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Research article

Non-chemical management of stem rot disease of Egyptian clover (*Trifolium alexandrinum* L.)

Manjeet Kaur, Ashlesha Atri* and Daljeet Singh

Punjab Agricultural University, Ludhiana-141004, India
*Corresponding author e-mail: ashlesha-atri@pau.edu

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Abstract

Stem rot of Egyptian clover (*Trifolium alexandrinum* L.) is considered as one of the major constraints for green fodder and seed yield. Berseem stem rot is incited by a soil borne pathogen *Sclerotinia sclerotiorum*. Botanical extracts of *Aegle marmelos*, *Melia azedarach*, *Murraya koenigii*, neem seed kernel extract (NSKE) and organic products like panchagavya, compost tea and poultry manure were evaluated for the management of stem rot disease. Botanical extracts were applied as foliar spray and seed treatment as well and organic inputs were applied as soil application and foliar spray. Among botanicals, application of *A. marmelos* showed a significant reduction in stem rot by 54.50% with 24.20% increase in green fodder yield. Among organic inputs, application of panchagavya significantly controlled the stem rot disease by 46.15% and enhanced the green fodder yield by 21.30% than untreated control. Application of panchagavya followed by *A. marmelos* leaf extracts along with significant disease control also enhanced green fodder yield during both seasons. Average disease control attained by application of panchagavya and *A. marmelos* was 54.50 and 46.15% and enhanced green fodder yield was 24.20 and 21.30%, respectively. The current study showed that plant extracts and organic inputs have potential for the management of stem rot of berseem.

Keywords: Botanicals, Egyptian clover, Management, Organic inputs, Stem rot

Introduction

Egyptian clover (*Trifolium alexandrinum*), locally known as berseem is a major annual *Rabi* season fodder legume crop, belongs to family Fabaceae and widely grown in areas of central, northern and north-west India (Satyapriya *et al*., 2013; Manjunatha *et al*., 2019; Kumar *et al.,* 2023). In India, it was introduced in 1904 and grown on near about 20 lakh hectares area followed by Egypt and Pakistan. It is widely grown in irrigated areas of Punjab, Rajasthan, Delhi, and other parts of northern and western parts of India. In Punjab, it is cultivated over an area of 2.30 lakh hectare area (Anonymous, 2021). Its growth and germination are favored by mild temperature but it could also be restricted by intense cold and frosty weather. It is highly admired by the farm animals and dairy farmers because of its succulent nature, fast growing habit and high nutritive value (Behari *et al*., 2003) as it contains 62% total digestible nutrients and 20% crude protein which make it more palatable (Iqbal and Iqbal, 2014; Manjunatha *et al*., 2017).

Sclerotinia species are pathogenic to many winter season crops. Its soil borne nature and sclerotia forming ability make it difficult to manage. Under favorable environmental conditions, when temperature rises above 21 °C and plant nutrients decline, white colour mycelium starts aggregating and form knot like structures on which oil drops appear and transformed into grey to black coloured sclerotia. These sclerotia help this pathogen to survive without its host during off season (Christias and Lockwood, 1973).

Now a day, use of fungicides for the management of berseem stem rot (Bhaskar *et al*., 2003; Iqbal and Iqbal, 2014) could be one of the options. However, soil borne pathogens are difficult to manage by using fungicides because of their endogenous growth habit and capability of the fungus to survive in soil under unfavorable conditions in the form of hard sclerotia for many years. However, efforts have been made for breeding stem rot resistant genotypes through interspecific hybridization with wild species (Roy *et al*., 2004; Malaviya *et al*., 2018) and germplasm screening has also been done for this disease but cultivar resistant to stem rot disease has not been identified yet (Malaviya *et al*., 2008). Use of chemical

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fungicides could lead to environment and soil health hazards and could also lead to decrease in effectiveness of fungicides due to cross resistance produced by the pathogens (Ishii, 2006; Aktar *et al*., 2009).

