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IN VITRO EFFICACY OF NATURAL COMPONENTS AND MANAGEMENT OF SUGARCANE WILT DISEASE CAUSED BY *FUSARIUM SACCHARI*

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ABSTRACT

The natural components were tested for their effectiveness in controlling sugarcane wilt disease under *In vitro* conditions. Eight components were used viz., garlic, neem, tulsi, giloe, aloe vera, lemongrass, sahjan, and bael. Extracts from all components were tested at 10%, 15%, and 20% concentrations using food poison techniques to control the sugarcane wilt pathogen *Fusarium sacchari* in the laboratory. All the tested natural components significantly inhibit the mycelial growth of pathogen as compared to control. Among the eight, garlic (72.53%) at 20% showed the highest inhibition, followed by neem and tulsi, which produced comparable results. The least inhibition was observed with bael (27.87%) at 20%, followed by lemongrass and sahjan. Giloe and aloe vera showed intermediate levels of mycelial growth inhibition. The present study found that in pot condition all the components reduce the settling mortality and disease incidence and these effectiveeffective natural components, when used at appropriate concentrations. Using natural components is safe and presents a good alternative to synthetic chemical fungicides.

Keywords: Sugarcane, *Fusarium sacchari*, Natural components, Efficacy, Management

Introduction

Sugarcane (*Saccharum officinarum* L.) is a large, perennial tropical grass in the Andropogoneae tribe of the Gramineae family, grown worldwide primarily for its sucrose content (Menossi *et al*., 2008). It is a polyploid plant and is mainly propagated vegetatively using stalks with buds (Croft *et al*., 2008). Sugarcane is a crucial crop grown in over 110 countries for sugar, ethanol, and bioenergy, primarily in tropical and subtropical regions. Covering about 26.34 million hectares, it produces around 1,859.39 million tons annually (Vamsi Krishna *et al*., 2023). Brazil leads with 38% of global production, followed by India with 22% (FAO, 2022). India, the second-largest producer, cultivates sugarcane on 5.15 million hectares, yielding 405.39 million tons per year (FAO, 2022). The crop accounts for about 80% of global sucrose production, valued at approximately US$150 billion annually (Ali *et al*., 2019), and involves around 12.34 million farmers and workers (Ram and Hemaprabha, 2020). India cultivates sugarcane on 58.85 lakh hectares, producing 490.53 million tonnes with a productivity of 83.3 tonnes per hectare. Uttar Pradesh leads in production with 27.95 lakh hectares, producing 224.24 million tonnes at 80.24 tonnes per hectare. Bihar ranks sixth in production, with 2.10 lakh hectares, 12.74 million tonnes, and a productivity of 60.62 tonnes per hectare (ISMA, 2024).

Being a valuable crop, sugarcane is significantly affected by a large number of diseases. Approximately 55 diseases affecting sugarcane have been reported in India (Rao *et al*., 2002; Rott *et al*., 2000). Diseases reduce about 10–15% of the nation's sugar production (Viswanathan & Rao, 2011). In Bihar, over 20 sugarcane diseases, including red rot, wilt, pokkah boeng, smut, leaf spot, and ratoon stunting, have been

reported. Recently, red rot and wilt have emerged as major concerns (Minnatullah *et al*., 2022). Wilt, affecting various commercial sugarcane varieties, is a major disease in the country, significantly reducing cane production and overall productivity. In Bihar, the incidence of wilt disease ranges from 5% to 80% across different sugar factory areas (Minnatullah *et al*., 2021 & 2022).

Synthetic chemicals have harmful effects on both the environment and living organisms, as their residues persist for extended periods. These fungicides impose significant financial burdens on farmers and contribute to the development of pathogen resistance to wilt disease due to their overuse. Therefore, exploring alternative methods for managing sugarcane wilt disease is essential. Natural components, which are by products of plant parts with active chemicals, can inhibit fungal growth and are environmentally friendly with no toxic effects on humans or the environment. In this study, eight different natural component extracts were tested for their efficacy against sugarcane wilt disease.

