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Evaluation of Potential Allelopathic relationship between Velvet bean (*Mucuna pruriens* var. *cochinchinensis*) and Speargrass (*Imperata cylindrica* (L.) Raeushel) with Selected methods

Authors must reduce the number of words of the abstract. Mucuna and Speargrass were written in 3 different forms: Mucuna, mucuna, mucuna (italic) and the same for Speargrass. Review!!!

ABSTRACT

Velvetbean *(Mucuna pruriens* var. *cochinchinensis)* has been reported to smother the growth of Speargrass [*Imperata cylindrical* (L) Raeushel] through competition for light and nutrients as well as having some potential allelopathic effect on speargrass. However, the mechanism of the allelopathic effect on speargrass is yet to be properly understood. Therefore three methods were evaluated at International Institute of Tropical Agriculture [illegible] Nigeria in 2009, to establish if mucuna exhibits allelopathic effect on [illegible]ion to the smothering effect. These methods included: (1) Mucuna fresh parts [(Leaves (L), Leaves + Vine (L+V), Vine (V) and Roots (R)] incorporation into pot planted to speargrass rhizome and a control pot without mucuna part incorporation; (2) Mucuna plant part extract (L, L+V, V and R) and control with ordinary water; and (3) Mucuna seed and speargrass rhizome interplant (One mucuna seed-interplant, Two mucuna seeds interplant and Three-mucuna seeds interplant) . Results indicated that every part of the mucuna plant has the potentials of being allelopathic to speargrass., Incorporating mucuna parts into pot planted with speargrass reduced both speargrass shoot height and number by a value ≥ 20 % and ≥ 50 % respectively. Similarly mucuna parts extract on the average reduced shoot and rhizome biomass by ≥ 60% and ≥ 50% respectively when compared to the control. The pot interplant method on the average showed a reduction of speargrass shoot and rhizome biomass by ≥ 20% and ≥ 16 % respectively. The effect of speargrass and mucuna interplant on speargrass shoot height was not variable between the speargrass-mucuna interplant and the sole speagrass control except at 12 WAP**.** This investigation revealed that in addition to the smothering effect of *Mucuna* on *speargrass*, it also exhibits allelopathic effect on *speargrass* and this could serve as a bio-control method for speargrass. All the mucuna parts studied has the potentials of exhibiting allelopathy effect on neighboring and susceptible plants. The allelopathy effect appears to be greater with the leaves or the vines or both.

Why uppercase? See experimental

Key words: Allelopathy, *mucuna* Imperata*, extract, methods, incorporation*

INTRODUCTION

Velvetbean [(*Mucuna pruriens* (L.) DC] is an annual legume that grows vigorously to provide early ground cover. This attribute of *Mucuna* spp has numerous agronomic advantages. The advantages of velvetbean ranges from providing green manure, nitrogen fixation, smothering of weed growth to reduction in the population of plant-parasitic

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Lorenzi (1984)

nematodes [1, 2] According to [3], velvetbean increases the yield of its companion crops and smothers or suppresses the growth of noxious weeds such as nutsedge (*Cyperus* spp.) and speargrass (*Imperata cylindrica* (L.) Raeuschel [4, 5]. The aspect of suppression of speargrass is of great importance in tropical production systems. This is because speargrass interference is a major challenge for crop production in sub-Saharan Africa with a great cost in both financially and physically resources. Speargrass is a strong competitor with crops. Crop yield losses vary as a function of crop type, cultural practices and environmental conditions. In corn, grain yield reductions of 80% [5, 6] have been reported. Velvetbean mechanism of suppression of noxious weeds such *Cyperus* spp, and *Imperata cylindrical,* is yet to be fully understood. Past studies with speargrass have implicated shading effect and competition for nutrient as the possible effect of smothering or suppression of this weed [7]. Authors ~~Others~~ have suggested possible allelopathic effect of mucuna on speargrass and other weeds [8, 9].

