EFFECT OF SEED TREATMENT ON GERMINATION AND INCIDENCE OF MICROORGANISMS IN SOYBEAN AT TWO TEMPERATURE REGIMES

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SUMMARY

Twelve soybean (*Glycine max* (L.) Merrill) lines were evaluated for germination ability and infection by *Bacillus subtilis* (Ehrenberg) Cohn., a bacteria, and total fungi at 30° and 38°C when the seeds were treated with antibiotic (streptomycin sulphate) and/or fungicide (Vitavax 200-FF). Non-treated seeds served as controls.

On the whole, seed treatments streptomycin sulphate, vitavax 200-FF, and combination of both did not improve germination percentages. However, seed treatments streptomycin sulphate and combination of streptomycin sulphate and vitavax 200-FF respectively, significantly improved the germination percentages in soybean cultivars "Centennial" and "Essex" at 38°C. At 30°C all three seed treatments significantly increased the percentage germination of the advanced breeding line N77—179, which initially had low germination.

Streptomycin sulphate lowered the percentage of seed infected by *Bacillus subtilis* at 38°C significantly meanwhile vitavax 200–FF significantly reduced the fungal infected seed at 30°C. However, the proportion of streptomycin sulphate treated seed infected with fungi was significantly greater at both temperatures and the incidence of *Bacillus subtilis* at both temperatures was higher on seed treated with vitavax 200–FF.

The presence of *Bacillus subtilis* increased and germination percentage declined significantly with increased incubation temperature from 30° to 38°C.

INTRODUCTION

The production of high quality soybean seed is not always possible in the sub-tropics and tropics and also in temperate latitudes. Seed-borne microorganisms perhaps are one of the major factors directly involved in the production of poor quality seed in soybeans (Sinclair, 1982). They can inhibit germination and thereby affect stand establishment. Their association with seed also leads to seed deterioration. Nineteen fungal species and one species of bacteria, *Bacillus* sp. were found in association with soybean seeds in India (Singh *et al.*, 1973). *Bacillus subtilis* and 38 genera of fungi were isolated from seed lots of 16 soybean cultivars in Ethiopia (Mengistu and Sinclair, 1979). The occurrence of the fungus *Phomopsis* spp. and *Bacillus subtilis* in soybean seed lots produced in Illinois state in U.S.A. was also documented (Floor, 1977; Sinclair, 1978).

A temperature of 30°C was reported as approximate optimum for soybean seed germination (Delouche, 1952). However, in one case as the temperature increased from 20° to 40°C the occurrence of *Bacillus subtilis* in soybean seeds increased and germination decreased (Tenne *et al.*, 1977). The similar pattern of behaviour was noticed in soybean seeds inoculated with a fungus, *Aspergillus flavus*, when the temperature was raised from 20° to 30°C (Dhingra *et al.*, 1973). A high percentage germination was obtained when soybean seeds were treated to protect the decay by seedborne bacteria (Ellis *et al.*, 1977; Hepperly and Sinclair, 1977; Schiller *et al.*, 1980) or fungi (Trika and Shanmugasundram, 1980).

The research reported here was conducted to assess the germination ability and the incidence of *Bacillus subtilis* and total fungi in the seed lots of 12 soybean lines at optimal 30°C and above optimal 38°C temperatures when the seeds were treated with antibiotic and / or fungicide.

MATERIALS AND METHODS

Seeds of 12 advanced breeding lines and cultivars were germinated at 30° and 38°C, after treatments with an antibiotic and / or a fungicide. The seeds for this study were harvested in October 1981 at the Texas A & M University Agricultural Research and Extension Centre, Beaumont, Texas, U.S.A. The seeds were kept in cold storage until tested. This was a factorial experiment (12 soybean lines x 4 seed treatments x 2 temperatures) arranged in a completely randomized design with five replications.

One hundred grams of seed from each line were treated with 125 mg of streptomycin sulphate or 0.3 ml carboxin (vitavax 200–FF) or both. Non treated seeds served as controls. Twenty-five seeds of each advanced breeding line or cultivar per treatment were placed on cellulose pads (kimpac) moistened with sterilized distilled water in 15 cm culture plates.

