EFFECTS OF FUNGICIDE APPLICATION FREQUENCY ON LATE LEAF SPOT SEVERITY, YIELD AND SOME PHYSIOLOGICAL PARAMETERS OF GROUNDNUT.

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SUMMARY

In 2016 cropping season, a trial was conducted to evaluate fungicide application frequency in the management of late leaf spot of groundnut induced by *Phaeiosariopsis personata* (Berk, & M. A. Curtis van Arx) in Samaru, North-West Nigeria. SAMNUT – 14, a variety of groundnut susceptible to the disease was subjected to five different frequencies [plants sprayed weekly (T_1), plants sprayed biweekly (T_2), plants sprayed every three weeks (T_3), plants sprayed every four weeks (T_4) and unsprayed plants (T_5)] of fungicide application. The experiment was in a Randomized Complete Block Design (RCBD) with three replications established at the Institute for Agricultural Research (IAR) Farm Samaru, Zaria. Natural infection in the field was relied upon as the source of inoculum. The parameters recorded were stand count at emergence, and at harvest, disease severity at 56, 63, 70, 77, 84, 91 and 98 Days After Sowing (DAS) based on 1 – 9 scale, Area under Disease Progress Curve (AUDPC), percent defoliation, pod and haulm yields, crude protein content and chlorophyll content were also computed. The results showed that spraying weekly was found to be most effective in reducing disease with minimum disease severity of 33.33 % and improved pod yield by 47.91 %. Biweekly application of the fungicide was also found to be effective with decrease in disease severity by 56.41 % and increase of 45.65 % in yield over untreated; and was most economical with cost: benefit ratio of 1:69.6. Crude protein and chlorophyll contents increased by 29.45 % and 78.57 % in the treated plants respectively compared to the untreated. It can be concluded from this study that spray of mancozeb + carbendazim at

biweekly interval was the best economical management option for late leaf spot of groundnut.

Keywords: Groundnut, *Phaeoisariopsis personata*, fungicide, crude protein, chlorophyll.

GROUNDNUT (*Arachis hypogaea* L.) also known as peanut, earthnut or monkey-nut is a member of the family Fabaceae and is used for human food and livestock feed (18). It is cultivated in the semi-arid tropical and sub-tropical regions of the world, mainly in developing countries of Africa and Asia (8). Groundnut is a key crop for small scale farmers especially in Africa and Asia where the crop serves as a valuable source of dietary protein, oil, and fodder for livestock. It contains 48-50% oil, 26-28 % protein, and a rich source of dietary fibre, minerals (calcium, potassium, phosphorus, magnesium) and vitamins (8). The crop is cultivated annually on about 24.63 million hectares worldwide with annual production of 41.27 million tons in shell and productivity of about 1.85 t ha^{-1} (4).

Groundnut yield and quality are severely constrained by a wide range of fungi, bacteria, viruses, and nematodes. Among the fungal diseases, early leaf spot (*Cercospora arachidicola* Hori) and late leaf spot (*Phaeiosariopsis personata* Van Arx) are the most prevalent, and occur throughout all groundnut growing regions (11). Late leaf spot is widely distributed throughout the world and can lead to yield loss of up to 80 % (13). It causes severe defoliation and reduces pod yield by more than 50 % if the crop is not protected with fungicides (19). The fungus penetrates leaf cells and withdraws their contents causing the cells to collapse and die, forming spots. Late leaf spot lowers yield by reducing the green leaf area available for photosynthesis leading to reduction in crude protein and chlorophyll contents and by stimulating abscission and extensive defoliation. Where fungicide application is normal practice for control during the crop season, pod yield losses are estimated at around 10%. However, much of the semi-arid tropics where fungicides are rarely used, losses in excess of 50% are common (16). It is therefore important that effective management of the disease be developed and applied (11). As a result, use of fungicide is the best alternative for effective management of the disease (11). Varying fungicide application frequency at suitable time of application will help reduce disease development thereby increasing pod and haulm yields.

