LR-5272
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RESEARCH ARTICLE

Legume Research- An International Journal

Quantification of Green Fodder Yield Losses Due to Diseases and Insect-pests Infecting Cowpea in North-Eastern and North-Western Regions of India

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10.18805/LR-5272

ABSTRACT

Background: Cowpea [*Vigna unguiculata* (L.) Walp.] is a leguminous crop cultivated for pulse, feed and fodder in tropical and sub-tropical regions worldwide. Although the cowpea is adapted to most of the regions in India, the crop is threatened by diseases like root rot, cowpea yellow mosaic virus, leaf spot and anthracnose and pests such as aphids and defoliators.

Methods: In this study, green fodder yield losses due to diseases and pests were quantified in fodder cowpea at five locations for three cropping seasons 2019-2021. There were two treatments one was protected with fungicides/biocontrol agents/biopesticides and another was unprotected. The incidence of diseases and pests was recorded in both treatments.

Result: The mean root rot incidence was 1.39 to 50.24 per cent at three locations, the severity of cowpea yellow mosaic virus ranged between 0.69 to 14.49% and the disease severity of foliar diseases was 0.75 to 40.40 per cent. Whereas aphid population was in the range of 3.93 to 30.26 aphids per twig and infestation on cowpea by defoliators was observed in the range of 1.31 to 45.64 per cent. The average green fodder yield losses were ranged between 11.06 to 46.21 percent with highest losses were recorded at Palampur location and lowest were estimated at Ludhiana location. The crop loss models were also prepared for disease and pest damage to assess the relationship between disease severity, pest infestation and green fodder yield of cowpea. The correlation was found negative/positive and highly significant at p=0.01 and p=0.05 between disease severity, pest damage values and green fodder yield losses at all five test locations. Regarding the management of diseases and pests, the focus should be on the timely application of management measures.

Key words: Cowpea, Diseases, Green fodder, Insect-pests, Yield losses.

INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] is a versatile, drought-tolerant and warm-weather legume belonging to the family Fabaceae. Cowpea is cultivated for pulse, feed, fodder and the recovery of soil fertility as green manure in tropical and sub-tropical regions worldwide (Gogile *et al.,* 2013). In India, cowpea is cropped in arid and semi-arid regions like Tamil Nadu, Andhra Pradesh, Karnataka, Haryana, West Uttar Pradesh, Delhi, Punjab, Rajasthan, Kerala and Maharashtra. The crude protein content in leaves is more than 30%, along with carbohydrates and minerals (iron, zinc, potassium, phosphorus), making it suitable for animal consumption (Biama *et al.,* 2020).

Although the cowpea is adapted to most of the regions in India, the crop is threatened by various diseases and pests. The major diseases that cause considerable losses in yield are collar rot, dry root rot, leaf spot and anthracnose. Significant yield losses upto 35 to 65% in cowpea have been reported due to root rots caused by *Rhizoctonia solani*, *Macrophomina phaseolina* and *Fusarium oxysporum* (Jha *et al.,* 2020). Cowpea mosaic virus is capable of causing 7-60% yield losses (Neya *et al.,* 2015). The major pests which severely damage cowpea during all growth stages are aphids (*Aphis craccivora*), defoliators such as tobacco caterpillar (*Spodoptera litura*) and Bihar hairy caterpillar (*Spilosoma oblique*). Defoliators infest growing plant tips, buds, leaves, stems. These pests have been reported to cause 65 to 100% yield losses (Anusha *et al.,* 2016).

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How to cite this article: Atri, A., Bhardwaj, N.R., Banyal, D.K., Landge, S., Dhal, A., Cheema, H.K. and Singh, D.P. (2025). Quantification of Green Fodder Yield Losses Due to Diseases And Insect-pests Infecting Cowpea in North-Eastern and North-Western Regions of India. Legume Research. 1-10. doi: 10.18805/LR-5272.

Submitted: 09-11-2023 **Accepted:** 04-11-2024 **Online:** 20-02-2025

Various strategies to manage diseases of pulse cowpea include use of resistant germplasm, biocontrol agents, biopesticides and chemicals (Ekhuemelo *et al.,* 2019; Modi and Tiwari, 2020; Enyiakwu *et al.,* 2021). Although, negligible data are available on the extent and nature of damage caused by these diseases and pests in fodder cowpea in different regions. Quantifying the extent of losses attributed to diseases and pests is essential. Thus, the present study was focused to determine yield losses due to leaf blights, root rots, mosaics, sucking pests and defoliators in fodder cowpea in North-Western regions of India.

