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Original Research Paper

Fungitoxic effect of crude neem extract against *Stemphylium vesicarium* and its influence on seed yield and quality of onion

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ABSTRACT

Stemphylium leaf blight, an emerging and economically devastating disease of onion crops, has prompted the search for eco-friendly alternatives due to stringent regulations with regard to synthetic fungicides. This study pioneers the comparative evaluation of *Azadirachta indica* crude extract against both six commercial fungicides (mancozeb, metiram + pyraclostrobin, difenoconazole, zineb, tebuconazole and kitazine) and two other botanicals (*Lantana camara* and *Pongamia pinnata*) under Indian field conditions. *In vitro* screening was conducted using the poisoned food technique at concentrations ranging from 100 to 2000 ppm, and field trials involved three foliar sprays at 10-day intervals on the onion var. Punjab Naroya. Under *in vitro* conditions, *A. indica* displayed >50% mycelial growth inhibition of *Stemphylium vesicarium* from concentrations as low as 250 ppm, outperforming all botanicals and most fungicides comparable only to triazole fungicides. Field trials demonstrated that *A. indica* (5%) reduced Stemphylium leaf blight disease severity by over 50%, with seed yield comparable to mancozeb and zineb. Notably, seeds treated with *A. indica* exhibited higher germination, improved vigour indices, and reduced electrical conductivity, indicating enhanced seed quality and membrane stability. By delivering disease control on par with leading synthetic fungicides without the associated environmental and resistance risks *A. indica* emerges as a potent, sustainable fungicidal alternative for onion seed production. The findings underscore its role in minimizing pesticide load while safeguarding both yield and seed quality, contributing to quality bulb and seed production.

Keywords: *Allium cepa*, fungicides, neem extract, seed quality, *Stemphylium vesicarium*

INTRODUCTION

Onion (*Allium cepa* L.), a valuable monocot belonging to the family Amaryllidaceae, stands as a pivotal vegetable crop cultivated in India. Its significance, however, is frequently marred by the pervasive influence of various pre-harvest and post-harvest challenges, especially biotic stresses. Among these, fungal diseases, such as damping off, Stemphylium leaf blight (SLB), downy mildew, basal stem rot, and purple blotch, cast a considerable shadow over onion seed production. Stemphylium blight, specifically caused by *Stemphylium vesicarium* (Wallr.) Simmons, has emerged as a notorious culprit, inflicting substantial losses on both seed and bulb crops. This disease has, in recent years, become a formidable barrier hindering the realization of the crop's full genetic potential (Shahnaz et al., 2013).

The initial symptoms of SLB manifest as small, yellowish-brown to tan, water-soaked lesions at the 3 to 4 leaf stage (Raghavendra Rao & Pavgi, 1975). As the disease progresses, infected leaves suffer extensive necrosis, originating from their tips. *S. vesicarium*, post-infection, produces host-specific toxins linked to necrosis (Singh et al., 2000). In advanced stages, desiccation and premature lodging of onion plants render them highly susceptible to secondary and post-harvest infections, with seed crops being more severely impacted than bulb crops.

Stemphylium blight is a pressing concern among onion pathogens, affecting virtually all cultivated varieties. Severely infected plants yield small, unmarketable bulbs, fetching lower prices (Raghavendra Rao & Pavgi, 1975).

To manage the disease, chemical fungicides offer a choice for the grower as it provides economical and effective management of Stemphylium disease. However, their continuous use has led to fungicide resistance, residual toxicity, along with the ill effect on health and the environment (Hay et al., 2019). In recent years, *Azadirachta indica* (neem) with its broad-spectrum antimicrobial properties and potent antifungal constituents offer a promising alternative chemical fungicides (Alzohairy, 2016). However, limited research on their comparative efficacy of *Azadirachta indica* crude leaf extract alongside commercial fungicides and other botanicals are evaluated under both *in vitro* and field conditions with respect to disease control, yield improvement, and seed quality enhancement. Based on gap the working hypothesis is crude leaf extract of *Azadirachta indica* possesses potent antifungal activity against *Stemphylium vesicarium* and, when applied as a foliar spray, can reduce disease severity, maintain or improve seed yield, and enhance seed quality parameters, and offers an eco-friendly alternative for SLB management.