Hence, there was a serious need to find out alternative ecofriendly management strategies which could manage such pathogens without harming environment and soil health. The plant extracts and organic amendments could be the best alternatives to control this fungal pathogen, which can complement the use of existing strategies. Antifungal activity of several botanical extracts and organic inputs have been well documented by various researchers against various phytopathogens especially soil borne pathogens (Sales *et al*., 2016; Ashlesha and Paul, 2017). Major role in plant extracts is well played by phytochemicals present in various plant parts like terpenoids, saponins, flavonoids, alkaloids and tannins (Rinez *et al*., 2013). Besides plant extracts, composts and manures could also be the alternative for synthetic fungicides like farm yard manure, poultry manure, cow dung manure, panchagavya, vermicompost and vermiwash which possess antifungal activities against wilt and root rot pathogens (Kerkeni *et al*., 2008; Sinha *et al*., 2010). Many workers have demonstrated that the use of plant based products and organic inputs provide resistance to plants against pathogens through activation of plants' own defense mechanism by accumulation of pathogenesis related proteins and induction of defense enzymes such as peroxidases, β-1, 3-glucanases and chitinases (Persaud *et al*., 2019). Thus the present study was undertaken to identify the suitable eco-friendly management strategy against stem rot disease infecting berseem crop.

Materials and Methods

Experimental site and design: The botanical extracts and organic inputs were evaluated under field conditions against stem rot pathogen during 2019-20 and 2020-21 seasons on BL 42 cultivar of berseem. The experimental fields were located at an elevation of 247 m above mean sea level (30°56'N latitude and 75°52'E longitude). The climate of Ludhiana is sub-tropical and semi-arid with very hot to dry and humid summer with average annual temperature of 23.1°C to 42.1°C and average annual rainfall of 705 mm. The experiment was conducted in randomized complete block design (RCBD) with three replications per treatment. The plot size was 3 x 3 m. The experimental crop received optimum weather conditions throughout the growing season.

Isolation and mass multiplication of pathogen inoculum: Berseem plants showing symptoms of stem rot caused by *S. sclerotiorum* were collected from the fields of Department of Plant Breeding and Genetics, Punjab Agricultural University (PAU), Ludhiana, Punjab, India. The diseased tissue along with healthy portion of berseem plant was cut into small pieces. The tissue was sterilized with 1.0% sodium hypochlorite for one minute and washed thrice using sterilized distilled water and transferred bits into Potato Dextrose Agar (PDA) plates. The plates were incubated at 25°C for 3 days. The pathogen was isolated and identified based on its morphology, mycelial growth and sclerotia formation (Ekins, 1993). Further, the pathogen was purified by hyphal tip method and its pathogenicity was also proven by following the *Koch's* postulates. The test isolate was maintained on PDA medium for further studies at -4°C. The pathogen was mass multiplied in maize sand meal medium (Dohroo, 1988).

Preparation of plant extracts: Leaves of *Murraya koenigii* (curry leaf), *Aegle marmelos* (bael), *Melia azedarach* (dereck) and *Cymbopogon citratus* (lemon grass) were collected from surrounding of PAU, Ludhiana. The collected plants were preferred over other plants due to their reported antifungal activity against plant pathogens (Pandey, 2015; Sales *et al*., 2016). Collected leaves were washed under running tap water and then dried for many days under shady conditions to reduce their moisture up to maximum possible content and to make them dry without losing essential nutrients. Leaf extracts were obtained by smashing leaves followed by dipping ground powder overnight in sterilized distilled water (1:2 w/v). These extracts were filtered through double layered muslin cloth and filtrate was used as a stock solution for further study.