MATERIALS AND METHODS

Study areas/experimental sites

The field trials were conducted during the rainy seasons for three years from 2019 - 2021 as part of the All India Coordinated Research Project (AICRP) on Forage Crops and Utilization in five regions of India in cowpea. The study locations were PAU, Ludhiana in Punjab state possesses a humid and subtropical climate. CSKHPKV, Palampur in Himachal Pradesh is a mid-hill region with a sub-humid climate accompanied by high rainfall, OUAT, Bhubaneswar in Odisha possesses tropical climatic conditions, MPKV, Rahuri in Maharashtra is in semi-arid region with tropical climatic conditions and IGFRI, Jhansi in Uttar Pradesh is characterized by hot and semi-humid climate (Fig 1).

Field trials

The experiments were laid out in a randomized complete block design (RCBD) with two treatments per location in seven replications. The plot size was 5× 5 m^2 and seeds of fodder cowpea variety "Bundel Lobia-1" were sown in rows 30cm apart at the rate of 525 g per treatment (75 g per plot). The trials were conducted by following the region-specific package of practices for cultivating fodder cowpea. The location wise details of all treatments are given in Table 1. Two foliar sprays were given with first spray immediately after disease appearance/pest attack and second spray was given at 15 days interval after the first one. The foliar sprays were applied with hand-operated knapsack sprayer with the dose of each fungicide and amount of water as mentioned in Table 1.

Disease assessment

The severity of root rot, cowpea mosaic virus, *Cercospora* leaf spot and anthracnose was assessed in each plot in 10 tagged cowpea plants. The percentage of diseased leaf area was assessed visually to rate the severity on cowpea plants and scoring was carried out after every foliar spray treatment at 35 and 55 days after sowing. The disease severities of cowpea yellow mosaic virus (YMV) were recorded on a 1-9 scale (Singh *et al.*, 1988), *Cercospora* leaf spot and anthracnose on a 5-point scale (Oladiran and Oso 1983). The recorded severities were

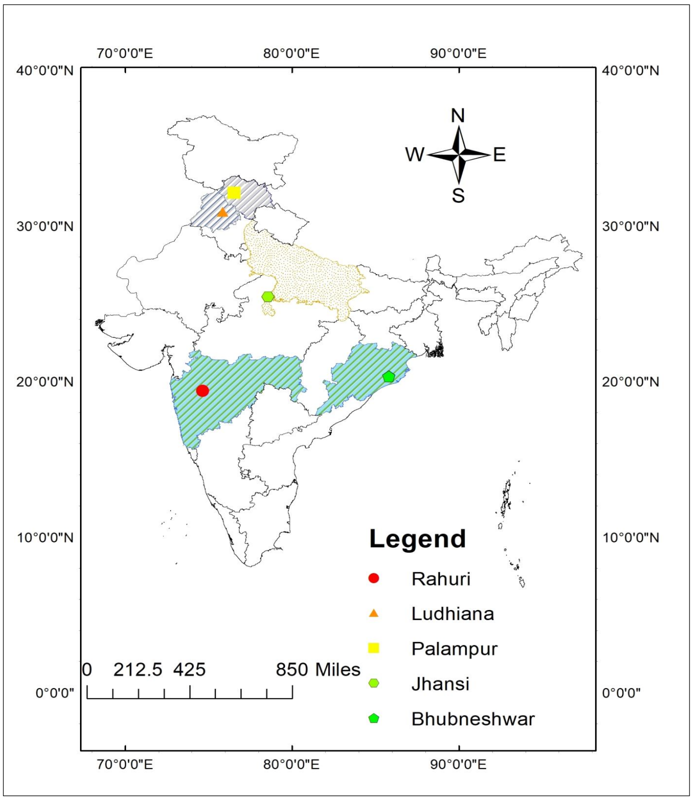

Fig 1: Map of India depicting the locations (states) of which data were considered for calculation of yield losses given as legends.

converted to percent disease severity index by using the given formula:

Disease severity index (%) =

$$\frac{\text{Sum of all ratings}}{\text{Total number of plants observed} \times \text{Maximum rating scale}} \times 100$$

Disease incidence (%) of root rot was calculated by counting diseased and healthy plants in each of the quadrants and the incidence value obtained using the formula below:

Disease incidence (%) =

$$\frac{\text{Number of diseased plants}}{\text{Total number of plants}} \times 100$$

Pest assessment

Observations on the incidences of aphids and defoliators were recorded on tagged plants. The sucking pests and defoliators were assessed based on the intensity of infestation (Kogan and Pitre, 1980). The aphid population was estimated using direct counts per stem or twig and the intensity of infestation on a 0-4 scale (Jayappa, 1984).