The present study was undertaken to evaluate the antifungal efficacy of crude leaf extract of *Azadirachta indica* against *Stemphylium vesicarium* under both laboratory and field conditions. Specifically, it aimed to compare its performance with selected commercial fungicides and other botanicals in inhibiting mycelial growth, reducing disease severity, and improving seed yield of onion. In addition, the study sought to determine the influence of *A. indica* application on seed quality parameters namely, seed germination, vigour indices, and seedling quality, with an aim to identifying an eco-friendly and sustainable alternative to synthetic fungicides for the effective management of Stemphylium leaf blight in onion.

MATERIALS AND METHODS

Experimental site and plant material

The present investigations on management of Stemphylium leaf blight using different fungicides and plant products were conducted both in the laboratory and field conditions at ICAR-Indian Agricultural Research Institute, New Delhi, during 2019-22 using a susceptible onion variety Punjab Naroya (Dangi et al., 2019). The following experimental treatment *viz*., T_1: mancozeb 75% WP 0.3%; T_2: metiram 55% + pyraclostrobin 5% WG 0.3%; T_3: difenoconazole 25% EC 0.1%; T_4: zineb 75% WP 0.2%; T_5: tebuconazole 25% EC 0.1%; T_6: sprays of kitazine 48% EC 0.2%; T_7: *Lantana camara* 5%; T_8: *Pongamia pinnata* 5%; T_9: crude leaf extract of *Azadirachta indica* 5%; T_{10}: check (without spray) were applied. Foliar application of fungicides and plant products was ensured as soon as the disease appeared and subsequent three applications at 10-day intervals.

In vitro antifungal assay using poisoned food technique

The seeds of 'Punjab Naroya' were obtained from Punjab Agricultural University, Ludhiana, India. Stemphylium blight pathogen was isolated from diseased onion leaves from the field of Division of Seed Science and Technology and confirmed through morphological and molecular characterization (Prasad et al., 2024). *In vitro* evaluation includes fungicides such as mancozeb 75% WP, metiram 55% + pyraclostrobin 5% WG, difenconazole 25% EC, zineb 75% WP, tebuconazole 25% EC and kitazine 48% EC and plant products such as *Lantana camara*, *Pongamia pinnata* and *Azadirachta indica* using poisoned food technique as described by Sharvelle (1961) with different concentration *viz*., 100, 250, 500,1000 and 2000 ppm.

Disease severity and seed yield

During field observation, per cent disease incidence was recorded by scoring five plants in each micro plot using a 0-5 scale. Further, the per cent disease index (PDI) was calculated with the scale using the formula given by Hussein et al. (2007).

$$\text{Percent of disease index} = \frac{\text{Sum of numerical values of disease ratings}}{\text{Number of fruit observed x maximum disease rating}} \times 100$$

During crop production, various growth parameters, including the seed weight (g) of each replication were recorded. Yield per acre was computed by using net plot yield data, and it was then converted to quintal per acre.

Seed quality parameters studies

The percentage of seed germination was recorded using four hundred seeds for each treatment by employing between paper methods with four replications. Ten normal seedlings were selected randomly from the germination test, and their shoot and root lengths were recorded for the calculation of seedling length. The mean value of seedling length was calculated and expressed in centimeter. For seedling dry weight (mg), randomly taken five normal seedlings were placed in a butter paper bag and dried for 24 hr in a hot air oven maintained at 70°C. The dry weight of the seedlings was recorded in an electronic balance and the average weight was computed and expressed in milligrams per five seedlings.

For electrical conductivity, 100 mg of seeds were soaked in 25 mL of distilled water for 24 hr at 20°C. The seed leachate was collected by decanting, and the electrical conductivity of seed leachate was measured at room temperature with a conductivity bridge meter along with distilled water as control and was expressed as milli siemens/cm (Dadlani & Agarwal, 1987). The vigour indices of seeds were calculated according to the method suggested by Abdul-Baki & Anderson (1973) and expressed as a pure number.