Materials and Method

The research was conducted in the laboratory of Plant Pathology at the Sugarcane Research Institute, Department of Plant Pathology, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, during 2023-24 planting season located at a latitude of 25.980°N and a longitude of 85.670°E. The study employed a completely randomized design using the poisoned food technique for screening natural components. Natural component extracts were evaluated at three different concentrations: 10%, 15%, and 20%. Each treatment was replicated three times. Diseased plants were brought to the laboratory from the Sugarcane Research Farm, Kalyanpur, Samastipur. The pathogen was isolated, maintained, and purified on oatmeal agar media at 27±1°C. After incubation, the purified growth of the fungus was observed and examined under a microscope to confirm the pathogen as *Fusarium sacchari*. Pathogenicity of the isolated fungus was tested by plug inoculation technique.

The eight natural components Neem leaf extract, Sahjan leaf, Tulsi leaf, Garlic bulb, Aloe vera pulp, Giloe leaf, Bael leaf, and Lemongrass leaf were collected locally and washed with sterilized distilled water. These components were then ground with 100 ml of water mixed with 100 g of plant material and filtered through muslin cloth to create a standard (100%) solution. To achieve the desired concentrations, the filtered extracts were diluted with distilled water. Specifically, 10%, 15%, and 20% concentrations were prepared by mixing 20, 30, and 40 ml of the extract with 80, 70, and 60 ml of sterilized distilled water, respectively. Double-strength concentrations of the natural components and media were prepared. The poisoned food technique was used to assess these phytoextracts against the wilt pathogen caused by *Fusarium sacchari*. Oatmeal-amended media, with varying concentrations of extracts, were inoculated with 5 mm discs of the pathogen (seven days old, actively growing, and from three isolates) in triplicate. Carbendazim were also inoculated with wilt pathogen and media without natural extracts served as the control. The plates were incubated at 27±1°C, and the colony diameter was measured in both treated and control plates after eight days. The percentage inhibition of mycelial growth was calculated using the formula as per given by Vincent (1947).

Table 1 : Description of the tested natural components

S. No	Local Name	Scientific Name	Family	Plant parts used	Concentration (%)
01	Neem	*Azadirachta indica*	Meliaceae	Leaves	10,15,20
02	Sahjan	*Moringa oleifera*	Moringaceae	Leaves	10,15,20
03	Tulsi	*Ocimum sanctum*	Lamiaceae	Leaves	10,15,20
04	Garlic	*Allium sativum*	Amaryllidaceae	Bulb	10,15,20
05	Aloe vera	*Aloe barbadensis*	Liliaceae	Pulp	10,15,20
06	Giloe	*Tinospora cordifolia*	Menispermaceae	Leaves	10,15,20
07	Bael	*Aegle marmelos*	Rutaceae	Leaves	10,15,20
08	Lemongrass	*Cymbopogan citratus*	Poaceae	Leaves	10,15,20

Management of sugarcane wilt disease caused by Fusarium sacchari through natural components in pot condition (In Vivo)

To manage wilt disease under natural conditions, an experiment was conducted using pots with three sugarcane varieties known to be susceptible (CoV 92102, Co 0238, and CoSe 95422) and one resistant variety (BO 153), with three replications. The experiment utilized natural components that had been evaluated in vitro. Concrete pots were thoroughly

cleaned, filled with sterilized soil, and left with a 10 cm gap from the top for watering. Each sugarcane variety was cut into one-bud setts and planted at a rate of 3 setts per and the extracts were applied to the pots at planting. Thirty days after planting, germination counts were recorded, and the pots were drenched again with the natural components. Settling mortality and disease incidence were assessed 45 days after planting. A third application of the natural components was made on the 45th day, and settling mortality percentage and disease incidence were recorded once more on the 60th day. Based on disease incidence, the varieties were evaluated for their resistance.

Results and Discussion

In vitro evaluation of natural components against sugarcane wilt pathogen Fusarium sacchari