Mean infestation index =

$$\frac{\text{Sum of numerical ratings} \times \text{Grade value}}{\text{Number of plants}}$$

Estimation of green fodder yield and yield losses

The cowpea crop was harvested at 65 days after sowing and green fodder yield (GFY) (q ha^{-1}) was recorded separately in protected and unprotected treatments. The green fodder yield losses were estimated by following the formula of Horn *et al.* (1995).

Yield loss (%) =

$$\frac{\text{Yield (q/ha) in protected treatment} - \text{Yield (q/ha) in unprotected treatment}}{\text{Yield (q/ha) in protected treatment} -} \times 100$$

Data analysis

All experimental data on different factors obtained from different regions were analyzed with SAS software (version 9.3) using analysis of variance (ANOVA) to assess the variability in yield losses due to diseases, pests and locations between the experimental repeats ($p \leq 0.05$). Means were compared using Fisher's Least Significance Difference (LSD) test ($\alpha \leq 0.05$).

RESULTS AND DISCUSSION

Impact of treatments on different diseases of cowpea

Incidence of root rot disease caused by *Rhizoctonia solani* presented in Table 2 was recorded in three geographically different locations during 2019, 2020 and 2021 (Fig 2).

(a) (b)

Fig 2: (a) Field view of root rot disease infecting cowpea and (b) Cowpea seedlings infected with root rot pathogen.

Table 1: Details of treatments.

Tr. no.	Details of treatment
T_1	Protected
	Seed treatment with tebuconazole 2DS @ 1 g/kg seed + Neem seed kernel powder (50 g/kg seed)
	For management of root rot and foliar diseases (anthracnose and leaf blight) of forage cowpea:
	➢Two foliar sprays of propiconazole @ 0.1% at 15 days interval.
	For management of defoliators in forage cowpea:
	➢Two foliar sprays of *Beauveria bassiana* @ 5 g/L (1×10^7 cfu/mL)
	For management of sucking pests and yellow mosaic virus incidence:
	➢Two sprays of imidacloprid 17.8 SL @ 0.3 ml/L at 15 days interval followed by two sprays of *Verticillium lecani* @ 5 g/L at 10 days interval.
T_2	Unprotected

Table 2: Disease incidence/ severity (%) of different diseases infecting cowpea and aphid population on cowpea from 2019 to 2021.

Treatments	Disease Incidence of root rot (%)**			Disease severity of YMV (%)			Severity of foliar diseases (%)			Aphids (number of aphids per twig)	
	Bhubaneswar	Ludhiana	Palampur	Rahuri[1]	Ludhiana	Palampur	Rahuri[2]	Palampur[3]	Bhubaneswar[3]	Rahuri	Bhubaneswar
T_1	4.34	1.39	14.29	0.69	6.69	1.10	0.75	10.90	12.69	4.69	3.93
	(11.96)*	(6.73)	(22.17)	(1.30)	(3.93)	(1.44)	(4.96)	(19.23)	(20.79)	(12.43)	(11.36)
T_2	20.40	6.25	50.24	1.57	14.49	4.33	1.31	33.05	40.40	27.41	30.26
	(26.80)	(14.46)	(45.12)	(1.60)	(2.77)	(2.31)	(6.54)	(35.06)	(39.43)	(31.38)	(33.33)
LSD at 5%	1.714	0.692	3.734	0.257	0.552	0.470	0.228	2.287	2.692	6.751	2.400
SE(m)	0.486	0.196	1.058	0.073	0.156	0.133	0.065	0.648	0.763	1.914	0.680
CV (%)	10.388	13.571	8.680	17.002	3.908	12.991	16.611	7.805	7.606	31.552	10.527

*Values in parenthesis are arc sine transformed;**Mean of three years; 1Values in parenthesis are square root transformed; 2Cercospora leaf spot; 3Anthracnose.