Vigour Index (VI) I = Germination (%) × total seedling length (cm)

Vigour Index (VI) II = Germination (%) × seedling dry weight (g)

Statistical analysis

The analysis of variance was calculated using a randomized block design and factorial completely randomized design analysis using OPSTAT software (HAU, Hissar). Statistical significance was tested using the F test. The critical difference was also used to test the difference among the treatment means.

RESULTS AND DISCUSSION

In the study, the efficacy of various treatments in inhibiting mycelial growth and managing Stemphylium leaf blight (SLB) of onion was comprehensively assessed using poisoned food technique. The findings reveal key insights into the effectiveness of these treatments.

Inhibition of mycelial growth

Significant reduction in mycelial growth of *S. vesicarium* were observed across different concentration among fungicides and plant products tested. Among all the treatments, tebuconazole (25% EC) and difenoconazole (25% EC) showed mycelial growth inhibition at relatively lower concentration i.e. 100 ppm (Table 1). Among botanicals, crude leaf extract of *A. indica* exhibited consistent inhibitory effect on the mycelial growth of *S. vesicarium* at all tested concentrations. Notably, *A. indica* exhibited over 50% inhibition of mycelial growth of *S. vesicarium* from as low as 250 to 2000 ppm outperforming other treatments both after 3rd and 7th day of incubation. Others fungicides, such as mancozeb and zineb 75% WP, showed limited effectiveness, with over 50% growth inhibition observed only at higher concentrations, starting from 1000 ppm. Alternative treatments, including *L. camara* and *P. pinnata* extracts, as well as chemical treatments like kitazine 48% EC and meitram 55%+ pyraclostrobin 5% WG, demonstrated less than 50% growth inhibition at all concentrations (Fig. 4).

The suppression of mycelial growth in *A. indica* resulted from the antifungal attributes found within its leaves. It includes bioactive constituents such as azadirachtin, nimbin, and various alkaloids, β-sitosterol, quercetin, and phenolic flavonoids which disrupt pathogen cell walls and metabolic processes and effectively hinder the growth of mycelia (Govindachari et al. 1998, Thawabteh et al., 2019). Kumar et al. (2012) reported that *Azadirachta indica* restricted 66.5% of *S. vesicarium* mycelial growth compared to the control. Similarly, Sobhy et al. (2014) found that *A. indica* and *Datura stramonium* exhibited the strongest antifungal activity against *Alternaria porri*, the causal agent of purple blotch, with *A. indica* extract reducing disease severity by approximately 70–75% under greenhouse conditions. Additionally, the concentration-dependent inhibition of *A. indica*, capable of suppressing over 50% of *S. vesicarium* growth from as low as 200 ppm, not only confirms its potent antifungal activity in line with earlier reports (Isman, 2006) but also demonstrates its superiority over traditional fungicides like mancozeb and zineb, underscoring its value as a safer and more environmentally friendly alternative to chemicals often challenged by resistance and ecological concerns (Fisher et al., 2012)

Disease severity and seed yield

From the field data presented in Table 1, Fig. 1 & 2, it can observed that triazole fungicides such as tebuconazole (25% EC) and difenoconazole (25% EC) when applied as foliar spray, led to a significant

Table 1 : *In vitro* evaluation of different fungicides and plant products on growth of *Stemphylium vesicarium*