. Hence, the objective of this ~~present~~ study was to explore three different ~~various~~ methods to establish the potential allelopathic effect of *Mucuna pruriens var. cochinchinensis* on ~~speargrass~~ insert scientific name of speargrass

2.0 MATERIALS AND METHODS

2.1 Study Location

This was a screen house experiments conducted twice at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, during the 2009 planting season. Results from repeated experiments were similar and therefore combined for analysis. Three methods were used in determining the allelopathic effect of velvetbean (***Mucuna pruriens* var. *cochinchinensis)*** on Speargrass (*Imperata cylindrica* (L.)Raeuschel).

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2.2 Allelopathy Methods Studied

2.2.1: Fresh mucuna parts incorporation method

improved this text.

This method was taken after [8]. . With this method mucuna leaves, (L), vines, (V), mucuna leaves + vines, (L+V) and roots, (R) (1.0% W\W in dry weight equivalent) were incorporated into the soil containing one speargrass stand per pot. The control treatment had no mucuna plant part(s) . All this including the control, (No mucuna) served as the five treatments that were replicated five times. The incorporation of the fresh *Mucuna* plant parts was done at 6 weeks after planting speargrass (WAP) and this practice was carried out weekly until 12WAP. Four parameters of speargrass shoot height, shoot number, shoot and rhizome dry weight were recorded at monthly (4 WAP) interval till 4 months after planting (16 WAP).

2.2.2 Mucuna Extract Velvetbean (*Mucuna pruriens* DC var. *cochinchinensis)* was grown out-of-doors in pots containing 35kg of river sand at the International Institute of Tropical Agriculture, Ibadan, Nigeria. Three months after planting, the main stem of each plant was cut at ground level, and the soil was washed gently from the roots. The mucuna plants harvested were partitioned into main and fine roots, leaf blades only (L), Leaf blade + vines (L+V), and vines (V) only and root only (R). The mucuna plant parts (L, V, L + V and R), were air-dried for 24 hours and 25g of each mucuna part was soaked in water in a flask for 48 hours at room temperature. The extract was strained through two layers of cheese cloth. The liquid extract of the mucuna plant parts (1L) were used in wetting the speargrass shoots in the pots [10]. Hundred percent stock solution of each mucuna plant part was used undiluted, and this was compared with ordinary water as control. The wetting was done every two days till 12 WAP. The treatments including the control were replicated five times. Four parameters of speargrass-shoot height shoot number, shoot and rhizome dry weights were recorded at 12 and 16 WAP.

2.2.3: Pot interplant method.

Pot interplant

~~This~~ was achieved by growing speargrass and mucuna together in one pot (donor and receiver plant together in control environment) [11]. Speargrass rhizome segment 5 cm

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long with one visible bud and node were planted at one segment per pot. The pots were filled with sterilized soil and planted with mucuna and speargrass to constitute the treatments as follows: treatment one (T1) had one mucuna seed planted with speargrass rhizome; treatment two (T 2) had three mucuna seeds planted around one speargrass rhizome segment; treatment three (T 3) had three mucuna seeds planted to one *Imperata* rhizome segment and the control had only one speargrass rhizome segment planted in it. These four treatments were replicated six times. The mucuna vines were staked so as to prevent the plant(s) from growing on the speargrass shoot. Thus the only access or contact of mucuna parts to the pot was through litter fall and possibly by the root or root exudate

Statistical Analysis

The experiment was repeated for each evaluation method, and the means of the experiments were treated as replicates in a completely randomized design for analysis of variance, and the means were compared using the protected LSD test at the 5% level of significance.

3.0 **RESULTS**

3.1 Fresh mucuna parts incorporation method

The results obtained showed that speargrass plant height, numbers of shoots were not significantly different from the control at 4WAP, (that is before the treatments were applied at 6 WAP) (Table 1 and Fig. 1). However, after incorporation of the mucuna treatments at 6WAP, the effect of the mucuna incorporation was visible at 8WAP where the numbers of speargrass shoots at the control were significantly higher than the number of shoots in the treatments that received mucuna parts. The effect on plant height did not differ significantly among the treatments (Figure 1) .At 12WAP, speargrass in no mucuna ????