The perusal of results indicated the significant differences in plots protected with fungicides or biocontrol agents and un-protected treatments with respect to cowpea root rot incidence. Least mean root rot incidence was observed at Bhubaneswar (4.04%) followed by Palampur (14.29%) as compared to un-protected plots that is 20.40 and 50.24 % respectively. However, at Ludhiana, root rot incidence was recorded in 2019 only and showed insignificant difference among the treated and untreated treatments.

Disease severity of yellow mosaic virus was assessed at three locations namely Rahuri, Ludhiana and Palampur (Table 2, Fig 3). Average minimum severity was recorded at Rahuri that is 0.69%, Palampur (1.10%) in protected plots as compared to un-protected ones that showed 1.57 and 4.33 % severity of yellow mosaic disease respectively during three cropping seasons. Also exhibited significant differences among both the treatments at $p<0.05$. Although at Ludhiana location, the yellow mosaic virus severity was higher (6.69%) than other locations but significantly lower than untreated plots (14.49%).

Two foliar diseases such as *Cercospora* leaf spot and anthracnose were observed at three locations on cowpea crop (Table 2, Fig 4). At Rahuri and Palampur, *Cercospora* leaf spot and at Bhubaneswar, anthracnose was prevalent in fodder cowpea. *Cercopsora* leaf spot severity was less at Rahuri in both the treatments (0.75 and 1.31%). Whereas, at Palampur, it was 10.90 and 33.05 % in protected and un-protected plots. At Bhubaneswar, anthracnose severity was 12.69% in protected and 40.40% in un-protected plots.

Defoliators and aphids

Aphid population was assessed in two locations Rahuri and Bhubaneswar on cowpea twigs (Table 2, Fig 5). Mean maximum number of aphids per twig on cowpea were found at Rahuri (4.69) on cowpea plants protected with biocontrol agent *Verticillium lecani* and 27.41 aphids on un-protected plants. At Bhubaneswar, average number of aphids were 3.93 and 30.26 per twig in treated and untreated plots respectively.

Damage caused by defoliators were found significantly less in plots protected with biocontrol agent *Beauveria bassiana* than un-protected plots (Fig 6 and 7). Lowest mean infestation by defoliators was recorded at Rahuri 1.31% against 3.55% in untreated plots followed by Bhubaneswar that is 1.61 and 4.94 % in treated and untreated treatments. At Ludhiana and Palampur locations, 2.10 and 4.86 percent damage was noticed in protected plots than un-protected plots (5.71 and 13.5%) respectively. Highest mean damage caused by defoliators was spotted at Jhansi (27.88%) in plots protected with *B. bassiana* than un-protected ones (42.36%). Foliar application of biocontrol agent provided significantly lesser percent infestation caused by defoliators in cowpea.

Impact of diseases and pests on green fodder yield losses

A significant effect of fungicides/pesticides and biocontrol agents on green fodder yield was noticed at all the test locations during three crop seasons with a significant increase in green fodder yield (Table 3). Highest mean green fodder yield was found at Ludhiana (535.3 q ha^{-1}) whereas at Bhubaneswar and Rahuri the green fodder yield was 346.7 and 316.1 q ha^{-1} respectively as compared to untreated plots that is 263.3 and 244.5 q ha^{-1}.

The incidence of diseases and insect-pests showed significant ($p<0.05$) effects on green fodder yield losses at Rahuri, Jhansi and Palampur except Ludhiana and Bhubaneswar where it was insignificant (Table 4). Maximum yield losses due to root, rot, YMV, foliar diseases and defoliators were 46.21 and 42.04% in 2020 and 2021 at Palampur. Whereas lowest losses due to diseases and pests were observed at Ludhiana during all three crop seasons that is 18.21, 11.06 and 16.38% due to root rot, YMV and defoliators. Yield losses were high at Jhansi in 2019 (25.20%) due to defoliators, at Rahuri in 2021 (29.19%) due to root, rot, YMV, foliar diseases, aphids and defoliators and at Bhubaneswar in 2020 (25.24%) due to root, rot, foliar diseases, aphids and defoliators respectively. Hence, average green fodder yield losses due to diseases and pests were higher at Palampur and Rahuri.