Treatment	Growth inhibition (%)											
	3rd day at different concentration (ppm)						7th day at different concentration (ppm)					
	100	200	500	1000	2000	Mean	100	200	500	1000	2000	Mean
T1	24.42 (34.74)	32.56 (28.34)	41.89 (39.67)	47.70 (17.87)	53.81 (19.43)	40.08 (28.01)	26.48 (37.32)	39.29 (32.00)	46.51 (47.00)	52.33 (19.94)	56.10 (21.83)	44.14 (31.62)
T2	6.06 (40.06)	8.80 (57.35)	12.30 (46.46)	22.09 (16.32)	33.98 (17.67)	16.65 (35.57)	13.06 (41.60)	16.32 (68.09)	20.27 (56.54)	27.71 (20.47)	37.67 (20.13)	23.01 (41.41)
T3	66.28 (40.17)	72.09 (62.77)	73.76 (59.51)	82.56 (19.56)	87.21 (16.91)	76.38 (39.78)	80.96 (43.10)	81.83 (71.75)	87.80 (68.35)	92.21 (25.31)	96.65 (20.95)	87.89 (45.90)
T4	23.27 (36.49)	37.74 (61.03)	42.17 (65.35)	51.04 (12.03)	59.95 (21.38)	42.84 (39.26)	38.37 (39.72)	43.02 (69.05)	62.79 (74.89)	66.28 (16.39)	79.07 (24.84)	57.91 (44.98)
T5	68.60 (44.05)	74.42 (60.54)	79.07 (63.29)	86.05 (16.29)	90.70 (46.00)	79.77 (46.04)	84.03 (45.75)	87.75 (69.19)	90.73 (72.38)	92.34 (20.71)	98.47 (49.61)	90.67 (51.53)
T6	2.99 (17.34)	5.81 (64.35)	8.08 (62.65)	11.12 (17.16)	17.78 (48.35)	9.16 (41.97)	5.53 (23.62)	8.63 (73.60)	12.36 (73.13)	15.67 (20.42)	27.33 (53.42)	13.91 (48.84)
T7	2.48 (26.13)	5.88 (34.18)	8.04 (67.70)	12.11 (16.68)	15.60 (48.02)	8.82 (38.54)	4.21 (30.30)	9.15 (43.49)	10.97 (76.20)	15.89 (18.85)	21.96 (50.66)	12.44 (43.90)
T8	4.74 (21.83)	6.04 (41.01)	7.94 (12.80)	14.34 (20.80)	16.35 (46.86)	9.88 (28.66)	7.34 (27.67)	8.64 (52.22)	12.25 (17.49)	16.84 (23.74)	22.16 (51.98)	13.45 (34.62)
T9	45.17 (21.98)	52.20 (42.15)	56.05 (18.14)	59.57 (13.80)	64.01 (51.33)	55.40 (29.48)	45.90 (27.71)	59.29 (49.59)	64.10 (22.77)	69.85 (17.58)	75.51 (57.52)	62.93 (35.04)
Factors				C.D.			SE(d)			SE(m)		
Days (A)				1.56			0.79			0.56		
Treatments (B)				2.47			1.25			0.88		
A x B				N/A			1.77			1.25		
Concentrations (C)				3.31			1.68			1.19		
A x C				N/A			2.37			1.68		
B x C				7.41			3.75			2.65		
A x B x C				N/A			5.31			3.75		

T_1: mancozeb 75% WP 0.3%; T_2: metiram 55% + pyraclostrobin 5% WG 0.3%; T_3: difenoconazole 25% EC 0.1%; T_4: zineb 75% WP 0.2%; T_5: tebuconazole 25% EC 0.1%; T_6: sprays of kitazine 48% EC 0.2%; T_7: *Lantana camara* 5%; T_8: *Pongamia pinnata* 5%; T_9: crude leaf extract of *Azadirachta indica* 5%; T_{10}: check (without spray)