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were not significantly (P≤0.05) taller than the rest of the treatment. but numbers of speargrass shoots were significantly lower in the mucuna incorporated plots when compared to the control plot ~~without mucuna~~. At harvest (16WAP), shoot and rhizome dry biomass of speargrass were significantly (P<0.05) higher in the control plot ~~without mucuna~~ than in the mucuna parts incorporated treatments. Mucuna parts did not differ significantly on their respective effect on the speargrass parameters measured. Fresh mucuna parts reduced speargrass shoot number to about 60% and the rhizome by ≥ 50% (Table 1). Although they were no differences between mucuna parts on their effect on speargrass but mucuna leave + vine incorporation appeared to have a greater effect on speargrass shoot and rhizome (Table 1). There were no significant (P >0.05) plant height differences between treatments before mucuna plant parts were incorporated at 4WAP. Similarly, there were no significant height differences among the treatments at 12 WAP, which was 6 weeks after mucuna plant parts were incorporated. However, at 16 WAP, which was 10 weeks after the mucuna parts were incorporated, significant height differences were observed between the control plot and the mucuna plots. In terms of speargrass height, the mucuna parts incorporation reduced mucuna height as follows: mucuna root and vine by 33.3%; mucuna leave + vine by 31.1% and mucuna leave by 21.5% when compared to the control ~~without mucuna~~. The result here indicates that fresh mucuna plant parts incorporation reduced speargrass height to ≥20 % of the control ~~without mucuna~~ (Figure 1).

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Table 1. Effect of soil incorporated mucuna plant parts on speargrass height, shoot number and biomass

	Shoot number per pot (WAP)				Dry biomass per pot (g) at 16 WAP	
Mucuna plant part	**4**	**8**	**12**	**16**	**Shoot**	**Rhizome**
No mucuna	1a	2a	2a	3.0a	2.175a	2.450a
Leaf + Vine(L+V)	1a	1b	1b	1a	0.475b	0.700b
Leaf (L)	1a	1b	1b	1a	0.575b	0.750b
Vine(V)	1a	1b	1b	1a	0.675b	1.175b
Root (R)	1a	1b	1b	1a	0.625b	0.925b
LSD(*P*=0.05)	0	0.5	0.5	0.8	0.684	0.917