Relationship between diseases, insect-pests and yield loss

The correlation was found negative/positive and highly significant at p=0.01 and p=0.05 between disease severity, pest damage values and green fodder yield losses at all five test locations (Table 5). At Ludhiana, correlation was found negatively significant between YMV and root rot that

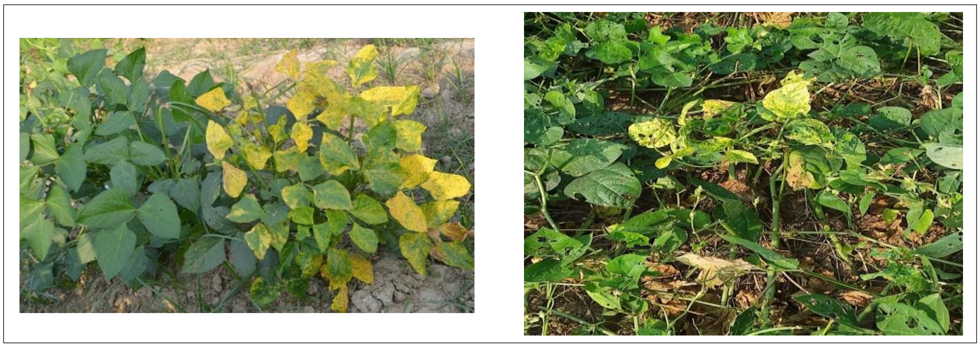

Fig 3: Cowpea plants infected with cowpea mosaic virus disease.

Fig 4: Cowpea anthracnose symptoms on plants and fields view of cowpea plants infected with anthracnose disease.

Fig 5: Infestation of aphids on cowpea plants.

is -0.634 ($p<0.05$) and -0.872 ($p<0.01$), whereas, for defoliators correlation was highly positive and significant ($p<0.0001$) r=0.980. The value of R^2 calculated was 0.402, 0.761 and 0.961 for YMV, root rot and defoliators respectively which indicated 40-96% variation in yield was due to incidence of YMV, root rot and defoliators. Likewise, at Rahuri, strong positively significant relationship was noticed between YMV, foliar diseases, defoliators, aphids and yield losses. The values of R^2 revealed that defoliators and diseases contributed >98% towards variation in GFY losses. At Bhubaneswar, a strong positive and significant relationship was found between aphids and yield losses (r=0.984) at $p<0.05$. A negative significant correlation was seen between defoliators and yield loss (r=-0.947) at $p<0.01$

Fig 6: Infestation of cowpea defoliators on the plants of cowpea.

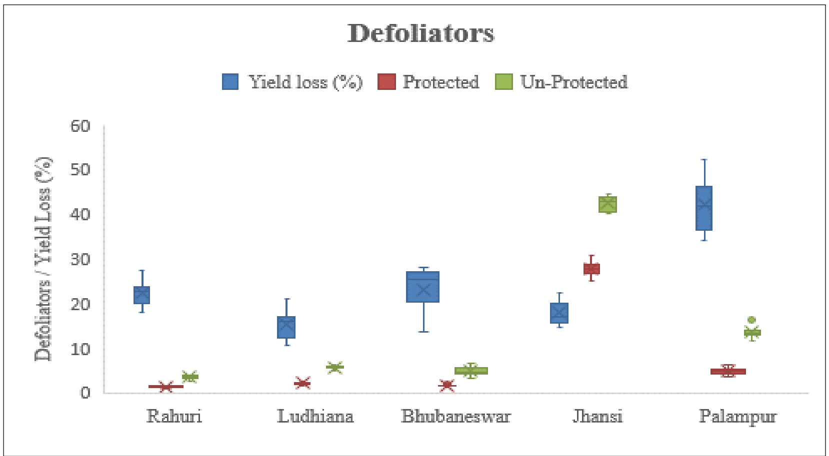

Fig 7: Effect of treatments on damage caused by defoliators with green fodder yield loss in cowpea at five locations.

Table 3: Effect of treatments on green fodder yield of cowpea from 2019 to 2021 at five locations.

Treatments	Mean green fodder yield (q/ha)				
	Rahuri	Ludhiana	Bhubaneswar	Jhansi	Palampur
T_1	316.19	535.35	346.70	154.00	169.57
T_2	244.58	453.07	263.38	126.19	97.52
LSD at 5%	13.064	18.682	20.120	4.783	13.180
SE(m)	3.703	5.296	5.703	1.356	3.736
CV (%)	3.494	2.835	4.947	2.561	7.402

Table 4: Impact on yield loss (%) from 2019 to 2021 at five locations.