Values in parenthesis are angular transformed value

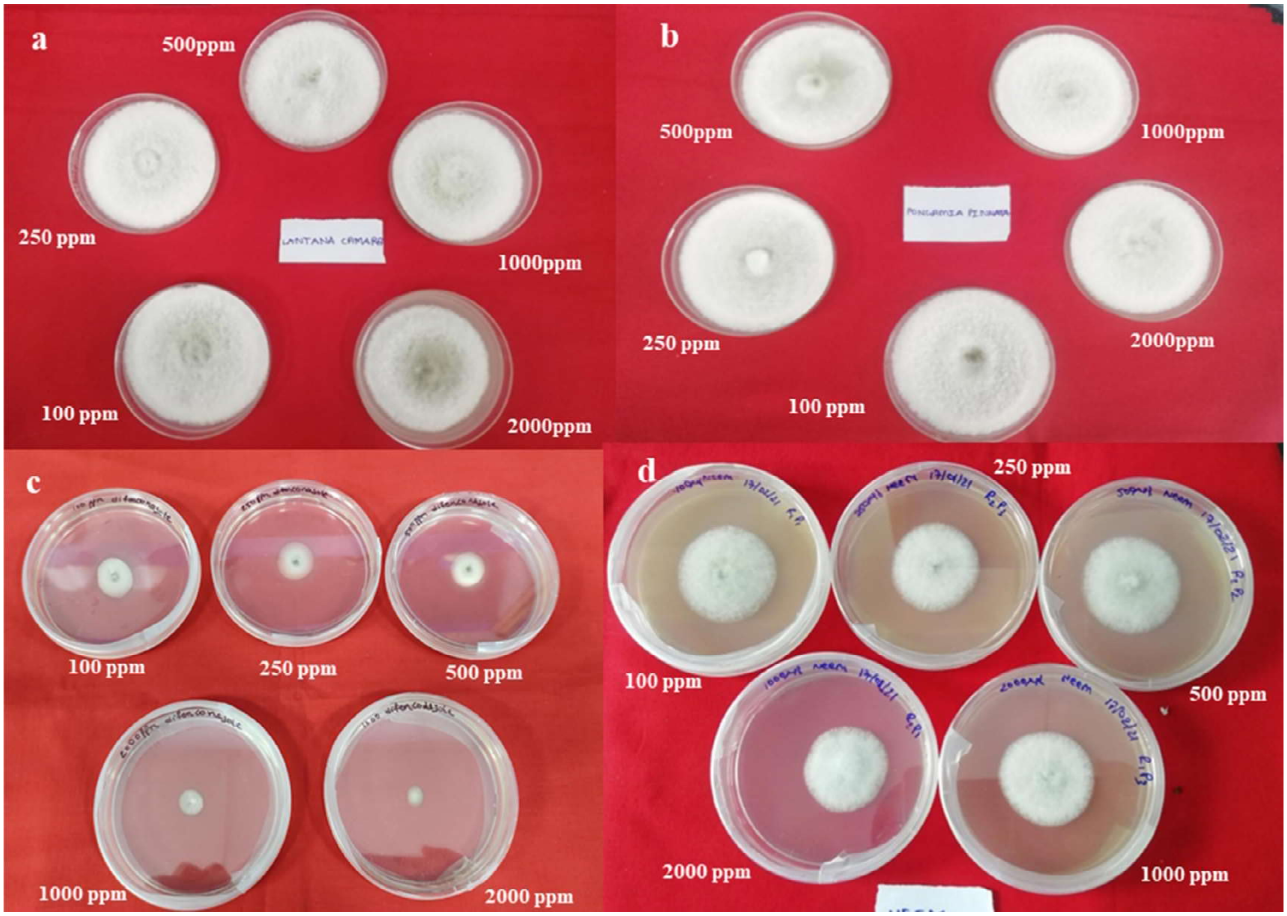

Fig 4 : Efficacy of a. *L. camara*, b. *P. pinnata*, c. difenoconazole, d. *A. indica*, and d. against *S. vesicarium* at different concentration under poisoned food technique

reduction in Stemphylium blight severity and increased seed yield per acre over other treatments. Remarkably, the foliar application of crude leaf extract of *A. indica* resulted in an effective reduction in disease severity by more than 50% and increased seed yield when compared to other treatments such as foliar application of mancozeb and zineb (75% WP), kitazine (48% EC) and meitram (55%) + pyraclostrobin (5% WG) as well as plant products including *L. camara* and *P. pinnata* extracts (Table 2).