The objective of the study was to assess the effects of late leaf spot on crude protein of seeds and chlorophyll contents of leaves of groundnut and use of fungicide application frequencies to manage the disease.

MATERIALS AND METHODS

Experimental site

The study was conducted in the Institute for Agricultural Research (IAR) Farm, Ahmadu Bello University Samaru, Zaria, situated at 11° 10ˈN, 07° 38ˈE and 685 m above sea level in the Northern Guinea Savanna zone of Nigeria.

Determination of the effects of frequency of fungicide application on severity of late leaf spot.

The experiment was established during 2016 rainy season at IAR Farm, the field was ploughed, harrowed and ridged at 0.75 m spacing before setting out the experimental plots. Two hundred and fifty gram (250 g) seeds of the groundnut variety were treated with metalaxyl 20 % + imidacloprid 20 % (Allstar®, 40 DS Jiangsu Kesheng Group Co., Ltd., China) at the rate of 10 g/ 4 kg seeds before sowing to protect seeds against soil-borne pests and damping off diseases to ensure seedling establishment. The groundnut seeds of SAMNUT 14 variety were sown in plots of four ridges 3 m long with 20 cm intra-row spacing. Two unplanted ridges between the plots and 2 m alleys between plots were left to minimize spray drift. The experimental field was 0.12 ha. Supplementary hoe weeding was done on 18th July, 2016 and 2nd August, 2016.

The variety, SAMNUT 14 which is known to be susceptible to late leaf spot (6) was subjected to five different frequencies (T_1, T_2, T_3, T_4 and T_5) of Mancozeb + Carbendazim application. The frequencies represented five (5) treatments. The treatments were laid out in a Randomized Complete Block Design (RCBD) and replicated three times.

Mancozeb + Carbendazim was applied using a knapsack sprayer at the rate of 2 kg ai/ha according to manufacturer's recommendation, starting at 60 days after sowing (DAS). Five randomly selected plants in each plot were tagged for data collection.

Data collected/calculated:

1. Stand count at emergence was taken at seven (14) days after sowing and stand count at harvest taken at three (3) days before harvest.
2. Disease severity scores were taken weekly using the scale described in Table 1 starting at 60 DAS to one week before harvest. The disease severity was computed using the

following formula as cited by Marley (12):

$$\text{Disease severity} = \frac{\text{sum of all disease ratings}}{\text{Total no. of plants assessed X Maximum disease score}} X\ 100$$

3. Disease severity values were used to calculate Area under disease progress curve (AUDPC) using the following formula adopted from Bharat *et al*. (3):

$$AUDPC = \sum_{i=1}^{n-1} \frac{Y_i + Y_{i+1}}{2} \times (T_{i+1} - T_i)$$

Where:
Yi = Disease severity (percent) at ith observation
Ti = Time (days) at ith observation
n = Total number of observations

Table 1: Description of the 9 – point scale for late leaf spot assessment

Description	Score	Disease Severity (%)
No disease	1	0
Lesion present largely on lower leaves; no defoliation	2	---+1 - 5
Lesion present largely on lower leaves; very few lesions on Middle leaves; defoliation of some leaflets evident on lower leaves.	3	6 - 10
Lesions are present on lower and middle leaves but severe on lower leaves; defoliation of some leaflets evident on lower leaves	4	11 - 20
Lesions are present on all lower and middle; 50% defoliation of lower leaves	5	21 - 30
Lesions severe on lower and middle leaves; lesions present ontop leaves but less severe; extensive defoliation of lower leaves; defoliation of some leaflets evident on middle leaves.	6	31 - 40
Lesions present on all lower leaves but less severe on top leaves; defoliation of all lower and some middle leaves.	7	41 - 60
Defoliation of all lower and middle leaves; lesions severe on top leaves and some defoliation of top leaves evident.	8	61 - 80
Defoliation of almost all leaves leaving bear stems; some leaflets may be present but severe leaf spots.	9	81 - 100

Subrahmanyam *et al*. (20)