Treatment details: A total of nine treatments were tested against the pathogen (Table 1). These botanical extracts and organic inputs were applied as soil application (10%), seed treatment (5%) and foliar spray (10%). Three foliar sprays were given starting from application of first spray 20 DAS, second and third after first and second green fodder cut at 50 and 80 DAS, respectively. Pathogen inoculum mass multiplied on sand maize meal medium had been added in the plots one week after the sowing of the crop.

Data recording: Data of disease severity was recorded at ten days after every foliar spray that is at 30 DAS, 60 DAS and 90 DAS. The stem rot damage on plants was assessed at 7 days interval using 0-9 scale of Mikaliūnienė *et al*. (2015) and percent disease severity (PDS) was estimated as per the formula given by McKinney (1923): PDS = {Σ ni x Si / (N x S)} x100, where, PDS = Per cent disease severity; Σ ni = Number of plants with stem rot symptoms; Si = Value of the score of symptoms; N = total number of tested plants; S = Maximum disease score; Disease intensity was measured with area under disease progress curve (AUDPC) calculated using Prescott *et al.* (1986) formula.

Statistical analysis: The analysis of all the experiments was done by using SAS software. ANOVA was used to compare significant difference among treatments at $P<0.05$. Means were compared using Fisher's Least Significant Difference (LSD) test ($\alpha<0.05$).

Results and Discussion

The extracts of botanicals like neem seed kernel extract, *A. marmelos*, *M. azedarach*, *C. citratus* and organic inputs *viz.*, panchagavya, compost tea and poultry manure were evaluated against stem rot disease under field conditions. The disease severity is presented along with disease control and green fodder yield (Table 2-3; Fig 1) indicating significant management of disease by all the treatments than untreated control.

***Effect on stem rot disease*:** The significant treatment which controlled disease more effectively and also increased green fodder yield was panchagavya followed by *A. marmelos*, *C. citratus*, neem seed kernel extract, poultry manure, *M. koenigii*, *M. azedarach.* Field trials were conducted during *Rabi* of 2019-20 and 2020-21. T1 (Soil application and foliar spray of panchagavya at 10%) showed significant disease control than untreated control. T1 showed maximum disease control (50.80%) with minimum disease severity (32.0%) after 90 days of sowing with minimum (1500) area under disease progress curve (AUDPC) during first year trial. In second year trial, disease control provided by panchagavya was 58.2% with minimum disease severity (30.0%) after 90 days of sowing along with minimum (1700) AUDPC values. Following panchagavya application, T6 (seed treatment and foliar spray of *A. marmelos* extract 10%) was the second best treatment which showed significant disease control of 41.1% with 1760 AUDPC showing disease severity of 38.3% at 90 DAS during first year trial. During second season, T6 showed significant disease control (51.2%) with 1920 AUDPC values and showing 35.0% disease severity at 90 DAS. The third best treatment was T8 (Seed treatment and foliar spray of *C. citratus* leaf extract at 10%). The T8 exhibited 39.3 and 36.7% disease severity with 1785 and 1950 AUDPC values and 39.5 and 48.8% disease control during first and second year, respectively. T2 (seed treatment and foliar spray of neem seed kernel extract at 5%) showed significant disease control (35.8%) at 41.7% disease severity (90 DAS) with 1885 AUDPC in first year trial. In second year trial disease controlled by T2 was 46.6% which showed 2025 AUDPC with 38.7% disease severity at 90 DAS. The per cent disease control showed by treatment T3 (soil application and foliar spray of poultry manure 5 t/ha) was 30.8% and recorded AUDPC was 1965 with 45.0% disease severity during *Rabi* 2019-20. In *Rabi* 2020-21, disease control by T3 was 44.2% and AUDPC was 2125 with 40.0% disease severity followed by treatment T5 (seed treatment and foliar spray of *M. koenigii* extract at 10%) found more significant than T7. The disease control with application of treatment T5 in the first year was 20.5% with 2055 AUDPC and