The results indicate that the foliar application of *A. indica* crude extract effectively reduces disease severity and increases seed yield, outperforming other plant-based products and even fungicides like mancozeb and zineb. This aligns with the observed field outcomes where *Azadirachta indica* crude extract effectively lowers purple spot disease severity and enhances seed yield better than several other botanicals and chemical fungicides (Abifah & Suryaminarsih, 2023). Thus, the foliar application of neem leaf extract is a notable, sustainable, and effective solution for controlling Stemphylium blight, not only in onion cultivation but also in other crops, offering a potential means to mitigate economic losses caused by SLB.

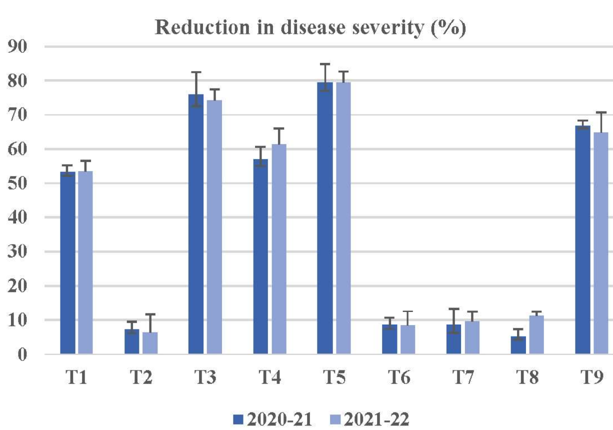

Fig. 1 : Effect of different fungicides and plant products on reduction of Stemphylium blight disease severity.

values are means ± standard errors of one experiment with three biological replicates

Table 2 : Effect of foliar application of different fungicides and plant products on Stemphylium blight disease severity and seed yield of onion during 2019-20 and 2020-21

Treatment	Disease severity (%)			Seed yield (q/acre)		
	2019-20	2020-21	Pooled	2019-20	2020-21	Pooled
T_1	35.55 (36.57)	36.44 (37.09)	35.99(36.84)	1.27	1.23	1.25
T_2	69.32 (56.91)	73.32 (58.94)	71.76(57.93)	0.80	0.77	0.79
T_3	18.22 (25.21)	20.00 (26.47)	19.11(25.85)	1.42	1.46	1.44
T_4	32.88 (34.91)	30.21 (33.32)	31.55(34.12)	1.33	1.30	1.31
T_5	15.55 (23.18)	16.00 (23.48)	15.77(23.38)	1.46	1.45	1.46
T_6	69.76 (56.34)	71.54 (57.75)	70.43(57.05)	0.64	0.72	0.68
T_7	69.32 (56.34)	70.65 (57.20)	69.98(56.78)	0.35	0.37	0.36
T_8	71.98 (58.07)	69.32 (56.36)	70.65(57.22)	0.32	0.32	0.32
T_9	25.33 (30.18)	27.55 (31.63)	26.44(30.91)	1.33	1.36	1.30
T_{10}	75.98 (60.96)	78.20 (62.17)	77.31(61.57)	0.18	0.18	0.18
SEm±	1.22	1.37	0.94	0.05	0.04	0.03
CD at 1%	3.66	4.08	2.70	0.13	0.12	0.09
CV (%)	4.82	5.62	5.33	7.93	7.34	5.26

T_1: mancozeb 75% WP 0.3%; T_2: metiram 55% + pyraclostrobin 5% WG 0.3%; T_3: difenoconazole 25% EC 0.1%; T_4: zineb 75% WP 0.2%; T_5: tebuconazole 25% EC 0.1%; T_6: sprays of kitazine 48% EC 0.2%; T_7: *Lantana camara* 5%; T_8: *Pongamia pinnata* 5%; T_9: crude leaf extract of *Azadirachta indica* 5%; T_{10}: check (without spray). Values in parentheses are angular transformed value

Table 3 : Germination percentage and electrical conductivity of Punjab Naroya during 2019-20 and 2020-21