4. Percent defoliation: Total number of leaflets and fallen leaflets on the main stem were counted at 90 DAS. Percent defoliation was calculated using the formula:

$$\text{Percent Defoliation} = \frac{\text{Number of fallen leaflets}}{\text{Total number of leaflets}} \text{ x } 100$$

5. Lesion number (total number of lesions on five randomly selected leaves)
6. Lesion size (measuring the diameter of five randomly selected lesion and the average determined) at 90 DAS.
7. Number of pods per plants for five selected plants per plot.
8. Pod and haulm yields
9. Nitrogen content of the samples was used to determined Crude Protein content of Groundnut Seeds using the formula:

$$\text{Nitrogen content of sample (\%)} = \frac{\text{volume of acid (ml) X Normality of standard acid}}{\text{weight of sample}} X\ 0.014\ X\ 100$$

Crude protein content (%) = nitrogen content × 6.25.
Where, 0.014 and 6.25 are constants.

10. Determination of chlorophyll content in leaves:

Extraction of chlorophyll Twenty – five (25) fresh leaf samples of SAMNUT 14 were collected randomly at 90 DAS from each plot to determine the chlorophyll content. From each sample, 1 g of the fresh leaves was taken and ground using laboratory mortar and pestle, 20 ml of 80 % acetone was added to the grounded leaves. It was then centrifuged at 10000 rpm for 5 minutes. Supernatant was collected, centrifuged till the residue becomes colourless. Absorbance of the solution was read at 645 nm and 663 nm against the solvent (acetone) blank.

Calculation of chlorophyll content The following formula adopted from Jyosthna *et al.* (9) was used to calculate the total chlorophyll, chlorophyll 'a' and chlorophyll 'b' and results were expressed as 'mg' of chlorophyll / g of fresh weight (mg/g).

Total chlorophyll = (20.2 x OD at 645 nm) + (8.02 x OD at 663 nm) x df
Chlorophyll 'a' = (127 x OD at 663 nm) – (2.69 x OD at 645 nm) x df
Chlorophyll 'b'= (22.9 x OD at 645 nm) – (4.68 x OD at 663 nm) x df
Where,
OD = Optical Density;
df = dilution factor.

11. Determination of cost – benefit analysis for using different application frequencies of fungicide to manage late leaf spot of groundnut.

The cost and benefit of using different application frequencies for the management of late leaf spot of groundnut during the 2016 cropping season was quantified. The cost: benefit ratio of each treatment was derived by subtracting the income of the untreated from the net income of each sprayed treatments and the products were divided by total cost of management for each treatment as described by Shabozoi *et al*. (17):

$$\text{Cost: benefit ratio} = \frac{\text{net income treatment} - \text{net income untreated}}{\text{cost of disease management}}$$

Data analysis

Data collected were subjected to analysis of variance (ANOVA), using SAS software version 9. Means were separated using Least Significant Difference (LSD) at 5 % level of significance.

RESULTS

Table 2 shows the effect of fungicide application frequency on stand count and yields of SAMNUT 14, 2016. The unsprayed plants (T_5) had the highest stand count at emergence (48.67) which was statistically similar to plants sprayed biweekly (48.33) and every four weeks (46.00), plants sprayed every three weeks recorded the least, but did not significantly differ from weekly and four weeks spray intervals. Plants sprayed biweekly recorded the highest stand count at harvest (47.33), followed by unsprayed plants which recorded 46.67 and did not differ significantly with the other treatments sprayed weekly and every four weeks. Plants sprayed every three weeks had the lowest stand count (40.67) at harvest. The highest pod yield was obtained on plants sprayed weekly which were statistically similar to plants sprayed biweekly and was significantly higher than those sprayed every three and four weeks. Unsprayed plants and plants sprayed every four weeks recorded the lowest yield and were statistically similar. The haulm yields did not differ significantly across all the treatments but plants sprayed weekly (5759.30 kg/ha) recorded the highest haulm yield.