Treatments	Yield loss (%)				
	Rahuri	Ludhiana	Bhubaneswar	Jhansi	Palampur
2019	16.94	18.21	22.08	25.20	37.76
2020	20.69	11.06	25.24	10.14	46.21
2021	29.19	16.38	22.06	18.50	42.04
LSD at 5%	7.670	NS	NS	6.119	5.510
SE(m)	2.462	2.758	3.918	1.964	1.769
CV (%)	29.243	47.959	44.821	28.958	11.140

Table 5: Correlation and Regression coefficient between yield loss and disease severity/incidence of diseases and insect-pests.

Location	YMV*	Root rot	Foliar diseases	Defoliators	Aphids
Ludhiana	Y = 23.33-0.857PDS r = -0.634 (<0.05)** R^2 = 0.402	Y = 35.55-1.759 PDS r = -0.872 (<0.01)** R^2 = 0.761	NP	Y = -4.882+0.359 PDS r = 0.980 (<0.0001)** R^2 = 0.961	NP
Rahuri	Y = -1.241+0.0867 PDS r = 0.987(<0.05)** R^2 = 0.975	NP	Y = -1.135+0.0849 PDS r = 0.958 (<0.05)** R^2 = 0.919	Y = 2.0343-0.0325 PDS r = -0.339 (<0.05)** R^2 = 0.115	Y = -6.673+0.51 PDS r = 0.912 (<0.05)** R^2 = 0.832
Bhubaneswar	NP	Y = 0.869+0.150 PDS r = 0.886 (<0.01)** R^2 = 0.786	Y = 9.253+0.1487 PDS r = 0.607 (<0.01)** R^2 = 0.369	Y = 2.526-0.039 PDS r = -0.947 (<0.01)** R^2 = 0.896	Y = 1.019+0.126 PDS r = 0.984 (<0.01)** R^2 = 0.969
Palampur	Y = 3.232-0.050 PDS r = -0.983 (<0.01)** R^2 = 0.967	Y = 33.44-0.456 PDS r = -0.950 (<0.05)** R^2 = 0.902	Y = 21.426-0.250 PDS r = -0.528 (<0.05)** R^2 = 0.279	Y = 16.879-0.286 PDS r = -0.809 (<0.01)** R^2 = 0.654	NP
Jhansi	NP	NP	NP	Y = 48.609-1.155 PDS r = -0.921 (0.410)** R^2 = 0.847	NP

*Average of three years, **t-test F-Probability, NP- Not present, PDS- Per cent disease severity, r- Pearson's correlation coefficient, R^2- Coefficient of determination.

with R^2 values of 0.969 and 0.896 respectively. Similarly, a strong positive correlation was detected between root rot and yield loss that is r=0.886 ($p<0.01$) with R^2 value of 0.886. For foliar diseases it was 0.607 ($p<0.01$) and R^2 value was 0.369. At Palampur, a high strong negative and significant relationship was detected between YMV and yield losses (r=-0.983) $p<0.01$ with R^2 value of 0.967 followed by root rot and defoliators that is r=-0.950 ($p<0.05$) and r=-0.809 ($p<0.01$) with 0.902 and 0.654 values of R^2. A negative and significant relation was observed between foliar diseases and yield losses r=-0.528 $p<0.05$). The value of R^2 indicated 65 to 95% variation in GFY was due to YMV, root rot and defoliators. Likewise, at Jhansi, defoliators showed non-significant negative relation with GFY losses (r=-0.921) and value of R^2 is 0.847.