Treatment	Germination (%)			EC (milli siemens/cm)		
	2019-20	2020-21	Pooled	2019-20	2020-21	Pooled
T_1	81.33 (64.44)	82.00 (64.98)	81.65 (64.71)	0.3721	0.3773	0.3747
T_2	73.33 (58.90)	77.00 (61.34)	75.16 (60.12)	0.4510	0.4598	0.4554
T_3	86.00 (68.00)	88.67 (70.32)	87.33 (69.16)	0.3007	0.3030	0.3019
T_4	81.00 (64.20)	78.67 (62.48)	79.83 (63.34)	0.3748	0.3830	0.3789
T_5	87.33 (69.14)	90.00 (71.55)	88.66 (70.34)	0.2815	0.2829	0.2822
T_6	73.00 (58.70)	76.00 (60.64)	74.5 (59.67)	0.4713	0.4672	0.4693
T_7	62.33 (52.12)	59.60 (50.56)	60.97 (51.34)	0.4722	0.4776	0.4749
T_8	64.00 (53.11)	60.00 (50.76)	62.00 (51.94)	0.4811	0.4738	0.4775
T_9	78.67 (62.50)	80.33 (63.66)	79.5 (63.08)	0.3577	0.3475	0.3526
T_{10}	52.00 (46.12)	50.00 (44.98)	51.00 (45.55)	0.5480	0.5399	0.5440
SEm±	1.09	1.01	0.77	0.01	0.01	0.01
CD at 1%	3.21	2.98	2.20	0.03	0.01	0.01
CV (%)	3.11	2.86	2.76	3.43	1.73	2.97

T_1: mancozeb 75% WP 0.3%; T_2: metiram 55% + pyraclostrobin 5% WG 0.3%; T_3: difenoconazole 25% EC 0.1%; T_4: zineb 75% WP 0.2%; T_5: tebuconazole 25% EC 0.1%; T_6: sprays of kitazine 48% EC 0.2%; T_7: *Lantana camara* 5%; T_8: *Pongamia pinnata* 5%; T_9: crude leaf extract of *Azadirachta indica* 5%; T_{10}: check (without spray). Values in parentheses are angular transformed value

Table 4 : Seedling length and seedling dry weight of Punjab Naroya during 2019-20 and 2020-21

Treatment	Seedling length (cm)			Dry weight (g)		
	2019-20	2020-21	Pooled	2019-20	2020-21	Pooled
T_1	9.70	9.40	9.55	0.024	0.024	0.0242
T_2	9.37	8.80	9.15	0.019	0.022	0.0206
T_3	9.73	9.60	9.67	0.026	0.026	0.0260
T_4	9.80	9.50	9.58	0.025	0.026	0.0252
T_5	9.70	9.50	9.60	0.026	0.026	0.0263
T_6	9.40	8.70	9.05	0.017	0.020	0.0182
T_7	9.60	9.03	9.00	0.017	0.020	0.0206
T_8	9.57	8.97	9.13	0.017	0.019	0.0180
T_9	9.23	8.67	8.85	0.021	0.020	0.0184
T_{10}	8.83	8.47	8.47	0.016	0.015	0.0153
SEm±	0.13	0.13	0.10	0.000	0.000	0.000
CD at 1%	0.38	0.38	0.29	0.001	0.001	0.001
CV (%)	2.34	2.45	1.96	1.89	2.09	1.41

T_1: mancozeb 75% WP 0.3%; T_2: metiram 55% + pyraclostrobin 5% WG 0.3%; T_3: difenoconazole 25% EC 0.1%; T_4: zineb 75% WP 0.2%; T_5: tebuconazole 25% EC 0.1%; T_6: sprays of kitazine 48% EC 0.2%; T_7: *Lantana camara* 5%; T_8: *Pongamia pinnata* 5%; T_9: crude leaf extract of *Azadirachta indica* 5%; T_{10}: check (without spray)

