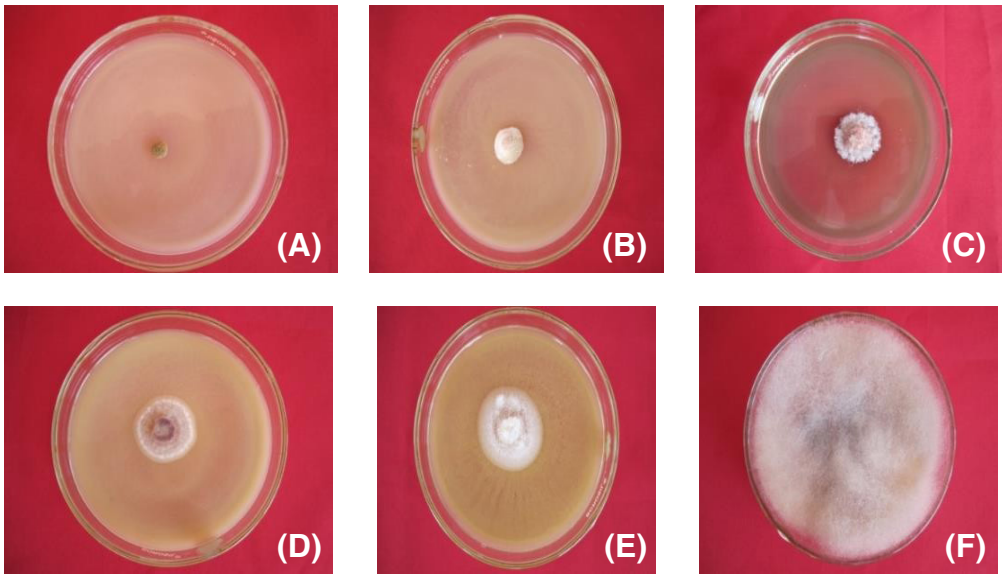

Figure 2: Effect of fungicides [(A) Hexaconazole, (B) Mancozeb, (C) Blitox, (D) Captan, (E) Ridomil and (I) Control] at 0.2% concentrations against the mycelial growth of *A. brassisicola*.

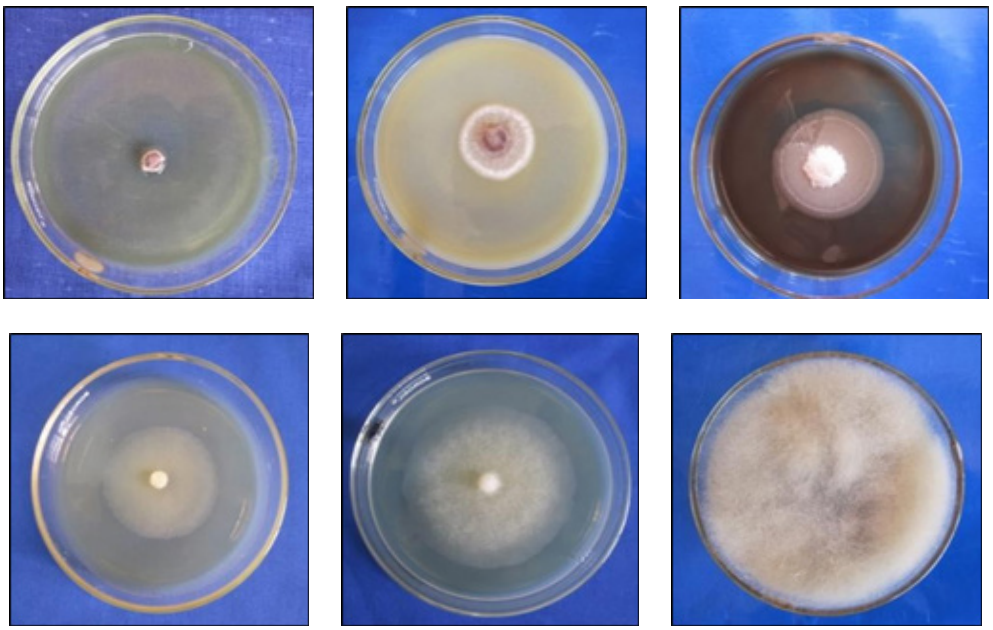

Figure 3: Effect of plant extracts [(A) *Azadirachta indica*, (B) *Ricinus communis*, (C) *Aloe vera*, (D) *Oscimum sanctum*, (E) *Cassia alata* and (I) Control] on the mycelial growth of *A. brassisicola* at 15% concentration.