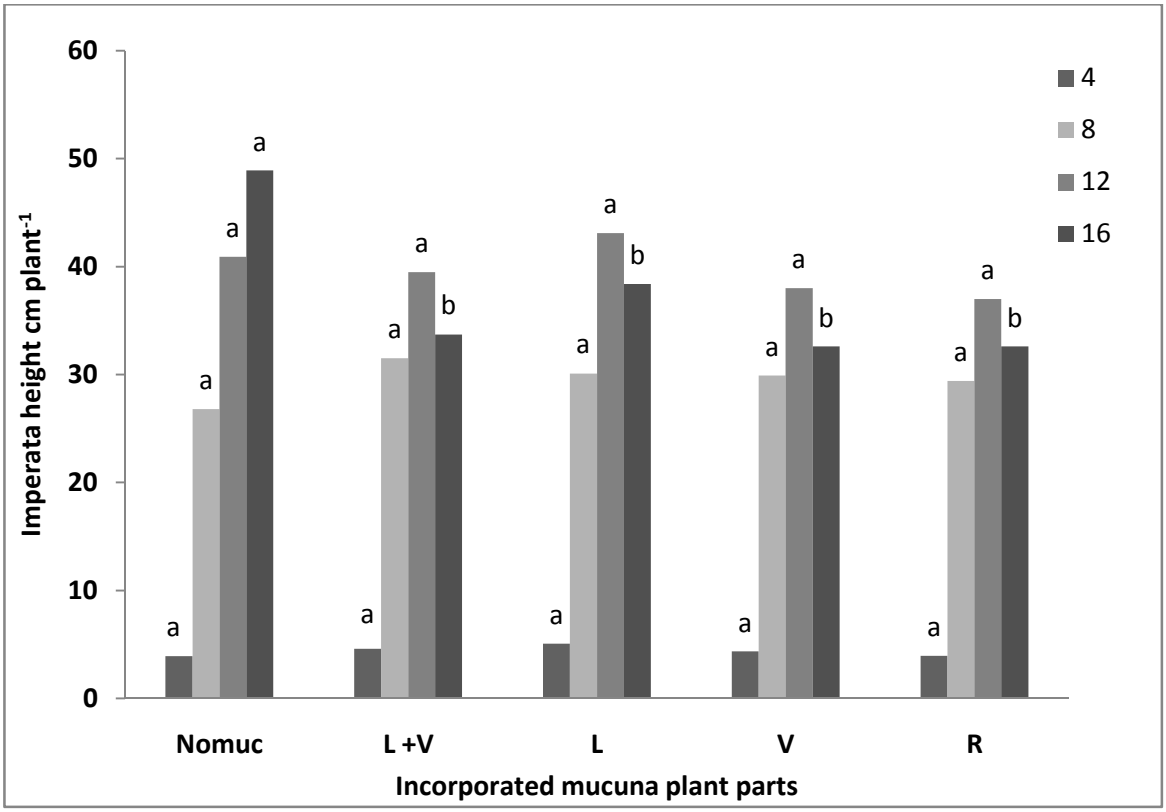

Figure 1: Effect of soil incorporated mucuna plant parts on ~~*Imperata*~~ speargrass height from 4 to 16 weeks after planting (WAP) (Mean values for height with the same letter on a bar are not significantly different at P <0.05)

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3.2 Effect of mucuna extract

The effect of mucuna extract on spear grass height, number of shoots, shoot dry weight and rhizome dry weight were determined**.** At 4WAP, speargrass shoot height in the control treatment was significantly (P≤0.05) taller than all the mucuna extract treatments except the mucuna root extract treatment (R) (P>0.05) In terms of speargrass shoots number there were not significantly (P>0.05) differences between the control and the various mucuna part extracts at 4WAP(Figure 2). The treatment effect started showing at 8WAP where speargrass height, and shoot number measured from the control treatments were significantly (P≤0.05) higher than those from the mucuna extracts. At 12 WAP, speargrass in the control plots were significantly (P≤0.05) taller than all the mucuna extract treatments except mucuna leave (L) + Vine (V) extract. Mucuna leave extract at 12WAP had a significant (P<0.05) effect in lowering speargrass height compared to the other mucuna part extract (Figure 2). Speargrass shoot number on the other hand, did not differ significantly with the mucuna extract treatments but had significantly higher number of speargrass shoot than the mucuna extract treatments (Table 2) At 16 WAP, both speargrass shoot height and number were significantly (P<0.05) lower in the mucuna extract treatments than in the control treatments ~~without mucuna~~. Mucuna extracts treatments did not differ significantly from each other in terms of their effect on shoot height and number, except for mucuna leave extract that had a significantly short speargrass shoot than the other extract treatments (Figure 2). Shoot and rhizome dry weight were (P<0.05) higher in the control treatments compared to the mucuna extract treatments, but mucuna extracts did not differ significantly on their effect on shoot and rhizome dry weight(Table 2). The effect of the mucuna part extracts on speargrass shoot/rhizome biomass reduction were in the following order of efficacy mucuna leaves (L)75.7%/62.5) >mucuna vines (V) (68.6%/61.3%)> mucuna leave + vine (L+V) (68.6%/55.3%) > mucuna root (R) (62.9%/51.8%) (Table 2). Thus averaged over mucuna extract parts, speargrass shoot and rhizome biomass were reduced by ≥ 60 % and ≥ 50% respectively when compared to the control without mucuna (Table 2). .

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183 **Table 2. Effect of mucuna plant part extract on speargrass height, shoot number and biomass**

	Shoot number per pot (WAP)				Dry biomass per pot (g) at 16 WAP	
Mucuna plant part	**4**	**8**	**12**	**16**	**Shoot**	**Rhizome**
No mucuna	1a	1.8a	1.3a	2.0a	1.750a	2.53a
Leaf + Vine(L+V)	1a	1b	1a	1b	0.550b	1.13b
Leaf (L)	1a	1b	1a	1.3b	0.425b	0.950b
Vine(V)	1a	1b	1a	1b	0.550b	0.980b
Root (R)	1a	1b	1a	1b	0.650b	1.22b
LSD(*P*=0.05)	0	0.34	0.35	0.35	0.29	1.10