The eight natural components extract namely Neem leaf, Sahjan leaf, Tulsi leaf, Garlic bulb, Aloe vera pulp, Giloe leaf, Bael leaf, and Lemongrass leaf were assessed *In Vitro* against the sugarcane wilt pathogen at concentration of 10, 15 and 20 per cent. All the tested plant extracts varied significantly in inhibiting the mycelial growth of the fungus at different concentration depicted in table 2 and figure 1. At 10% concentration, the highest mycelial growth inhibition of the wilt pathogen *Fusarium sacchari* was exhibited by Garlic (29.85%), followed by Neem (25.28%), Tulsi (22.46%), Giloe (20.35%), Aloe Vera (19.26%), Lemon grass (17.54%), and Sahjan (15.63%). The minimum mycelial growth inhibition was observed with Bael (13.79%). At 15% concentration, the highest mycelial growth inhibition of the wilt pathogen *Fusarium sacchari* was exhibited by Garlic (52.64%), followed by Neem (49.56%), Tulsi (37.65%), Giloe (31.47%), Aloe Vera (29.52%), Lemon grass (26.67%), and Sahjan (24.54%). The minimum mycelial growth inhibition was observed with Bael (21.46%). At 20% concentration, the highest mycelial growth inhibition of the wilt pathogen *Fusarium sacchari* was exhibited by Garlic (72.53%), followed by Neem (69.34%), Tulsi (56.82%), Giloe (52.46%), Aloe Vera (44.72%), Lemon grass (39.85%), and Sahjan (34.65%). The minimum mycelial growth inhibition was observed with Bael (27.87%). The mycelial growth inhibition with Carbendazim @0.1% (used as a positive control) was 100%, while there was 0% mycelial growth inhibition in the control without any components (OMA media only) at 8 days after inoculation. The results of Apet *et al*. (2015) support the findings of the current study, both highlighting garlic as the most effective botanical treatment against the soil-borne pathogen *Ceratocystis paradoxa*. This effectiveness is attributed to garlic's antifungal compounds, such as phenols, tannins, and alkaloids. Similarly, research by Chaudhary *et al*. (2010) and Chaudhary *et al*. (2019) demonstrated garlic's high efficacy against various pathogens, including notable inhibition of *Fusarium oxysporum* and *Fusarium udum*. Ghante *et al*. (2018) also observed garlic's strong performance among botanicals against *Fusarium udum*. The antifungal properties of garlic are linked to active compounds like diallyl disulfide and diallyl trisulfide, as detailed by Singh *et al*. (1979, 1980) and Cavallito and Bailey (1944).

Management of sugarcane wilt disease in pot condition by using natural components

The data presented in Table 3 illustrates the effects of natural components on germination percentages, settling mortality, and disease incidence. On the 30th day after planting, the sugarcane variety CoV 92102 had the highest germination rate with garlic (21.56%), followed by neem (20.85%), tulsi (17.63%), giloe (15.38%), aloe vera (13.43%), lemongrass (12.78%), sahjan (12.17%) and Bael resulted in the lowest germination percentage (11.68%). The control with carbendazim achieved a germination rate of 29.68%, while untreated pots had a rate of 9.48%. Likewise, sugarcane variety Co 0238, garlic led to the highest germination (28.90%), with neem (28.15%), tulsi (25.83%), giloe (25.18%), aloe vera (24.95%), lemongrass (24.32%), sahjan (23.36%) following and bael resulted in the lowest germination percentage (22.84%). Carbendazim as a control had a germination rate of 32.74%, and untreated pots had 21.63%. In CoSe 95422, garlic again achieved the highest germination rate (29.23%), followed by neem (26.74%), tulsi (26.16%), giloe (25.95%), aloe vera (25.32%), lemongrass (24.72%), and sahjan (23.95%). The lowest rate was with bael (23.23%). The control with carbendazim had a 34.52% germination rate, compared to 23.74% for untreated pots. In BO 153, garlic yielded the highest germination (36.57%), followed by neem (36.14%), tulsi (35.57%), giloe (35.12%), aloe vera (34.86%), lemongrass (34.25%), and sahjan (33.27%). Bael had the lowest germination rate (33.04%). Carbendazim as a control had a germination rate of 39.95%, and untreated pots had 30.56%, as shown in Table 3.

On the 45th day after planting, settling mortality was recorded. For CoV 92102, garlic treatment resulted in the lowest mortality (15.35%), followed by neem (15.82%), tulsi (18.73%), giloe (21.56%), aloe vera (24.62%), lemongrass (25.75%), sahjan (27.28%) and the highest mortality was observed with bael (28.43%). Carbendazim as a control showed 8.36% mortality, while untreated pots had 32.65%. In Co