Ten plates were prepared for each soybean line x seed treatment combination. Five plates of each combination were incubated at 30°C and five at 38°C. The number of seeds germinated and decayed by *Bacillus subtilis* and fungi were recorded after five days of incubation. The seeds were considered germinated when the length of the radicle was one and one-half times the length of the imbibed seed and were apparently healthy. Analysis of variance was made on all data and means were compared by the Duncan's Multiple Range Test (Duncan, 1955). Simple correlations were examined where applicable.

RESULTS AND DISCUSSION

The seed treatments streptomycin sulphate, vitavax 200–FF, and the combination of both did not improve germination percentages (Table 1). Instead the seed treated with either streptomycin sulphate or vitavax 200–FF showed slight decreases in germination percentages at 30°C and major decreases at 38°C compared to untreated control. Only 1.2% of the total variation in germination was explained by seed treatment whereas 50.8% of the variation was explained by temperature. Streptomycin sulphate significantly lowered the percentage of seed infected by *Bacillus subtilis* at 38°C and vitavax 200–FF significantly reduced the proportion of fungal infected seed at 30°C. However, the proportion of streptomycin sulphate treated seed infected with fungi was significantly greater at both temperatures and the incidence of *Bacillus subtilis* at both temperatures was higher on seed treated with vitavax 200–FF. This might have resulted from bacterial suppression by streptomycin sulphate giving the fungi competitive advantage and vice versa when fungi were controlled. Increase of *Bacillus subtilis* on vitavax treated seed and fungi on streptomycin sulphate treated seed might account for the declines in germination percentages at 30° and 38°C. However, germination percentages of seed treated with the combination of streptomycin sulphate and vitavax 200–FF were the same as those of the untreated seed at both temperatures. Streptomycin sulphate has been reported as phytotoxic to soybean seedlings (Hepperly and Sinclair, 1977). The reduction of fungal infection and increase of bacteria in seed treated with a fungicide was observed earlier (Trika and Shanmugasundram, 1980).

However, the response of some soybean lines was different from that of most entries. Germination percentage of seed treated with streptomycin sulphate was significantly higher and infection percentage by *Bacillus subtilis* was significantly lower than the untreated seed for the cultivar Centennial at 38° C (Tables 2 and 3). Combination of streptomycin sulphate and vitavax 200-FF significantly increased the germination percentage and decreased the percentage of infection of *Bacillus subtilis* in seed of the cultivar Essex at 38°C. At 30°C, all three treatments significantly increased the percentage germination of the advanced breeding line N77—179, which initially had low germination (Table 2). Vitavax 200-FF significantly decreased the presence of total fungi in this cultivar at 30°C (Table 4) and streptomycin sulphate and the combination at 30°C reduced the percent of seed infected by *Bacillus subtilis* (Table 3). This is consistent with the report of Ellis *et al.* (1977), who made similar observation with low germinating soybean cultivar "Dare". Improvement in germination by fungicidal seed treatment was also reported (Trika and Shanmugasundram, 1980).

The different behaviour of soybean lines to seed treatments resulted from the interaction, which was significant at the 1% level. The presence of *Bacillus subtilis* increased and the germination percentage declined significantly when the incubation temperature was raised from 30° to 38°C. The incidence of total fungi decreased with the increase of incubation temperature (Figure 1). An increase of seed infection by *Bacillus subtilis* and concomitant decrease in germination was also observed earlier by Sinclair (1978) and Tenne *et al.* (1977).

Coefficients of correlation were negative and statistically significant at the 1% level between germination percentage and percent seed infection by *Bacillus subtilis* at both 30° and 38°C, and percentage germination and percent of seed infected by total fungi at 30°C (Table 5). Thus, germination was inversely related to seed infection by *Bacillus subtilis* and total fungi at 30°C and only by *bacillus subtilis* at 38°C- Similar relationships were reported by several other workers (Ellis *et al.*, 1977; Floor, 1977; Sinclair, 1978; Tenne *et al.*, 1974; Wallen and Cuddy, 1960) earlier.

CONCLUSION

From the results of this investigation it appears that in general neither antibiotic nor fungicidal seed treatment nor combination of both improves seed germination at optimal and above optimal germination temperatures in soybeans. However, the response of soybean lines to seed treatments is found highly variable. In some cases, antibiotic and/ or fungicidal seed treatment seems to improve the germination remarkably.