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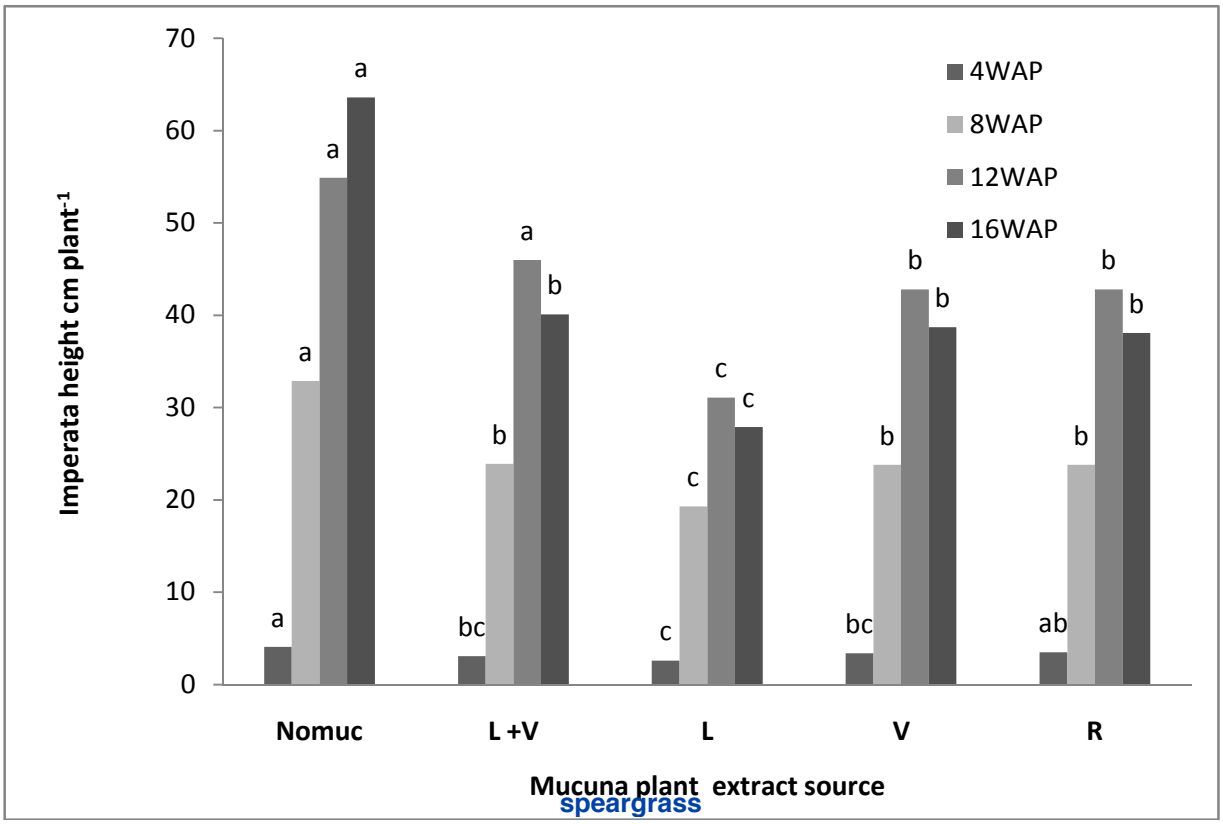

185
186 **Figure 2: Effect of mucuna plant parts extracts on ~~*Imperata*~~ height from 4 to 16 weeks after planting**
187 **(Mean values for height with the same letter on a bar are not significantly different at P <0.05)**

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3.3 Pot interplant screening method