Table 1. Details of treatments

Sl. No.	Details of treatment
T1	Soil application + foliar spray of panchagavya (10%)
T2	Soil application + foliar spray of Neem seed kernel extract (5%),
T3	Soil application + foliar spray of Poultry manure@ 5t/ha
T4	Soil application + foliar spray of Compost tea (10%)
T5	Seed treatment + foliar spray of *Murraya koenigii* extract (10%)
T6	Seed treatment + foliar spray of *Aegle marmelos* extract (10%)
T7	Seed treatment + foliar spray of *Melia azedarach* extract (10%)
T8	Seed treatment + foliar spray of *Cymbopogon citratus* extract (10%)
T9	Control

Table 2. Effect of different treatments on disease incidence under field conditions during 2019-20

Treatments	Disease severity (%)			Disease control (%)	Green fodder yield (q/ha)*	Increase in yield (%)	Seed yield (q/ha)*	AUDPC
	30 DAS	60 DAS	90 DAS					
T1	9.80 (18.259)^{f}	31.70 (34.217)^{f}	32.00 (34.429)^{e}	50.80	580.0^{a}	24.70	4.50^{a}	1500^{e}
T2	19.20 (25.950)^{d}	38.30 (38.229)^{ef}	41.70 (40.182)^{d}	35.80	531.7^{c}	14.30	3.60^{d}	1885^{cde}
T3	19.30 (26.011)^{d}	41.70 (40.182)^{de}	45.00 (42.104)^{cd}	30.80	516.7^{d}	11.10	3.50^{de}	1965^{cde}
T4	25.00 (29.985)^{b}	51.70 (45.938)^{b}	55.00 (47.860)^{b}	15.40	480.0^{ef}	03.20	3.07^{fg}	2570^{b}
T5	21.80 (27.795)^{c}	45.00 (42.104)^{cd}	48.70 (44.248)^{bc}	20.50	515.0^{d}	10.80	3.39^{e}	2055^{cd}
T6	13.50 (21.530)^{f}	33.30 (35.237)^{f}	38.30 (38.229)^{de}	41.10	570.0^{ab}	22.60	4.20^{b}	1760de
T7	22.90 (28.565)^{c}	50.00 (44.982)^{bc}	53.30 (46.904)^{b}	18.00	486.7^{e}	04.70	3.20^{f}	2280^{bc}
T8	15.90 (23.466)^{e}	36.70 (37.243)^{ef}	39.30 (38.824)^{de}	39.50	561.7^{b}	20.80	3.93^{c}	1785de
T9	33.40 (35.267)^{a}	60.00 (50.769)^{a}	65.00 (53.740)^{a}		465.0^{f}		2.97^{g}	3622.5^{a}
CD (P<0.05)	1.933	6.397	7.485		14.055		0.164	438.666
SEM	0.639	2.115	2.475		4.648		0.054	145.071
CV (%)	5.516	8.492	9.159		5.925		2.611	11.643

*10 quintals (q) = 1 ton; AUDPC: Area under disease progress curve; Data in parenthesis are arcsine transformed; DAS: Days after sowing; Mean values followed by the same superscript letter(s) within the column are not significantly different at P<0.05 according to LSD (Fisher's Least Significant Difference)

48.7% disease severity and during the second cropping season disease control was 44.2% with 2225 AUDPC and 40.0% disease severity. T7 (seed treatment and foliar spray of *M. azedarach* extract 10%) controlled disease by 18.0% with recorded AUDPC of 2280 and 53.3% disease severity at 90 DAS during 2019-20. During 2020-21, disease control was 27.9% and AUDPC was 2525 with 51.7% disease severity observed in plots treated with treatment T7. The least effective treatment was T4 (soil application and foliar spray of compost tea 10%) which showed maximum AUDPC (2570 and 2875) with maximum disease severity (55.0 and 53.0%) and minimum disease control (15.4% and 23.3%) during all two berseem growing seasons, respectively.