Antibiotic seed treatment is found reducing the incidence of bacterial infection meanwhile increasing the seed infection by fungi and vice versa when the seed is treated with fungicide.

The above optimal temperature, as 38°C in this case, seems to reduce seed germination drastically. The incidence of bacterial infection increased tremendously at this temperature as compared to 30°C, which is considered to be the optimum temperature for seed germination in soybeans.

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Table 1. The influence of streptomycin sulphate, vitavax, and the combination of both on germination and seed infection by microorganisms at two incubation temperatures.

Seed	*Streptomycin sulphate*		*Vitavax*		*Streptomycin sulphate and Vitavax*	
	30°C	*38°C*	*30°C*	*38°C*	*30°C*	*38°C*
Germinated°	97.0†	64.8	93.8	17.9*	99.4	100.5
Infected with						
B. subtilis°	97.0	22.2*	116.4	168.5*	93.5	85.2
total fungi°	269.6*	315.0*	48.8*	97.1	157.3*	97.9

° Percent of untreated control.

† Figures are means of 12 lines,

* Significantly different from control at P=0.05 based on Duncan's Multiple Range Test (Duncan, 1955).

Table 2. **Germination of seed from 12 soybean lines treated with streptomycin sulphate, vitavax, and the combination of both at two incubation temperatures.**

Soybean line	*Streptomycin sulphate*		*Vitavax*		*Streptomycin sulphate and Vitavax*	
	30°C	*38°C*	*30°C*	*38°C*	*30°C*	*38°C*
N77–179	269.6*	100.0	152.1*	100.0	182.6*	100.0
Centennial	106.0	242.5*	96.5	52.6	94.8	137.5
F77–1797	99.1	10.4	96.6	0.0	102.6	116.5
Cobb	98.3	51.3	91.4	5.3	87.9	0.0
R74–511	95.6	100.0	73.9	100.0	69.6	0,0
D78–5502	94.5	67.8	94.5	40.7	105.5	81.4
D77–6166	91.6	115.2	95.0	100.0	98.3	134.8
D76–9454	86.8	105.2	110.9	0.0	105.5	96.0
Essex	84.2	100.0	65.0	100.0	80.9	200.0*
D78–3238	81.5	0.0	75.0	0.0	119.8	0.0
D77–6057	78.7	100.0	108.5	0.0	133.3	100.0
N77–1602	58.0	100.0	97.7	100.0	91.7	100.0

°Figures are in percentages relative to the untreated control.
*Significantly higher than control at P=0.05 (Duncan's Multiple Range Test).

Table 3. **The effect of streptomycin sulphate and the combination of streptomycin sulphate and vitavax on the proportion of seed infected with Bacillus subtilis for 12 soybean lines incubated at two temperatures.**

Soybean line	*Percent of the Untreated Control*			
	Streptomycin sulphate		*Streptomycin sulphate and Vitavax*	
	30°C	*38°C*	*30°C*	*38°C*
D77–6057	0.0*	96.8	63.6*	68.0*
D78–5502	0.0*	100.0	50.0*	151.0
Centennial	0.0*	18.2*	142.0	115.4
Essex	11.1*	61.2*	81.4	51.2*
N77–179	12.7*	122.8	55.0*	120.0
N77–1602	17.4*	93.6	176.0	69.2*
Cobb	20.0*	63.3*	360.0	145.2
F77–1797	28.6*	194.4	42.8*	118.9
D76–9454	31.8*	112.4	113.6	101.9
R74–511	33.3*	99.2	93.0	106.4
D78–3238	50.0*	93.6	25.0*	65.6*
D77–6166	600.0	143.0	400.0	68.9*

*Significantly lower than control at P=0.05 based on Duncan's Multiple Range Test.

Table 4. The effect of vitavax and the combination of vitavax and streptomycin sulphate on fungal infection in seed of 12 soybean lines during germination at two incubation temperatures.