The effect of *mucuna* and speargrass interplant on spear grass height, number of shoots, at 4WAP were not significantly (P≤0.05) different from the control treatment ~~without mucuna interplant~~ (Table 3). However, at 8WAP (Table 3),speargrass shoots were significantly shorter (about 13%) in the mucuna-speargrass treatments compared to the control ~~without mucuna~~ but there were no significant differences between shoot number in the control treatment and the mucuna-speargrass interplant treatments. The treatment effect was conspicuous at 12WAP where the control was significantly (P≤0.05) higher than other treatments for plant height, and number of shoots, except one mucuna seed-speargrass interplant treatment. The mucuna treatments on the other hand did not differ on their effect on speargrass (Table 3). The effect of the mucuna-speargrass interplant on speargrass dry biomass at 16WAP was not significantly different from the control ~~without mucuna~~. However, both speargrass shoot and rhizome dry weight were about 10-36 % and 17 % higher respectively in the control treatment than in the mucuna interplant treatment (Table 3).

Table 3. Effect of mucuna and speargrass interplant on speargrass height, shoot number and biomass

	Plant height (cm)				Shoot number per pot (WAP)				Dry biomass per pot (g) at 16 WAP	
Mucuna + Imperata interplant	**4**	**8**	**12**	**16**	**4**	**8**	**12**	**16**	**Shoot**	**Rhizome**
No mucuna interplant	4.8a	37.4a	60.3a	65.2a	1a	1.0a	2.5a	2.5a	2.05a	2.40a
T1(One mucuna seed interplant	4.8a	32.6a	41.8ba	37.5b	1a	1a	1b	1b	1.85a	2.00a
T2(Two mucuna seed interplant	5.0a	32.7a	38.8b	35.8b	1a	1a	1b	1.0a	1.85a	2.00a
T3(Three mucuna seed interplant	4.8a	32.7a	41.0b	34.5b	1a	1a	1b	1b	1.30a	2.00a
LSD(*P*=0.05)	0.86	2.60	18.77	10.60	0	0	1.13	1.13	1.11	1.11

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DISCUSION

This study provides evidence on the allelopathic potentials of mucuna. All speargrass attributes measured (shoot height, number, dry biomass and rhizome dry biomass) were inhibited to a varying degree by the various mucuna either by soil incorporation, extract of parts or by interplanting with speargrass. Our result showed that parts of mucuna used for treatment had significant effects on all studied germination attributes of speargrass. This is evidenced in the fact that the treatment without mucuna parts, produced the highest shoot height, shoot number and dry biomass. This inhibition is a sign of the effect of mucuna on speargrass and similar effect have been reported by other research [8], who attributed this inhibitory effect to ~~a~~ the compound L-*DOPA* in mucuna. Authors did not use L-Dopa as a positive control and to prove this assumption, why? The effect of the incorporation of the mucuna plant parts started showing two weeks after incorporation into the sand and by 16WAP; the speargrass shoot had turned brown (loss of chlorophyll content). It was also observed in the mucuna extract method, that the growth of speargrass was reduced after the addition of the liquid content from the mucuna plant parts. Unlike, the first method, the treatment effect started showing loss of chlorophyll content around 10WAP (or 4 weeks after incorporation (WAI). The Pot screening method (mucuna-speargrass interplant), showed little or no significant difference from the control. This may be due to the experiment being in controlled environment and /or the duration of the experiment which may not have been enough for the mucuna leachate to have negative effect on the growth and development of the speargrass shoot. It is also possible to assume that mucuna allelopathy may be a function of both root exudates and leave litter accumulation and decomposition. Thus the duration of the experiment may not have been enough to allow for sufficient interplant interaction and litter effect for the interference to manifest properly. In all the methods adopted for

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mucuna

the present study, the fresh ~~*Mucuna*~~ plant part incorporation and extract methods were observed to be more effective in determining the allelopathic potentials of mucuna on

These comments have been written above. Very repetitive.

speargrass. In these methods, the fresh mucuna plant parts were able to retard the growth of speargrass and the extracts inhibited the formation of shoots and roots of *Imperata* rhizomes. This observation is supported by earlier research which reported that the main effect of allelopathic compound is delaying of radical and shoot growth [12]. It has also been reported that most of the growth inhibitory effect of mucuna is associated with compound *L*-DOPA in both the plant parts and seed [8, 9]. The concentration in mucuna parts tends to vary, but mucuna seed have been reported to contain more between 6-9% [13]. Our result ~~here~~ have confirmed earlier report that weed suppression ability of mucuna may be as a result of competition for light and nutrients, as well as allelopathy. This result indicates that every part of mucuna plant has some allelochemicals with the potentials of being allelopathic to sensitive neighboring plants. Allelopathic effect of fresh mucuna leaves on *Phaseolus vulgaris* has been reported, where growth reduction averaged 40 % [8]

of what? Re-write.