Seed germination and seedling quality

Seeds obtained from foliar applied plants of Punjab Naroya were used to study the effect of *A. indica* on seed quality parameters like germination, seedling length, seedling dry weight, vigour index I and II and electrical conductivity in comparison to different fungicides and plant products sprayed plants (Table 3 & 4). The results showed that, across all treatments, the mean germination percentage, seedling length, and dry weight were notably higher than the control group. Additionally, seeds obtained from the *A. indica* crude extract at 5% concentration outperformed kitazine 48% EC and meitram 55%+pyraclostrobin 5% WG, *L. camara*, and *P. pinnata*. Moreover, it was at par with mancozeb 75% WP and zineb 75% WP in terms of higher germination rates, vigour indices, and lower electrical conductivity (Fig. 2 & 3).

The study shows that *A. indica* crude extract at 5% concentration promotes higher germination rates, robust seedling vigour indices, and lower electrical conductivity due to less abnormal seedling development and increased seed length and dry weight compared to other treatments Gupta et al. (2012) reported that neem leaf additives help preserve onion seed viability by preventing fungal growth and insect infestation during storage, leading to improved seed germination and reduced fungal prevalence. Further, antifungal and insecticidal nature of *A. indica* plays a role in reducing fungal inoculum penetration, stabilizing membrane integrity, and decreasing permeability. Sundaram et al. (1999) also reported the positive impact of neem extract on seed quality. The overall results underscore the potential of *A. indica* as an eco-friendly alternative in the management of fungal diseases in agriculture. This aligns with the

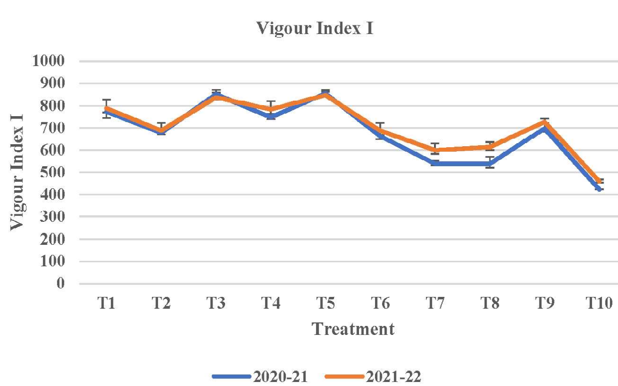

Fig. 2 : Effect of different fungicides and plant products on seed vigour index I of Punjab Naroya.

values are means ± standard errors of one experiment with three biological replicates

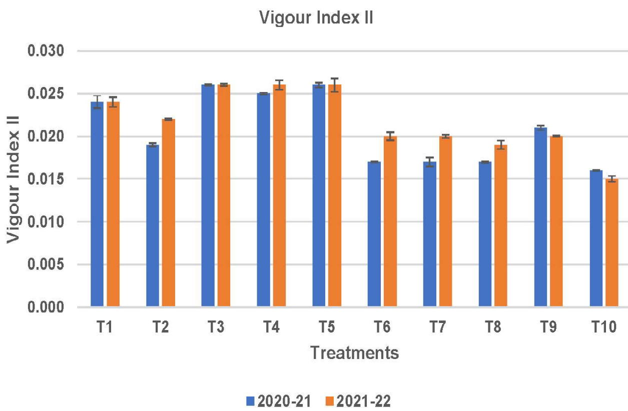

Fig. 3 : Effect of different fungicides and plant products on seed vigour index II of Punjab Naroya.

values are means ± standard errors of one experiment with three biological replicates

growing global emphasis on sustainable and environmentally conscious farming practices (Dhaliwal et al., 2015).

CONCLUSION

This study establishes crude leaf extract of *Azadirachta indica* as a natural, effective, and eco-friendly solution for managing Stemphylium leaf blight in onion. Its efficacy was comparable to, or better than, conventional fungicides in reducing disease severity while also improving seed yield and quality parameters. Moreover, its potential extends to other crops as a plant-based treatment within integrated disease management programs. Adoption of neem-based solutions can reduce pesticide dependence, minimize the environmental impact associated with chemical fungicides, and support residue-free agricultural production.

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