Soybean line	*Percent of the Untreated Control*			
	Vitavax		*Vitavax and Streptomycin sulphate*	
	30°C	*38°C*	*30°C*	*38°C*
D76–9454	0.0*	100.0	36.4*	100.0
R74–511	0.0*	100.0	141.9	100.0
D77–6057	2.9*	100.0	132.4	100.0
D78–3238	6.3*	100.0	237.5	100.0
N77–179	6.4*	100.0	137.1	100.0
N77–1602	14.3*	100.0	35.7*	100.0
D78–5502	28.6*	66.0	71.4	100.0
Cobb	100.0	100.0	100.0	100.0
Centennial	100.0	100.0	100.0	100.0
F77–1797	100.0	100.0	200.0	75.0
D77–6166	100.0	100.0	75.0	100.0
Essex	100.0	100.0	620.0	100.0

*Significantly lower than control at P=0.05 according to the Duncan's Multiple Range Test.

Table 5. Coefficients of correlation between germination percentages and percent of seed infection with microorganisms at two incubation temperatures.

Percent seed infection	*Germination percentage*	
	30°C	*38°C*
Seed infected with		
B. subtilis	–0.709**	–0.889**
total fungi	–0.599**	–0.074

**Significant at 1% level.

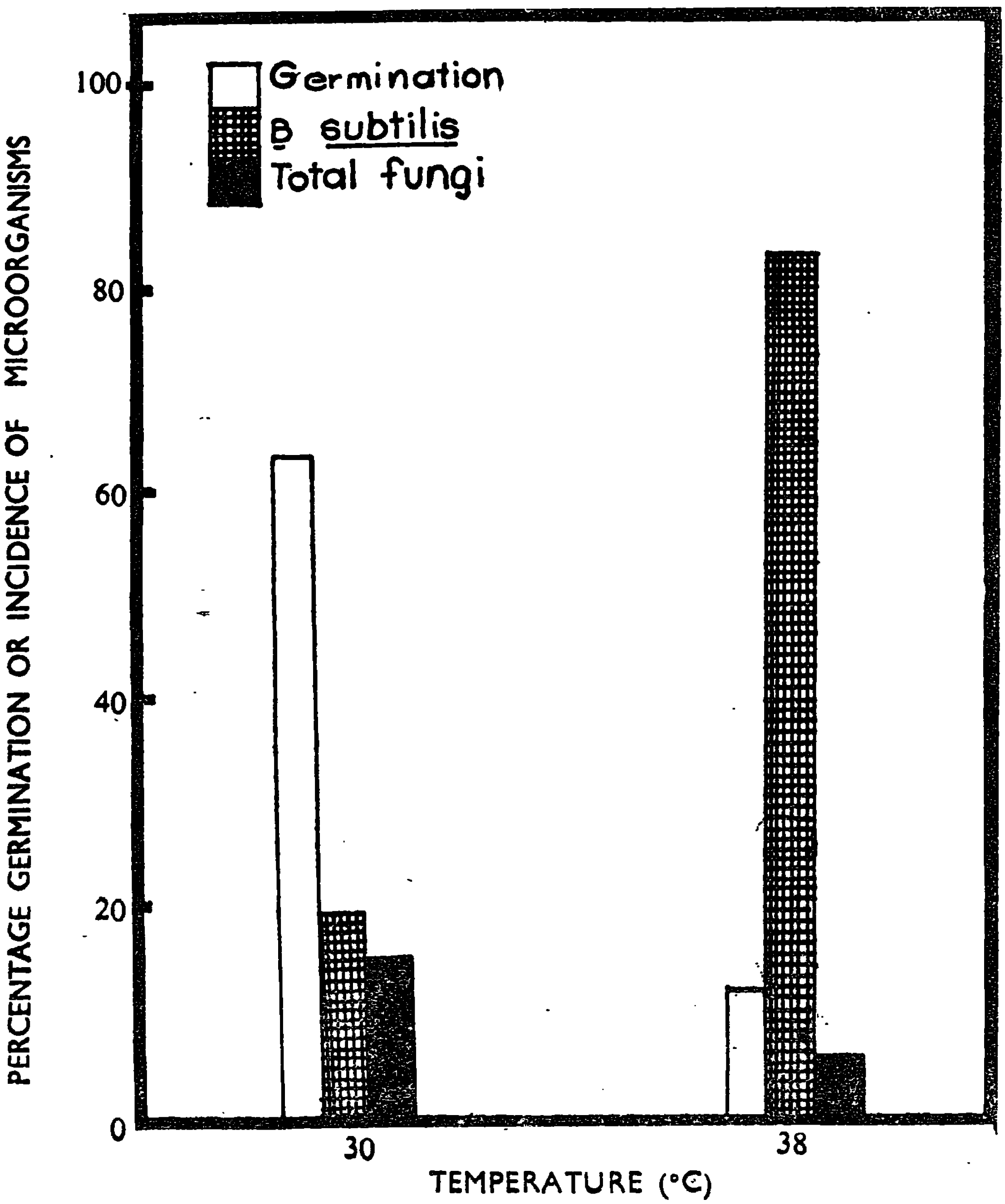

Fig. 1. Mean percentage germination and seed associated with *Bacillus subtilis* and total fungi at two incubation temperatures.

AGRONOMIC STUDIES IN CEYLON SPINACH
(Basella Spp)

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ABSTRACT

Generating guidelines giving proper management practices to obtain increased yields is a requirement for inducing increased spinach cultivation among farmers. Agronomic studies were performed to determine nitrogen requirements, frequency of harvesting, effect of trellising and use of organic manure in two species of Ceylon spinach.

Five nitrogen levels and three harvesting frequencies were investigated in a split-plot experiment. Results revealed that 140 kg N/ha together with a 20 day harvest frequency gave best results.

An experiment was also conducted with trained and untrained vines, using two species of spinach, *Basella alba* and *B. rubra* in a split-plot design. Three manurial treatments were also incorporated into this experiment.

Combined use of 70 kg N/ha with 12 tons of cattle manure/ha gave marked increase in yield over single applications of 140 kg N/ha or 12 tons of cattle manure/ha. *B. rubra,* the red type, gave significantly higher yields and was also more fertilizer responsive than the green type. Urea/cattle manure combination gave significantly higher yields with untrained vines than with trained vines. There was no additional yield increase due to trellising.

INTRODUCTION