Table 3 shows the effect of fungicide application frequency on late leaf spot disease severity, lesion number, lesion size, percent defoliation and number of pods per plant. Disease severity at 98 DAS was highest in unsprayed plants, followed by plants sprayed every three and four weeks which were statistically similar and recorded 68.99 % and 75.99 % respectively. Plants sprayed biweekly recorded 56.41 % which was higher than those sprayed weekly. The plants sprayed weekly recorded the least severity of 33.33 %. The unsprayed plants had the highest lesion number which was not significantly different from those sprayed every four weeks, but significantly higher ($p \geq 0.05$) than plants sprayed every three, two and one weeks respectively, which were significantly different from each other. The biggest lesion size was recorded in plants sprayed weekly (7.63) which was statistically higher than those sprayed biweekly, every three and four weeks which were all statistically similar. Unsprayed plants had the lowest lesion size. Percent defoliation differed significantly across all the spray frequencies. Plants sprayed weekly had lowest percent defoliation, followed by those sprayed biweekly, every three and four weeks. The highest percent defoliation was observed in unsprayed plants. Plants sprayed weekly produced the highest pod number which was statistically similar to that of plants sprayed biweekly followed by plants sprayed every three and four weeks, the least pod number was recorded for unsprayed plants.

The effect of fungicide application frequency on severity of late leaf spot on groundnut is presented in Figure 1. Plants sprayed weekly recorded the lowest disease severity across all the assessment period which increases with an increase in the frequency of spray, whereas unsprayed plants recorded the highest disease severity. The Figure 2 shows the calculated values for Area under disease progress curve over time. Unsprayed plants recorded the highest area under disease progress curve of (2011.8) followed by plants sprayed every four weeks (1966.58) and those sprayed every three weeks (1779.66) which was higher compared to the plants sprayed biweekly. The least estimate of area under disease progress curve was observed in plants sprayed weekly.

Table 4 shows the effect of fungicide application frequency on crude protein and chlorophyll contents of groundnut seed and leaf. Crude protein and chlorophyll contents increased significantly with an increase in the spray frequency across all the treatments. The highest crude protein and chlorophyll contents were recorded for plants sprayed weekly and the lowest was observed in unsprayed plants.

Table 5 shows the cost and benefit analysis of using different application frequencies for managing late leaf spot on SAMNUT 14. All the sprayed frequencies (T_1 – T_4) had superior financial costs and benefit compared to the untreated (T_5). Groundnut yield in all the treatments resulted in revenue that exceeded the cost of the plants protection even in the unsprayed plots. The highest cost benefit ratio (1:69.6) was obtained from plants sprayed biweekly followed by plants sprayed weekly (1:42.9), plants sprayed every three weeks recorded cost benefit ratio of 1:29.9 and the lowest was observed in plants sprayed every four weeks (1:5.4).

Table 2: Effect of fungicide application frequency on germination, pod and haulm yields of groundnut

Frequency of spray (weeks)	Stand count at emergence	Stand count at harvest	Pod yield (kg/ha)	Haulm yield (kg/ha)
T_1	46.67[ab]	46.33[abc]	888.90[a]	5759.30[a]
T_2	48.33[a]	47.33[a]	851.90[ab]	5611.10[a]
T_3	44.00[b]	40.67[c]	574.10[bc]	5055.60[a]
T_4	46.00[ab]	41.00[bc]	463.00[c]	4481.50[a]
T_5	48.67[a]	46.67[ab]	444.00[c]	4237.00[a]
SE±	1.24	1.81	86.96	527.63

Means with the same superscript in a column are not significantly different at 5 % level of significance ($P \leq 0.05$) using Least Significant Difference (LSD)

T_1 = Plants sprayed weekly, T_2 = Plants sprayed biweekly, T_3 = Plants sprayed every 3 weeks, T_4 = Plants sprayed every 4 weeks; T_5 = Unsprayed plants.