0238, garlic had the lowest mortality (10.48%), followed by neem (10.74%), tulsi (11.46%), giloe (11.64%), aloe vera (11.86%), lemongrass (12.15%), and sahjan (12.43%). The highest mortality was with bael (12.78%). Carbendazim showed 6.34% mortality, and untreated pots had 15.46%. For CoSe 95422, garlic achieved the lowest mortality (10.02%), followed by neem (10.35%), tulsi (11.16%), giloe (11.37%), aloe vera (11.65%), lemongrass (12.04%), and sahjan (12.17%). The highest mortality was with bael (12.68%). Carbendazim had 5.28% mortality, and untreated pots had 13.43%. In BO 153, garlic resulted in the lowest mortality (3.63%), followed by neem (3.96%), tulsi (4.20%), giloe (4.26%), aloe vera (4.35%), lemongrass (4.38%), and sahjan (4.53%). The highest mortality was with bael (4.69%). Carbendazim showed 2.87% mortality, while untreated pots had 6.78%, as illustrated in Table 3.

On the 60th day after planting, settling mortality was assessed again. For CoV 92102, garlic had the lowest mortality (14.98%), followed by neem (15.42%), tulsi (18.54%), giloe (21.13%), aloe vera (24.36%), lemongrass (25.47%), sahjan (26.97%), and the highest mortality was with bael (26.09%). Carbendazim as a control showed 8.05% mortality, and untreated pots had 33.53%. In Co 0238, garlic achieved the lowest mortality (10.06%), followed by neem (10.23%), tulsi (11.15%), giloe (11.24%), aloe vera (11.43%), lemongrass (11.96%), sahjan (12.05%), and the highest mortality was with bael (13.34%). Carbendazim showed 6.07% mortality, and untreated pots had 16.12%. For CoSe 95422, garlic had the lowest mortality (9.84%), followed by neem (10.17%), tulsi (11.05%), giloe (11.18%), aloe vera (11.35%), lemongrass (11.83%), sahjan (11.94%) and the highest mortality was with bael (12.26%). Carbendazim had 4.92% mortality, and untreated pots had 13.95%. In BO 153, garlic had the lowest mortality (3.52%), followed by neem (3.84%), tulsi (4.16%), giloe (4.19%), aloe vera (4.27%), lemongrass (4.29%), sahjan (4.45%) and the highest mortality was with bael (4.56%). Carbendazim as a control had 2.82% mortality, while untreated pots had 7.08%, as shown in Table 3. These findings were similar to a report of Singh *et al*., 1979. Gohil and Vala (2003) also found that carbendazim is more effective than mancozeb in improving sugarcane germination and controlling *Fusarium moniliforme* wilt under pot culture conditions. Similar results were reported by Goodall *et al*. (1998).

Conclusion

Fusarium sacchari poses a significant economic threat to global sugarcane production, causing major reductions in yield, quality and quantity. While, various chemical fungicides are available to manage this pathogen, their widespread use can lead to health risks and environmental damage. In this study, garlic and neem showed a high percentage of inhibition against the pathogen among eight tested natural components. These natural substances have the potential to serve as effective, eco-friendly and might offer alternatives compared to harmful chemical, as they have minimal environmental and health impacts. The current research is limited to laboratory and pot conditions, so further *In Vitro* and *field* trials are needed.

Table 2: Efficacy of natural components extract on mycelial growth inhibition of wilt pathogen *Fusarium sacchari.*

S.No	Treatment	Colony diameter (mm)			Growth inhibition (%)			Mean
		10%	15%	20%	10%	15%	20%	
1	Neem	67.24	45.39	27.69	25.28 (30.18)	49.56 (44.75)	69.34 (56.38)	48.06
2	Sahjan	75.93	67.91	58.81	15.63 (23.29)	24.54 (29.69)	34.65 (36.06)	24.94
3	Tulsi	69.78	56.12	38.86	22.46 (28.29)	37.65 (37.85)	56.82 (48.92)	38.97
4	Garlic	63.13	42.62	24.72	29.85 (33.12)	52.64 (46.51)	72.53 (58.39)	51.67
5	Aloe	72.66	63.43	49.75	19.26 (26.03)	29.52 (32.91)	44.72 (41.97)	31.16
6	Giloe	71.68	61.67	42.78	20.35 (26.81)	31.47 (34.12)	52.46 (46.41)	34.76
7	Bael	77.58	70.68	64.91	13.79 (21.80)	21.46 (27.60)	27.87 (31.87)	21.04
8	Lemon grass	74.21	65.99	54.13	17.54 (24.76)	26.67 (31.09)	39.85 (39.14)	28.02
9	Carbendazim @ 0.1%	0.00	0.00	0.00	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
10	Check	90	90	90	0	0	0	0
	SEm (±)	1.26	1.14	0.92				
	CD (5%)	3.76	3.41	2.74				
	CV	3.31	3.53	3.54				