Effect on seed and green fodder yields: Maximum increase in green fodder yield was observed with the application of panchagavya during both the cropping seasons by 24.70 and 23.70 per cent followed by application of T6 which enhanced the green fodder yield by 22.60 per cent (570.0 q/ha) and 20.00 per cent (583.0 q/ha) and yielded 4.20 q/ha and 3.90 q/ha seed yield in the first year and second year trial, respectively. T8 yielded green fodder 561.7 q/ha and 570.0 q/ha with 3.93 q/ha and 3.87 q/ha seed yield in the first year and second year trial, respectively. T2 enhanced green fodder yield by 14.30% and 14.80% with seed yield of 3.60 q/ha and 3.37 q/ha during *Rabi* 2019-20 and 2020-21, respectively. Seed yield of 3.50 q/ha and 3.30 q/ha with green fodder yield of 516.7 q/ha and 540.0 q/ha was increased by T3 treatment in the first and second year, respectively. During first year and second year trial, green fodder yield increased by 10.80% and 10.80% and recorded seed yield was 3.39 q/ha and 3.20 q/ha in case of treatment T5, respectively. T7 increased green fodder yield by 4.70% and 9.00% and recorded seed yield was 3.20 q/ha and 2.97 q/ha during both the years, respectively. Minimum green fodder yield was 480.0 and 506.7 q/ha, recorded with the application of treatment T4 during both the berseem growing seasons but significantly better than the untreated control.

The exploration of different ecofriendly approaches comprising of plant extracts and organic inputs has become mandatory for the management of stem rot disease in sustainable manner. Panchagavya can be applied as a foliar application or soil application (Natarajan, 2002). It provided resistance against pests and plant diseases and also played significant role in yield increase (Tharmaraj *et al*., 2011).

Table 3. Effect of different treatments on disease incidence under field conditions during 2020-21

Treatments	Disease severity (%)			Disease control (%)	Green fodder yield (q/ha)*	Increase in yield (%)	Seed yield (q/ha)*	AUDPC
	30 DAS	60 DAS	90 DAS					
T1	13.30 (21.127)^{e}	28.30 (32.128)^{c}	30.00 (33.147)^{c}	58.20	601.7^{a}	23.70	4.03^{a}	1700^{d}
T2	20.70 (27.001)^{cd}	33.30 (35.237)^{c}	38.70 (38.176)^{bc}	46.60	558.3^{c}	14.80	3.37^{b}	2025^{cd}
T3	21.00 (27.247)^{cd}	35.00 (36.223)^{bc}	40.00 (39.132)^{bc}	44.20	540.0^{d}	11.00	3.30^{b}	2125^{cd}
T4	26.30 (30.848)^{b}	45.00 (42.104)^{b}	53.00 (46.981)^{ab}	23.30	506.7^{e}	04.20	2.90^{cd}	2875^{b}
T5	23.70 (29.077)^{bc}	36.70 (37.243)^{bc}	40.00 (39.132)^{bc}	44.20	539.0^{d}	10.80	3.20^{bc}	2225^{cd}
T6	17.30 (24.463)^{de}	31.70 (34.217)^{c}	35.00 (36.223)^{bc}	51.20	583.3^{b}	20.00	3.90^{a}	1920^{cd}
T7	23.70 (29.094)^{bc}	38.30 (38.176)^{bc}	51.70 (45.948)^{b}	27.90	530.0^{d}	09.00	2.97^{cd}	2525^{bc}
T8	17.30 (24.359)^{de}	33.30 (35.204)^{c}	36.70 (36.833)^{bc}	48.80	570.0^{bc}	17.20	3.87^{a}	1950^{cd}
T9	36.50 (37.151)^{a}	66.70 (54.969)^{a}	71.70 (58.044)^{a}		486.3^{f}		2.83^{d}	4025^{a}
CD (P<0.05)	5.905	10.525	11.263		15.587		0.308	587.772
SEM	1.953	3.481	3.725		5.155		0.102	194.381
CV (%)	15.233	15.576	15.501		1.635		5.224	14.179