Table 3: Effect of fungicide application frequency on late leaf spot disease severity, lesion number, lesion size, percent defoliation and number of pods per plant

Frequency of spray (weeks)	Disease severity (%) at 98 DAS	Number of lesions	Lesion size (cm)	Percentage defoliation (%)	Number of pods/plant
T_1	33.33[d]	1.60[d]	7.63[a]	6.96[e]	33.33[a]
T_2	56.41[c]	10.60[c]	3.51[b]	14.78[d]	30.33[ab]
T_3	68.99[b]	17.60[b]	3.18[b]	31.64[c]	26.67[bc]
T_4	75.99[b]	34.60[a]	2.91[b]	41.81[b]	25.00[bc]
T_5	87.04[a]	37.00[a]	1.97[c]	49.26[a]	21.33[c]
SE±	2.65	0.79	0.23	2.10	1.99

Means with the same superscript in a column are not significantly different at 5 % level of significance ($P \leq 0.05$) using Least Significant Difference (LSD).

T_1 = Plants sprayed weekly, T_2 = Plants sprayed biweekly, T_3 = Plants sprayed every 3 weeks, T_4 = Plants sprayed every 4 weeks; T_5 = Unsprayed plants.

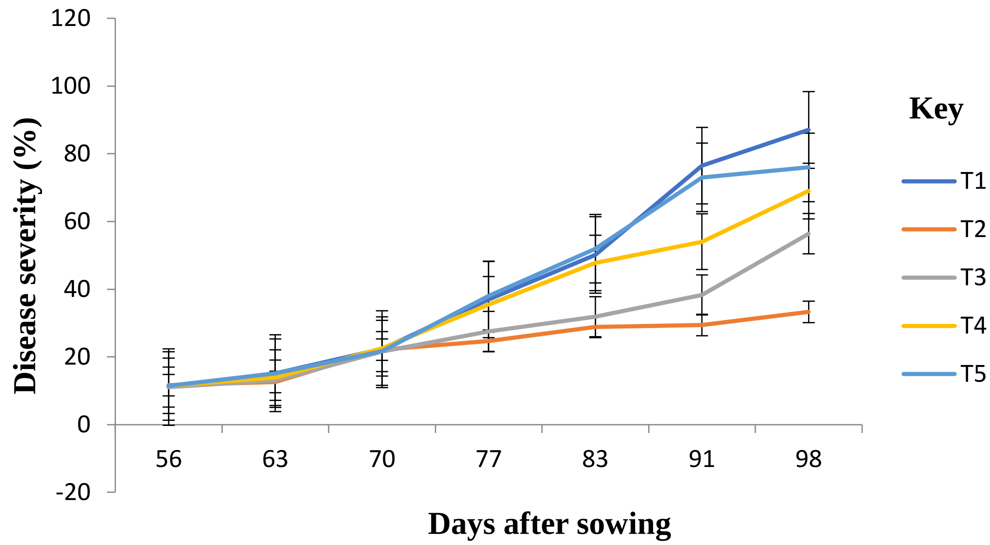

Figure 1: Effect of fungicide application frequency on severity of late leaf spot on groundnut at Samaru, 2016.

T_1 = Plants sprayed weekly, T_2 = Plants sprayed biweekly, T_3 = Plants sprayed every 3 weeks, T_4 = Plants sprayed every 4 weeks; T_5 = Unsprayed plants.