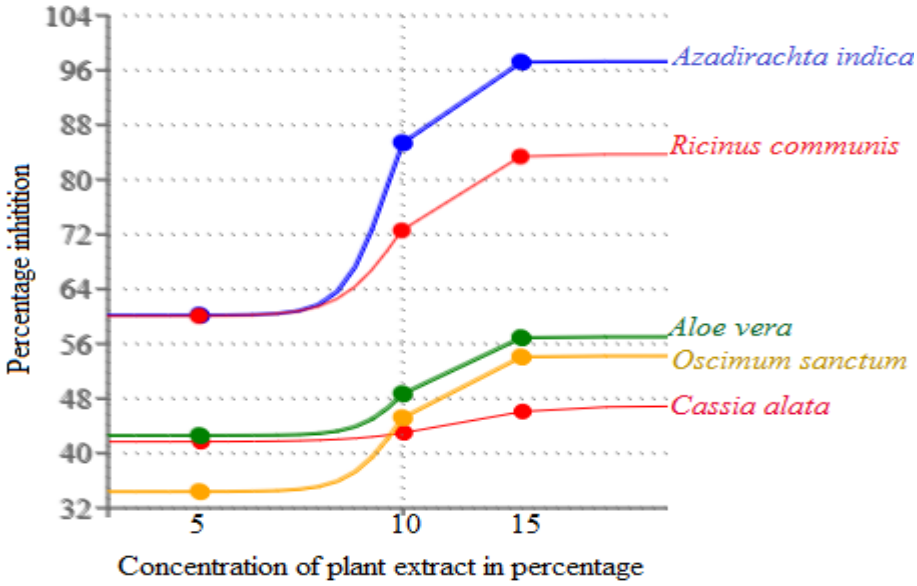

Figure 4: Sigmoid plot of percentage inhibition of mycelial growth of *A. brassisicola* at 5, 10 and 15% of plant extract.

A. brassicicola using five fungicides and five aqueous plant extracts at different concentrations showed a percentage inhibition of the pathogen ranging from as low as 41.8%. Similar reports were also reported by Ahmad and Ashraf (2016) while studying the *in vivo* and *in vitro* management of leaf spots of *Brassica campestris* L. (mustard) caused by *Alternaria brassicae*.[7] Hexaconazole was found highly effective fungicides for controlling the growth of *A. brassicicola* with 100% efficacy at a low concentration of 0.1% w/v. Among the different concentrations tested 0.2% (81.3%) was very effective followed by 0.15% (79.1) whereas least inhibition was observed in 0.1% concentration (75.6%). Among the treatments, the means were found to be statistically significant. This finding is supported by the findings of Kiran *et al.* (2018), where hexaconazole at 0.05, 0.1 and 0.2 % concentrations could completely inhibit *A. brassicicola* fungal mycelium growth.[12] Use of commercial fungicides such as Mancozeb and Hexaconazole at 0.2 and 0.05% against Alternaria blight disease in rapeseed

mustard in a field trial showed an increase in yield of 85.5 and 113.6%, respectively, over control.[4] Tu 2015 reported that *in vitro* use of hexaconazole completely inhibits growth of *A. brassicicola*.[13] Kantwa *et al.* (2014) and Biswas and Ghosh (2018) reported that Mancozeb could be used as an effective fungicide against *Alternaria* sp. which is at par with our findings.[14,15]

Among the five plant extracts used, the anti-fungal effects of the leaf extracts of *R. communis* and *C. alata* against the mycelial growth of *A. brassicicola* were not reported earlier. The present investigation is in close conformity with the findings of Pun *et al.* 2020 who reported that during the use of plant extract, maximum fungal growth was inhibited by leaf extracts of *A. indica* (80.15%) *in vitro* against *A. brassicicola* causing leaf spot of cabbage.[16] Gupta *et al.* (2019) reported that the effectiveness of neem plant extract against the fungal pathogen *A. brassicola* at concentrations of 15% and 25% and the percentage mycelial inhibition observed were 65.55 and 68.88, respectively.[17] An earlier report by Khalse *et al.* 2017 on the effect of plant extract from eucalyptus, lantana and datura leaves extract against the mycelial growth of *Alternaria brassicae* showed percentage inhibition ranging between 52-58% at 10% concentration.[8] A similar result was also observed by Chamoli *et al.* (2020) that maximum percent of mycelial growth inhibition was found by neem, garlic and tulsi extracts against *A. brassicicola* causing leaf spots of cabbage.[18]

As per the findings of Shrestha *et al.* (2005) commercial fungicides such as mancozeb and iprodione could be used as sprays in fields for controlling alternaria blight, whereas as per the findings of Saharan *et al.* (2015) an intergrated approach of fungicides with plant extracts or biocontrol agents for the effective management of alternaria blight disease in mustard.[5,6]

Although the leaf extracts of *A. indica* and *R. communis* showed a good result in inhibiting the mycelial growth of *A. brassicicola*, the concentration of the extract was too high to be applicable in a field trial, and hence, a blending of commercial fungicides at low concentrations along with these leaf extracts could help in achieving good results in terms of yield and cost efficacy while reducing the use of chemical fungicides and increasing the use of plant extract-based fungicides.

CONCLUSION

In the present study, it can be concluded that Hexaconazole is the drug of choice to control fungal leaf spot in cabbage is caused by the pathogen *A. brassicicola*. Plant extract of *A. indica* and *R. communis* showed high percentage inhibition against mycelium growth for the pathogen as well as low IC_{50} value thus suggesting it could be used as an alternative to chemical fungicide to control leaf spots in cabbage.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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Not Applicable.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not Applicable as the study was performed in plants/ *in vitro*.