*10 quintals (q) = 1 ton; AUDPC: Area under disease progress curve; Data in parenthesis are arcsine transformed; DAS: Days after sowing; Mean values followed by the same superscript letter(s) within the column are not significantly different at P<0.05 according to LSD (Fisher's Least Significant Difference)

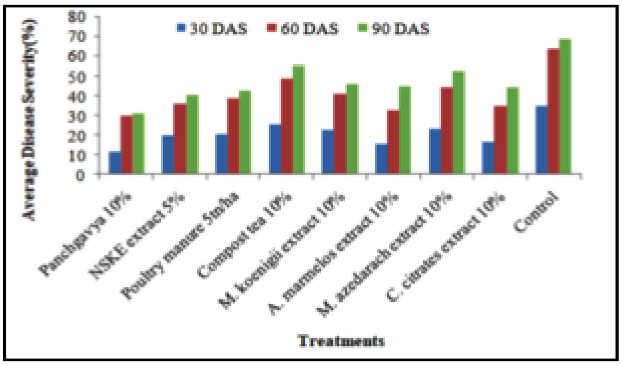

Fig 1. Efficacy of different botanical extracts and organic products at different concentrations under field conditions against stem rot of berseem

Fertilizer properties along with biopesticidal properties of panchagavya, enhance crop growth and yield and also control the disease (Somasundaram and Amanullah, 2007). Complete inhibition of mycelial growth of *S. sclerotiorum, S. rolfsii* and *R. solani* was also reported by application of panchagavya (Dogra, 2006). Atri *et al*. (2021) found that soil application of panchagavya (10%) and *A. marmelos* extract (5%) provided >50 per cent inhibition of stem rot of berseem caused by *S. trifoliorum*. *A. marmelos* showed antifungal activities against various soil borne plant pathogens like *S. sclerotiorum, S. rolfsii* and *F. solani* (Meena *et al*., 2016). Various compounds have been reported in *A. marmelos* such as propanoic acid, flavones, hexadecanoic acid, quinolenes and -caryophyllene which might be one of the reasons behind antifungal activity of *A. marmelos* leaf extracts (Vardhini *et al*., 2018; Nair *et al*., 2020). Persaud *et al*. (2019) observed reduction in sheath blight disease of rice by 18 per cent when rice plants were treated with water extracts of *A. marmelos, A. indica* and *C. flaxousus*. *A. marmelos* application at 5 per cent concentration under *in-vivo* and under *in-vitro* conditions decreased disease severity of stem rot of berseem (Atri *et al*., 2021). Adegoke and Odesola (1996) observed growth inhibition of *Aspergillus flavus* and *A. fumigatus* by using powder extracts of *C. citratus.* Antifungal activity of *C. citratus* extract is due to presence of some volatile essential components (Wang *et al*., 2005) and presence of citral was also observed by Mitchell *et al*. (2010) which were found main antimicrobial substance present in *C. citratus*. Some workers have reported the mechanisms behind the antifungal properties of compost tea were competition for space and nutrition (Al-Mughrabi *et al*., 2008), parasitism (El-Masry *et al*., 2002) induction of systemic resistance and antibiosis (Zhang *et al*., 1998).

Conclusion

Organic inputs and plant extracts showed highest potential against stem rot of berseem along with enhancement in green fodder and seed yield. Seed treatment followed by foliar application of panchgavya (10%) and *A. marmelos* extract exhibited least stem rot severity under field conditions during both the cropping seasons. These inputs showed to enhance the production of green fodder by 24% and 21%, respectively. The remarkable reduction in stem rot severity and increase in green fodder indicated development of new sustainable eco-friendly management strategy that will reduce the dependency of farmers on chemicals thereby reducing the cost of cultivation and enhancing the profit.

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