Ceylon spinach, *Basella alba* and *Basella rubra* is a very productive leafy vegetable which is suitable for both home-gardens and market production in the humid tropics. *Basella alba,* the green species, has greater consumer preference since *Basella rubra* is red in colour and resembles the foliage of the beet. Ceylon spinach is a rich source of vitamins A and C, calcium and iron. A brief review presented by Winters (1964) describes its place of origin, botanic characters, and its usage.

Spinach is mainly grown in home gardens of the low country wet zone while large scale cultivation is confined to the Colombo District, where farmers cultivate it along with other leafy vegetables. The possession of a C_4—cycle photosynthetic parthway (IBPGR, 1977) very similar to that of amaranth may render the crop suitable for propagation in the drier areas of the country.

Spinach cultivation could only be enhanced by generating sufficient research information giving management practices necessary to obtain increased yields. Hence, studies were undertaken to determine suitable agronomic practices to be adapted in spinach production. Two separate studies were carried out, the details of which are reported.

1. Nitrogen Requirements and Frequency of Harvesting

Nitrogen is of prime importance in the production of leafy vegetables. It promotes vegetative growth to a greater degree than root growth. Also, nitrogen darkens the green colour of the leaves and improves succulence. Further, a system of harvesting has to be adopted in order to obtain quality product. Many green vegetables have low consumer preference when they are harvested too late. The increase in fibre content and the development of stronger flavours are the primary reasons for the reduction in quality of the over-mature leaves and stem. Therefore, the time of harvesting and frequency of harvesting are important factors to be considered in spinach production.

2. Species, Trellising and Effect of Manuring

Trellising and the use of organic manure are common practices prevalent among home gardeners. Inorganic fertilizers are hardly used by this sector. On considering farmers who grow for market production they may face difficulties in the use of trellising, since it increases cost of production. Therefore, it is necessary to assess the economic advantage in the use of trellises.

Organic materials in the soil improve soil structure and lead to good drainage, aeration and nutrient availability. In contrast to mineral fertilizers, organic manure releases nutrients into the soil for relatively long continuous periods. Yet, the use of mineral fertilizers may be of value in increasing production.