Authors must relocate this last phrase. Out of context.

CONCLUSION:

Various? I can found just three!!!

This study through the various methods of assay used has provided evidence of the potential allelopathic effect of Velvetbean (*Mucuna spp*) on the growth of speargrass. The results also point to the fact that the allelopathic effect maybe more with the leaves and vines.

REFERENCES

Review the format of all references. Some mistakes were highlighted.

1. Vargas-Ayala R and Rodrıguez-Kabana R, Bio-remediative management of soybean nematode population densities in crop rotations with velvetbean, cowpea, and winter crops. Nematropica 2001; 31:37–46

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2. Fujii Y, Allelopathy of hairy vetch and mucuna: their application for sustainable agriculture. in Biodiversity and Allelopathy: from Organisms to Ecosystems in the Pacific, ed. by Chou CH, Waller GR and Reinhardt C. Academia Sinica, Taipei, 1999; pp. 289–300.

3. Lorenzi, H. Consideracoes sobre plantas daninhas no plantio directo. In. plant Direto no Brazil. Eds. Torradao, P.V. and Aloisi R.R., Fundacao Cargill, Campinas, 1984; pp. 24 - 35

4. Inga, A Zasada, Waldemar Klassen, Susan LF Meyer, Maharanie Codallo and Aref A Abdul-Baki. Velvetbean (*Mucuna pruriens*) extracts: impact on *Meloidogyne incognita* survival and on *Lycopersicon esculentum* and *Lactuca sativa* germination and growth. Pest Manag. Sci. 2006; 62:1122–1127 (2006)

5. Udensi, E.U., I.O Akobundu, A.O. Ayeni and D. Chikoye. Management of *Imperata cylindrica* with Velvetbean (*Mucuna pruriens* Var. Utilis) and herbicides. Weed Technol.1999; 13: 201 – 208.

6. Koch, W., F. Grobmann., A. Webber., J. Lutzeyer and I.O. Akobundu. Weeds as companion of maize / cassava cropping systems. *In* M. Von open, stand ortgemaesse lanwirtschaft in West Africa. Stuttgart, Germany: Universitaet Hohonhein 1990; pp. 219-244.

7. Chikoye, D., F. Ekeleme, and **U.E.Udensi**. Cogongrass suppression by intercropping cover crops in corn/cassava systems. ***Weed Sci. 2001; 49: 658–667***.

8. Fujii, Y., T. Shibuya and T. Yasuda. Allelopathy of velvetbean: Its discrimination and identification of L-DOPA as a candidate of allelopathic substances. Japan Agricultural Research Quarterly 1992; 25: 238 – 247

9. Fujii Y. Screening of allelopathic candidates by new specific discrimination, assessment methods for allelopathy, and the inhibition of L-DOPA as the allelopathic substance from the most promising velvet bean (*Mucuna pruriens*). Bull. Nat. Inst. Agro-Environ. Sci. 1994; 10:115-218.

10. Putnam, A.R. Weed Management in Agro-ecosystem: Ecological Approaches (ALLELOPATHY: Problems and opportunity in weed management) 1988; pp. 77- 88

11. Putnam, A.R. and Duke W.B. Biological suppression of weeds evidence for allelopathy in accessions of cucumber. Science 1974; 185; 370 – 372

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12. El-Khatib, A.A., Hegazy, A.K. and Galal, H.K. Does allelopathy have a role in the ecology of *Chenopodium murale*? Annals of Botany Fennici 2004; 41:37-45

13. Rehr, S.S., Janzen, D.H. and Feeny, P. P 1973. *L*- DOPA in legume seeds: a chemical barrier to insect attack. Science 1973; 181: 81 - 82