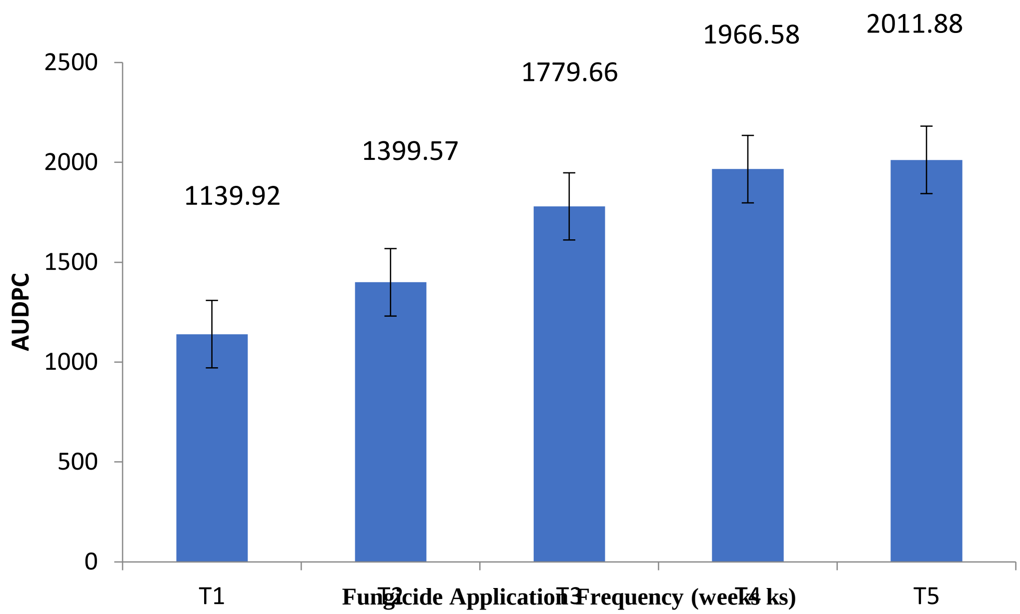

Figure 2: Cumulative area under disease progress curve for varying fungicide application frequency.

AUDPC = Area under disease progress curve.

T_1 = Plants sprayed weekly, T_2 = Plants sprayed biweekly, T_3 = Plants sprayed every 3 weeks, T_4 = Plants sprayed every 4 weeks; T_5 = Unsprayed plants.

Table 4: Effect of fungicide application frequency on crude protein content of groundnut seed and chlorophyll determination

Frequency of spray	Crude protein Content	Chlorophyll 'a'	Chlorophyll 'b'	Total Chlorophyll
T_1	33.18^a	0.90^a	0.50^a	1.40^a
T_2	29.92^b	0.60^b	0.40^b	1.00^b
T_3	28.05^c	0.60^b	0.40^b	1.00^b
T_4	24.69^d	0.40^c	0.20^c	0.70^c
T_5	23.41^e	0.20^d	0.10^d	0.30^d
SE±	0.00	0.86	1.36	1.22

Means with the same superscript in a column are not significantly different at 5 % level of significance ($P \leq 0.05$) using Least Significant Difference (LSD).

T_1 = Plants sprayed weekly, T_2 = Plants sprayed biweekly, T_3 = Plants sprayed every 3 weeks, T_4 = Plants sprayed every 4 weeks; T_5 = Unsprayed plants.

Table 5: Cost and benefit analysis of using different application frequencies for managing late leaf spot on SAMNUT 14

Variables	T_1	T_2	T_3	T_4	T_5
Spray regimes (times)	7	4	3	2	0
(A) Total yield (kg/ha)	888.9	851.9	574.1	463.0	444.4
(B)Total income (₦/ha)	133,335	127,785	86,115	69,450	66,660
(C) Protection cost (₦/ha)	1,515.5	866	649.5	433.0	0.00
(D) Net Benefit (₦/ha) (B - C)	131,819.5	126,919	85,456.5	69,017	66,660
(E)Benefit over untreated (₦/ha)	65,159.5	60,259.0	18,805.5	2,357.0	-
(F) Cost:Benefit Ratio (E/C)	1:42.9	1:69.6	1:28.9	1:5.4	-
ROI (B/C)	87.99	147.56	132.59	160.39	-

ROI = Return on Investment

T_1 = Plants sprayed weekly, T_2 = Plants sprayed biweekly, T_3 = Plants sprayed every 3 weeks, T_4 = Plants sprayed every 4 weeks; T_5 = Unsprayed plants.