In order to determine the relevance of these "small farmer practices" to larger production areas two species of Ceylon spinach were tested under different management conditions.

MATERIALS AND METHODS

1. Nitrogen Requirements and Frequency of Harvesting

Five nitrogen levels, 0, 80, 100, 120, 140 kg per ha and three harvesting frequencies at 15, 20 and 25 day intervals were investigated in a split-plot experiment with harvesting frequencies as main plots. The treatments were replicated four times.

Normal land preparation was carried out to prepare the plots. A basal dressing of phosphorus (60 kg P_2O_5 / ha) and potassium (100 kg K_2O / ha) was applied to the planting holes. In relevant treatments one half of the total quantity of nitrogen was also applied to planting holes. Planting was done 30 cm. apart by placing 2 seeds per hill. Two weeks following germination, seedlings were thinned out leaving one plant per hill. The remaining nitrogen was applied one month after germination. The first common harvest was performed 60 days after germination by cutting the stem at 20 cm from the soil surface and another two more harvests were made at appropriate intervals.

2. Species, Trellising and Effect of Cattle Manure

The treatments used in a split plot design experiment with four replicates were as follows :

i. Main plots : (*i*) Vine untrained.
(*ii*) Vine trained on to 45 cm high bamboo frame.

ii. Sub plot combinations of :

Varieties: (*i*) *Basella alba* var. Ceylon gaint spinach
(*ii*) *Basella rubra*.

Manuring: (*i*) 140 kg N / ha as urea
(*ii*) Well rotted cattle manure (12 tons / ha).
(*iii*) Well rotted cattle manure (12 tons / ha) and 70 kg N / ha as urea.

Land preparation, planting and thinning-out were done as in the previous experiment. Cattle manure and half of the nitrogen fertilizer were applied as basal to the appropriate plots. The remaining half of nitrogen was used as a top dressing 30 days after germination. Untrained vines were harvested at 72, 95 and 118 days after germination. Following the 3rd harvest flowers were produced in both varieties and no economic yields were obtained. In the other treatments, vines were trained on 45 cm tall bamboo frames and harvests were made at 85, 128 and 171 days after germination.

RESULTS AND DISCUSSION

1. Nitrogen Requirements and Frequency of Harvests

Significantly higher yields were obtained when spinach was harvested at 20 day intervals than at 25 days (Table 1). The difference between 20 and 15 day intervals and 15 and 25 day intervals was not significant.

Table 1. Effect of Frequency of Harvesting

Frequency of harvesting in days	*Mean green yield kg / ha*
15	16620.4
20	18611.1
25	13564.8
L. S. D. (p=0.05)	3148.1
C. V.	18.20%

Leaves and shoots harvested at 15 day intervals did not show sufficient growth and were too tender. Insufficient growth and food storage may be reasons for lower yields obtained at 15 day harvesting intervals even though 5 harvests were carried out during the 60 day period instead of the 3 harvests possible using the 20 day intervals. It was also observed that plants harvested at 15 day intervals produced extensive flower clusters leading to the stunted growth of plants. Based on this it may be concluded that 20 day interval of harvesting is more advantageous. The leaves and stems harvested at 25 day intervals were over matured and fibrous and could not meet the consumer requirements. There was a negligible amount of flowers in the 20 and 25 day intervals harvesting treatments.

Yields were increased with the increased use of nitrogen fertilizer (Table 2). 140 kg nitrogen was significantly superior to 0 and 80 kg N / ha. but not to 100 and 120 kg N / ha. 100 kg/ha and 120 kg N / ha were superior to only 0 nitrogen treatment.

Table 2. Effect of Nitrogen on Green Spinach Yield

Nitrogen kg / ha.	*Mean green spinach yield kg / ha.*
0	7314.8
80	13657.4
100	18148.1
120	18240.7
140	24027.8
L. S. D. (p=0.05)	7916.6
C. V.	20.12%

The nitrogen x frequency of harvesting interaction was significant (Table 3). If an application of 140 kg N / ha is done, significantly high yield is obtained when harvesting is done at 20 days rather than at 15 or 25 day intervals. Differences in other treatment combinations also show some advantage with higher fertilizer levels.

Table 3. Nitrogen x Frequency of Harvesting Interaction.