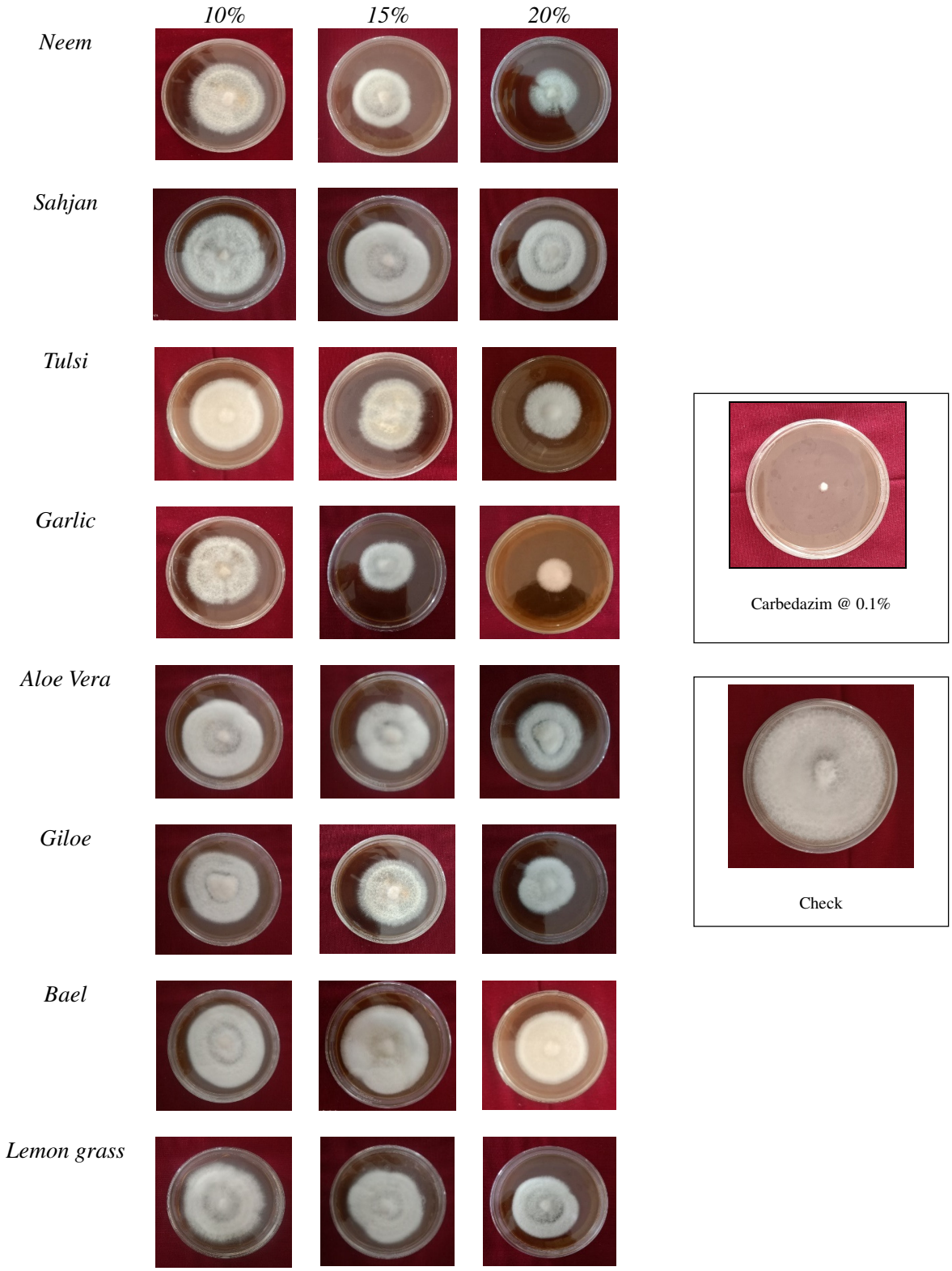

Plate 1: Reduction of mycelial growth of *Fusarium sacchari* upon various treatment of natural components

Table 3 : Management of Sugarcane wilt disease in pot condition by various natural components