PATIENT CONSENT

Not Applicable as the study was performed in plants/ *in vitro*.

ABBREVIATIONS

mL: Millilitre; **mt:** Metric tone; **IC_{50}:** Inhibitory Concentration required for 50% inhibition of mycelium growth.

SUMMARY

Five fungicide and five aqueous botanical extracts was used to assay the mycelium growth inhibitory effect against leaf spot causing fungal pathogen *Alterneria brassicola* in cabbage. Hexaconazole effectively inhibited the mycelial growth of *A. brassicicola* at various concentrations, while Ridomil showed minimal inhibition. Aqueous extract of *A. indica* showed the highest inhibition at 10 and 15% concentrations, whereas, the extracts of *Aloe vera*, *Oscimum sanctum* and *Cassia alata* showed a lesser percentage inhibition effect.

REFERENCES

1. Bose TK, Kabir J, Das P, Joy PP, editors. Tropical horticulture, Naya Prokash. Calcutta; 2001. p. 449-632.
2. Website information fao.org [FAOSTAT]. FAO statistics database. Food and Agriculture Organization of the United Nations. p. 2019; updated 2023, cited. Available from: http://www.fao.org/faostat/en/#data/QV.

3. Singh HK, Singh RB, Kumar P, Singh M, Yadav JK, Singh PK, *et al*. Alternaria blight of rapeseed-mustard: a review. J Environ Biol. 2017;38(6):1405-20. doi: 10.22438/jeb/38/6/MS-248.
4. Choudhary CS, Mishra AK, Singh RS, Mukherjee U, Pandey A. Management of alternaria blight of Indian mustard in Bihar. Int J Curr Microbiol Appl Sci. 2018;7:1053-6.
5. Shrestha SK, Munk L, Mathur SB. Role of weather on Alternaria leaf blight disease and its effect on yield and yield components of mustard. Nepal Agric Res J. 2005;6:62-72. doi: 10.3126/narj.v6i0.3366.
6. Saharan GS, Mehta N, Meena PD, Dayal P. Alternaria diseases of crucifers: biology, ecology and disease management. Singapore: Springer; 2016.
7. Aqeel A, Yaseen A. *In vitro* and *in vivo* management of Alternaria leaf spot of *Brassica campestris* L. J Plant Pathol Microbiol. 2016;7(7).
8. Khalse KD, Lal AA, Simon S. Efficacy of bio-agents and plant extracts against the alternaria leaf spot of cabbage (*Alternaria brassicae*). J Pharmacogn Phytochem. 2017;6(4):1980-2.
9. Nene YL, Thapliyal PN. Fungicides in plant disease control, Oxford and IBH Publishing Co. Pvt. Ltd. Calcutta; 1993. p. 531-50.
10. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors. Nature. 1947 Jun 21;159(4051):850. doi: 10.1038/159850b0, PMID 20343980.
11. Nene YL. Fungicides in plant disease control. Fungicides Plant Dis Control. 1971.
12. Kiran GV, Thara SS, Pavan S. Physiological characterisation of *Alternaria brassicicola* causing Alternaria leaf spot in cabbage. Int J Curr Microbiol Appl Sci. 2018;7(10):2727-34. doi: 10.20546/ijcmas.2018.710.317.
13. Tu VD. Studies on Alternaria leaf spot (*Alternaria brassicicola* (Schw.) Wiltshire) of cabbage (*Brassica oleracea* var. capitata L.) [Ph.D. thesis]. Bengaluru: University of Agricultural Sciences; 2015.
14. Kantwa SL, Tetarwal JP, Shekhawat KS. *In vitro* effect of fungicides and phyto-extracts against *Alternaria alternata* causing leaf blight of groundnut. IOSR JAVS. 2014;7(6):28-31. doi: 10.9790/2380-07612831.
15. Biswas M, Ghosh T. Evaluation of phyto-extracts, biological agents and chemicals against the development of *Alternaria brassicae in vitro* and *in vivo*. Eur J Med Plants. 2018;22(3):1-9. doi: 10.9734/EJMP/2018/40412.
16. Pun LB, Chhetri K, Pandey A, Poudel R. *In vitro* Evaluation of Botanical Extracts, Chemical Fungicides and *Trichoderma harzianum* Against *Alternaria brassicicola* Causing Leafspot of Cabbage. Nep Hort. 2020;14(1):68-76. doi: 10.3126/nh.v14i1.30612.
17. Gupta P, Chandra R, Dubey P. Bio-efficacy of plants extract against *Alternaria brassicicola* causing Alternaria leaf spot of cabbage. J Pharmacogn Phytochem. 2019;8(1):2332-4.
18. Chamoli V, Ravi S, Verma SK, Singh AK. *In vitro* Appraisal of Botanicals and Bioagents against *Alternaria brassicicola* Inciting Alternaria Leaf Spot of Cabbage. Int J Curr Microbiol Appl Sci. 2020;9(10):2461-6. doi: 10.20546/ijcmas.2020.910.294.

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