The present study provides an insight into GFY losses caused by major location specific diseases such as YMV, root rot, *Cercospora* leaf spot, anthracnose and pests like aphids and defoliators at five geographically different Indian test locations. Similar studies have been described to assess the losses due to diseases and pest incidence in pod and seed yield of cowpea (Baoua *et al.,* 2021). However, scanty reports are available with respect to the losses in green fodder yield of fodder cowpea. High severity of cowpea mosaic increased the seed yield losses to more than 64% as recorded by Neya *et al.* (2015). Ganiyu *et al.* (2018) observed 23.67-46.67% incidence of anthracnose in plots treated with neem plant extracts as compared to control (80%) with more than 40% loss in pod number and seed yield. In the conducted studies, root rot incidence and foliar disease severity were ranged between 1.39 to 50.24% and 0.75 to 40.40% respectively in fodder cowpea. In this context, several workers have demonstrated the effect of plant extracts against anthracnose pathogen infecting cowpea (Lemos de Silva *et al.,* 2015; Enyiukwu *et al.,* 2021). Application of fungicides belonging to group triazoles have been found effective against anthracnose with 16.94% disease severity (Dabbas *et al.,* 2015). The previous reports suggesting 3.59-9.43% pod damage by defoliators in cowpea (Nghia and Srivastava, 2015). The losses in yield due to sucking pests like *A. craccivora* and *Bemisia tabaci* were observed in the range of 35% in cowpea by Anandmurthy *et al.* (2020). Our study also found that the use of BCAs namely *B. bassiana* and *V. lecanii* against sucking pests and defoliators followed by foliar spray of imidacloprid reduced the damage caused by these pests in cowpea. Swarnalata *et al.* (2015) noticed that the application of imidacloprid and *V. lecanii* to be the most effective treatments against cowpea aphids. Additionally, Saranya *et al.* (2010) and Sahayaraj and Namachivayam (2011) used *V. lecanii* fungus for spraying the cowpea crop for the management of aphids. Ozdemir *et al.* (2020) evaluated the two BCAs *Metarhizium anisopliae* and *Beauveria bassiana* against cowpea weevil and suggested that both the biocontrol agents showed reduction in aphid population. As presented in results, the yield loss caused by root rot, YMV, foliar diseases, aphids and defoliators were ranged between 11.06 to 46.21% over three seasons and five locations. These results confirmed that cowpea yield was reduced due to the attack of insects and caused yield losses over 90% (Raheja, 1976). Booker *et al.* (2005) noticed more than 66% yield loss in cowpea due to cowpea mosaic virus.

Disease and pest incidence was positively or negatively and significantly ($p<0.05$) correlated with GFY loss in all the locations. Nabirye *et al.* (2003) demonstrated the effect of thrips injury on cowpea and reported a significant negative relationship between density of thrips and cowpea yields. Also, detected more than 63% losses in cowpea yield as a result of thrips attack. Odulaja and Oghiakhe (1993) worked out the yield loss caused by *Maruca testulalis* pod borer in cowpea by using crop loss model. Alghali (1992) reduced the losses caused by thrips, sucking bugs of cowpea and recorded 50-100% reduction in the yield. According to our results, diseases and pests were both negatively and positively significantly correlated with yield losses. While according to Baoua *et al.* (2021) the correlation between predominant diseases (root rot, anthracnose and leaf spot), pests (*Aphis craccivora* and *Maruca vitrata*) and yield was significantly negative. In the study of Harouna *et al.* (2018), the cowpea defoliator was found responsible for reducing the yields by 17.5-26.5%. Similarly, losses caused by pod borers have been estimated between 20 and 82% (Zakari *et al.,* 2019). Likewise, yield losses due to aerial blight, anthracnose and phoma blight in soybean were 41.0, 64.8 and 51.7% respectively (Pawan *et al.,* 2023; Manzoor *et al.,* 2023).

CONCLUSION

The present study provides estimates of green fodder yield losses due to diseases and pests in fodder cowpea. It also suggested lower incidence of diseases and pests in plots protected with fungicides and biocontrol agents than unprotected plots. The green fodder yield losses increased with increase in severity of diseases or incidence of pests and result in huge green fodder yield reduction. The correlation was found negative/positive and highly significant between disease severity, pest damage values and green fodder yield losses at all the test locations. Regarding the management of diseases and pests, the focus should be on timely application of management measures.

ACKNOWLEDGEMENT

The authors thank Indian Council of Agricultural Research (ICAR) for its support through AICRP on Forage Crops and Utilization.

Authors' contributions

N. R. Bhardwaj designed the experiment. Ashlesha Atri, D. K. Banyal, Sandip Landge and Arabinda Dhal conducted the field experiments for three years. Ashlesha, D. K. Banyal, Harpreet K. Cheema and N. R. Bhardwaj collected

the data. Ashlesha Atri compiled, analyzed the data and wrote the manuscript. Devinder Pal Singh reviewed the manuscript.

Data availability

The datasets used during the current study are available from the corresponding author on reasonable request.

Declarations

Funding

The study was not funded by any agency.

Compliance with ethical standards

The manuscript has not been submitted elsewhere.

Human and animal rights

This study did not involve human participants and/or animals.

Informed consent

All authors consent to this submission.

Conflict of interest

The authors declare that they have no conflict of interest.

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