S. No	Components	CoV 92102					Co 0238					CoSe 95422					B0 153				
		30th	45th		60th		30th	45th		60th		30th	45th		60th		30th	45th		60th	
		Germination(%)	Disease Incidence	Mortality (%)	Disease Incidence	Mortality (%)	Germination (%)	Disease Incidence	Mortality (%)	Disease Incidence	Mortality (%)	Germination (%)	Disease Incidence	Mortality (%)	Disease Incidence	Mortality (%)	Germination (%)	Disease Incidence	Mortality (%)	Disease Incidence	Mortality (%)
1	Neem	20.85	3.41	15.82	3.39	15.42	28.15	3.16	10.74	3.12	10.23	28.74	3.08	10.35	3.06	10.17	36.14	0.94	3.96	0.84	3.84
2	Sahjan	12.17	3.92	27.28	3.87	26.97	23.36	3.52	12.43	3.47	12.05	23.95	3.23	12.17	3.21	11.94	33.27	1.16	4.53	1.12	4.45
3	Tulsi	17.63	3.57	18.73	3.53	18.54	25.83	3.29	11.46	3.24	11.15	26.16	3.15	11.16	3.11	11.05	35.57	1.10	4.20	1.06	4.16
4	Garlic	21.46	3.29	15.35	3.24	14.98	28.90	3.11	10.48	2.98	10.06	29.23	3.02	10.02	2.95	9.84	36.57	0.72	3.63	0.65	3.52
5	Aloe Vera	13.43	3.78	24.62	3.72	24.36	24.95	3.40	11.86	3.32	11.43	25.32	3.21	11.65	3.18	11.35	34.86	1.14	4.35	1.10	4.27
6	Giloe	15.38	3.64	21.56	3.61	21.13	25.18	3.37	11.69	3.28	11.24	25.95	3.19	11.37	3.14	11.18	35.12	1.13	4.26	1.09	4.19
7	Bael	11.68	3.98	28.43	3.95	26.09	22.84	3.57	12.78	3.50	13.34	23.23	3.24	12.68	3.22	12.26	33.04	1.18	4.69	1.14	4.56
8	Lemon grass	12.78	3.83	25.75	3.77	25.47	24.32	3.46	12.15	3.38	11.96	24.72	3.22	12.04	3.20	11.83	34.25	1.14	4.38	1.11	4.29
9	Carbendazim	29.68	3.16	8.36	3.11	8.05	32.74	3.00	6.34	2.92	6.07	34.52	2.91	5.28	2.89	5.95	39.95	0.41	2.87	0.37	2.82
10	Control	9.48	4.00	32.65	3.98	33.53	21.63	3.82	15.46	3.73	16.12	22.34	3.53	13.43	3.50	13.95	30.56	1.40	6.78	1.38	7.08
	SE(±m)	0.42		0.48		0.46	0.60		0.22		0.21	0.63		0.20		0.19	0.87		0.08		0.06
	CD (5%)	1.25		1.43		1.37	1.79		0.66		0.64	1.87		0.61		0.57	2.58		0.25		0.19
	CV	4.43		3.83		3.74	4.04		3.34		3.33	4.13		3.26		3.09	4.32		3.36		2.70