Levels of Nitrogen	*Frequency of harvest in days*		
kg/ha	*15*	*20*	*25*
0	8750.0	6944.4	6203.7
80	15509.3	15972.2	9490.7
100	18657.4	17592.6	18287.0
120	17592.6	19027.8	18148.1
140	22546.3	33657.4	15925.9
L. S. D. (p=0.05)	6990.7		

Nitrogen may thus play a vital role in increasing yield of this crop and the most obvious result that emerges from this study is that with 140 N / ha the best frequency of harvest is 20 days.

2. Species, Trellising and Effect of Cattle Manure

The main plot treatments of training were not statistically significant showing that there is no additional advantage in yield rendered by trellising (Table 4). However comparatively higher yields were obtained when the vines were untrained. It was also assessed that the time taken for the completion of harvest was delayed by 53 days in trained vines when compared to untrained vines. Hence, it follows that the latter practice is more advantageous. Although trellising is practiced by farmers, the only advantage that could be expected from this practice is in obtaining of a clean harvest.

Table 4. Effect of Vine Training Methods

Method of training	*Mean yield kg / ha*
Trained	25347.2
Untrained	35763.9
L. S. D. (p=0.05)	N.S.
C. V.	23%

Considering the species, results were significant (Table 5). Red spp. gave significantly higher yields showing its superiority over the green type. This confirms observations from reports of the Division of Horticulture, Central Agricultural Research Institute, Gannoruwa in 1978 (K. D. A. Perera unpublished). The yields obtained for red spinach were higher with all manurial combinations and the increase in yields was over 10,000 kg / ha except with cattle manure alone. Higher production of axilary branches and late flowering were the advantageous characteristics observed in the red species.

Table 5. Weight of Spinach, Stem and Leaves in kg / ha.

Variety	*Urea*	*Cattle Manure*	*Urea+ cattle manure*	*Total*	*Mean*
Green Cey-spinach	15,903	18,629	37,014	71,546	23,849
Red Cey-spinach	28,090	24,418	59,462	111,970	37,323
Total	43,993	43,047	96,476		
Mean	2199.7	21,524	48,238		

L. S. D. Varieties (p=0.01) = 11,576 kg / ha.

L. S. D. Manuring (p=0.01) = 14,178 kg / ha.

Combined use of 70 kg N / ha with 12 tons of cattle manure / ha gave marked increase in yield over single application of 140 kg N / ha or 12 tons cattle manure / ha. This is clearly seen in both the green as well as the red type. Single treatment of urea and cattle manure does not result in statistically observable differences in yield with both varieties.

Considering species differences and response to fertilizer, it is evident that *Basella rubra* shows very good response to fertilizer use in all instances. It was observed that the growth of the green spp. was very slow during the first month. This observation could explain the inefficient use of nitrogen by early growth phase, probably resulting in loss of nitrogen by leaching.

It has been reported that addition of well rotted cattle manure could lead to improved conditions in the physical properties of the soil, enabling more efficient use of mineral fertilizers by plants (FAO, 1978). This could explain the marked yield increases obtained on combined application of inorganic and organic fertilizers over the application of 140 kg N/ha since the cattle manure subscribed about 72 kg N/ha and the total N in the combined cattle manure + urea treatment was about 140 kg N/ha given as urea only.

Training × variety and training × variety × manuring interactions gave no significant differences. The training × manuring interaction was significant (Table 6).

Table 6. Training and Manuring Effects on Yield of Spinach in kg / ha

Manuring	*Trellis*	*Untrained*
Urea	16537	26510
Cattle manure	24123	19870
Urea + Cattle Manure	35434	61042

L. S. D. for interaction $p=0.05$ = 14889 kg / ha

CV for sub plots =4.7%

Significantly higher yields were obtained with untrained vines using the urea / cattle manure combination. The same was not true when urea and cattle manure were applied separately. When plants were not trained, pruning was begun at an early stage of growth (72 days after germination) and subsequent harvesting was at 23 day intervals. With trained vines initial harvesting was delayed to 85 days after germination and 43 day harvesting frequency was observed. Early pruning of untrained vines would have led to better vegetative growth thereby resulting in more efficient utilization of soil nutrients leading to higher yields.

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