DISCUSSION

The increase in frequency of mancozeb + carbendazim spray particularly at weekly and biweekly intervals resulted in a significant increase in pod and haulm yields compared to those sprayed at later weeks. This result agrees with the findings of Naab *et al*. (14) who reported that application of foliar sprays of benomyl was effective in controlling early and late leaf spots and improved groundnut biomass and pod yield by 39 % and 75 % respectively. Ndedu (15) also reported that, irrespective of time spraying commenced, spraying at 7 – 14 days interval resulted in a significantly lower disease severity for both early, late leaf spots and rust than spraying at 21 days interval. Similar results were also obtained for pod and haulm yields.

The study also revealed significant decrease in disease score, fewer lesions, but larger lesion size on leaflet with increase in the frequency of fungicide spray. The fewer lesion number recorded on the groundnut varieties treated with mancozeb + carbendazim shows that the fungicide is efficient in inhibiting the spread of the fungus. This agrees with the findings of Ambang *et al*. (2) who reported that after two or more sprays of METPS and benomyl, there was a significant reduction in the evolution of lesion size. The findings agree with Ndedu (15) who reported that higher rates of mancozeb formulations resulted in lower disease scores and fewer lesions on the leaflet.

The severities of late leaf spot and Area under disease progress curve (AUDPC) also varied in all the spray frequencies evaluated which increases with an increase in severity. This result agrees with Izge *et al*. (7) who in a study found a lot of variability existing among the groundnut varieties evaluated in all characters, probably due to their inherent level of resistance to attack by the pathogen.

The result also showed significant reduction in percent defoliation with increase in the spray frequency. Increase in spray frequency reduces the susceptibility of groundnut to late leaf spot thereby lowering the percent defoliation. This might be due to the failure of the fungus to successfully invade the host tissue, resulting in low infection frequency. This low percentage of leaf damage resulted in low percent defoliation. This finding agrees with Hossain *et al*. (5) who reported that moderately resistant groundnut genotypes to leaf spots and rust had lower percentage defoliation. The study also showed clearly that severe late leaf spot infection caused significant reduction in crude protein and chlorophyll contents in all the five spray frequencies evaluated. This could have been to the interference of the fungus with the photosynthetic activity causing higher number of lesions thereby reducing the net leaf area available for photosynthesis and resulting in reduced chlorophyll contents required for normal synthesis of the various nutrients needed by the plants and consequent reduction in pod yield of the crop. On the other hand, the fungus might have also utilized the protein synthesis by the plants for their growth and development thereby reducing the amount left in the infected leaves. The reduction in protein and chlorophyll contents in the plants due to destruction of leaves by the disease have been reported earlier (1).

Crude protein, chlorophyll ‘a’, chlorophyll ‘b’ and total chlorophyll increased with an increase in the spray frequency of fungicide. This confirmed earlier report by Lalithakumari *et al.* (10) that the effects of systemic fungicide on the physiological response of groundnut plants against early and late leaf spots reduce the disease incidence and increased the protein, total nitrogen and phenols and decrease total sugar contents. Jyosthna *et al*. (9) also reported decrease in total chlorophyll, chlorophyll ‘a’ and chlorophyll ‘b’ due to late leaf spot infection which was more pronounced in susceptible cultivars than the resistant ones.

The cost benefit ratio shows that plants sprayed weekly gave higher yield which resulted in higher income than all the other treatments but had higher cost for plant protection. However, plants sprayed biweekly gave higher cost benefit ratio of 1:69.6 than all the other treatments. The results agree with Shabozoi *et al.* (17) who reported that the highest usable yields of tomato with greater benefit obtained in chlorothanil or mancozeb at 7 and 14 days interval were primarily due to suppression of *Alternaria* spp. and other fruits rots.

CONCLUSION

Use of varieties that are resistant or tolerant and timely application of fungicide are essential for optimum yield in that they play important roles in lowering disease severity and the level of late leaf spot infection. However, biweekly application of the fungicide was found to be effective in reducing disease with minimum value of disease severity of 56.41 % and increase in yield by 45.65 %, crude protein by 29.45 % and total chlorophyll by 78.57 % over unsprayed, and was most economical with cost: benefit ratio of 1:69.6.

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