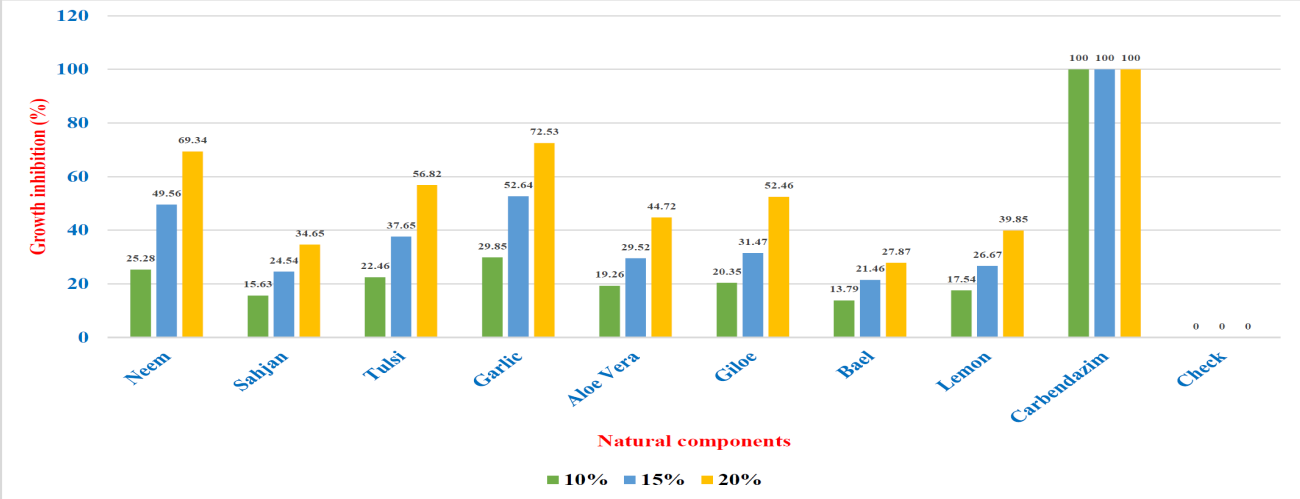

Fig. 1 : Effect of natural components on mycelial growth inhibition

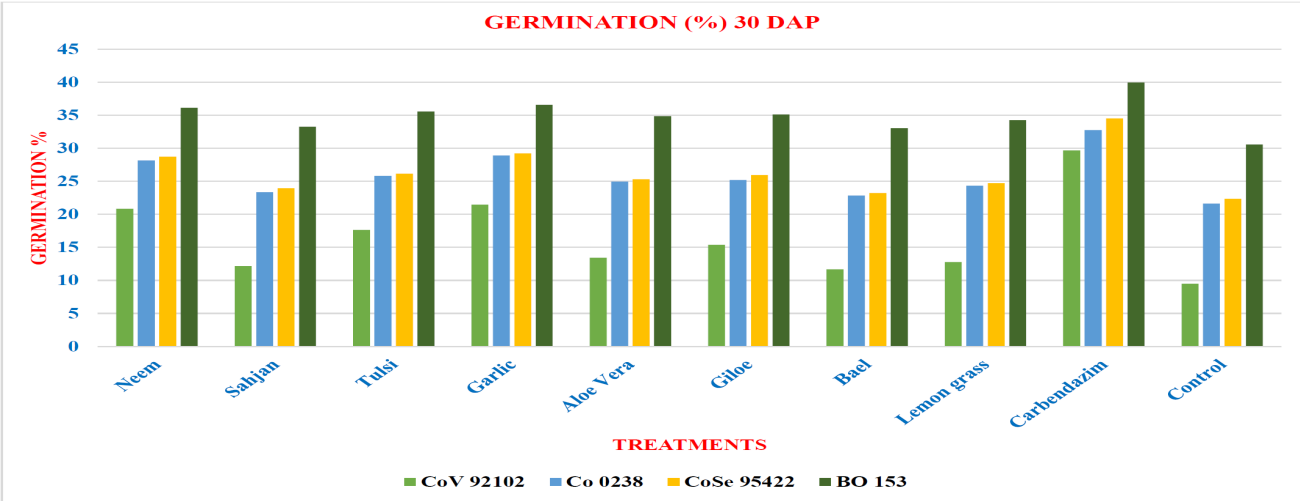

Fig. 2 : Germination percentage 30 days after planting (DAP)

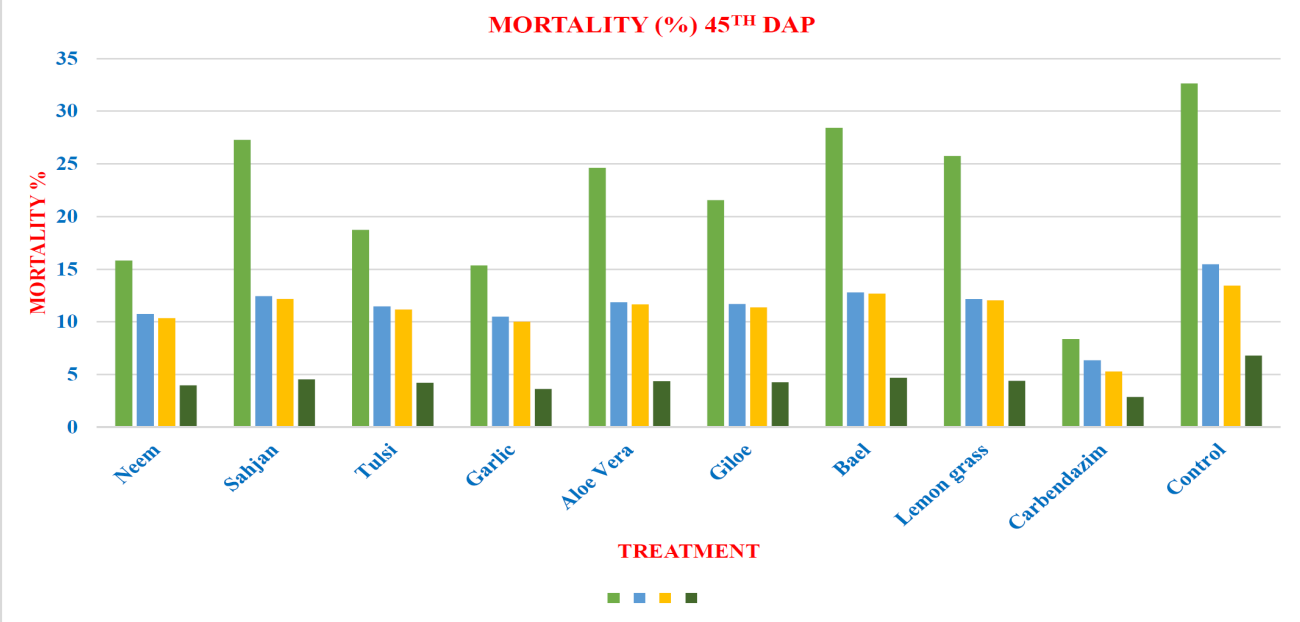

Fig. 3 : Settling mortality percentage 45 days after planting (DAP)

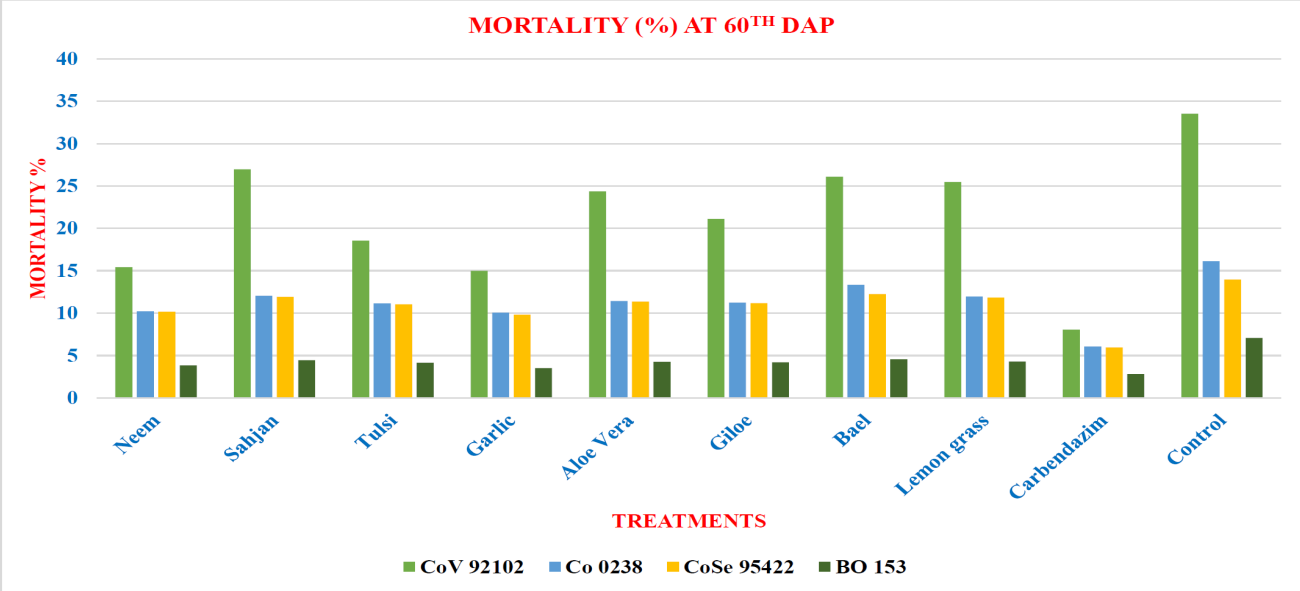

Fig. 4 : Settling mortality percentage 60 days after planting (DAP)

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