

**ELSAID MOHAMED ELSAID MOHAMED**

**POTATO GROWTH AND PRODUCTIVITY UNDER DIFFERENT  
BIOLOGICAL NITROGEN FERTILIZATION STRATEGIES**

Thesis submitted to Universidade Federal de Viçosa, as a fulfillment of post-graduate program requirements in plant production to obtain the degree of *Doctor Scientiae*.

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## **DEDICATION**

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## **BIOGRAPHY**

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## ABSTRACT

ELSAID, Mohamed Elsaid Mohamed , D.S. Universidade Federal de Viçosa, February, 2013. **Potato growth and productivity under different biological nitrogen fertilization strategies.** Advisor: Ricardo Henrique Silva Santos. Co-advisors: Paulo Cezar Rezende Fontes and Luiz Alexandre Peternelli.

Organic residues were reported to improve agricultural systems sustainability. Meanwhile, the extensive use of organic residues in potato cropping systems remains destitute of quantitative information on their effect on nitrogen supply, N use efficiency and economic returns. Additionally, knowledge of potato growth patterns preceding organic residues incorporation is still scarce. Thus, the current study aimed to explore the influence of different organic residues as well as management practices on potato growth and productivity. Three successive experiments were conducted in the winter seasons of 2009 to 2012. The first experiment (Exp.1) studied the effect of combining either compost or the green manure sunhemp (*Crotalaria juncea* L.) with four compost extract (CE) concentrations compared to mineral nitrogen (250 kg ha<sup>-1</sup>). Treatments were arranged in a factorial scheme (2×4 +1), employing split-plot in RCBD with four replicates. Potato nitrogen status was evaluated employing SPAD index and leaf N-total content after CE application. Dry matter production, N recovery efficiency and N distribution between foliage and tubers were quantified before senescence. Fresh tuber yield components as well as nitrogen use efficiency (NUE) were evaluated. Additionally, the possibility of yield prediction through nutritional indices was tested. The second experiment (Exp.2) evaluated the efficiency of split application of the GM sunhemp N doses. Ten treatments (2×4+2) in a factorial scheme as 8 treatments were based on sunhemp as a nitrogen source. GM nitrogen full dose incorporation (FI) and split application (SD) combined with 4 N doses (75, 150, 225 and 300 kg ha<sup>-1</sup>). Additionally, 2 control treatments as recommended mineral nitrogen (MN 250 kg ha<sup>-1</sup>) and zero N. Treatments were arranged in a split-plot in RCBD with four replicates. Three nutritional indices (SPAD, N-total and total chlorophyll (CHLT) were tested at 21 DAP in the fourth expanded leaf. Moreover, dry matter production, N recovery efficiencies and N partitioning between foliage and tubers were quantified before senescence. At harvest, tuber yield components as well as NUE were evaluated. Furthermore, feasibility of nutritional indices to forecast yield was tested. The third experiment (Exp.3) studied dry matter and nitrogen as well as tuber yield accumulation patterns over the season. The experiment consisted of 3 N doses (100, 200 and 400 kg ha<sup>-1</sup>) from the GM sunhemp,

recommended mineral nitrogen ( $250 \text{ kg ha}^{-1}$ ) and zero N treatment. Four sampling events were realized, from 35 DAP to 80 DAP. Thus, split-plot in RCBD with 4 replicates was adopted. Harvest index, nitrogen recovery, nitrogen harvest index and nitrogen apparent recovery was evaluated at the last sampling event. In addition, yield and NUE were evaluated at maturity. Results of Exp.1 showed that integration of CE with compost and sunhemp resulted in similar yields to MN fertilizer. The GM sunhemp increased potato N content and recovery than compost. Inversely, compost augmented yield and N use efficiency. Furthermore, compost extracts stimulated potato growth and productivity. The undertaken nutritional indices seemed to be viable for N status diagnoses and yield prediction under organic fertilization. Exp.2 results demonstrated that the GM sunhemp provided potato with adequate N supply. Additionally, potato presented lower SPAD values and were not affected by GM split N supply. However, CHLT and N-total were similar to those of mineral N fertilizer. Dry matter production was higher than mineral N. Conversely; N recovery was lower than under mineral nitrogen. In addition, N recovery efficiency and apparent N recovery associated with sunhemp N dose and were higher than MN. Yield and its components were similar or greater than mineral N and were increased by split GM nitrogen supply. As well as DM production was increased by 9.3%. The GM sunhemp presented higher nitrogen use efficiency than mineral nitrogen with superior efficiency under split GM application. The employed nutritional indices presented feasibility to predict potato yield under green manuring, particularly when GM incorporated before planting. Exp.3 results demonstrated that N from the GM sunhemp was not a limiting factor during potato peak growth and stimulated potato growth and yield accumulation early in the season which was greater than mineral fertilizer. Furthermore, the accumulated tuber yield (at 80 DAP) exceeded 90% of tuber harvest. Potato presented elevated yields and nitrogen productivity under green manuring. Thus, economic yield and early harvest can be attained by substituting mineral N fertilizer by green manure

**Key words;** *Solanum tuberosum* L, organic fertilization, nitrogen nutrition, growth and productivity

## RESUMO

ELSAID, Mohamed Elsaid Mohamed, D.S. Universidade Federal de Viçosa, fevereiro de 2013. **Crescimento e produtividade da batata sob diferentes estratégias biológicas de adubação nitrogenada.** Orientador: Ricardo Henrique Silva Santos. Co-Orientadores: Paulo Cezar Rezende Fontes e Luiz Alexandre Peternelli.

A utilização de resíduos orgânicos favorece a melhoria da sustentabilidade dos sistemas agrícolas. No entanto, a sua utilização no cultivo da batata é carente de informações quantitativas sobre o seu manejo no fornecimento de nitrogênio, eficiência de utilização de N e do retorno econômico. Além disso, os padrões de crescimento ainda não estão bem estabelecidos. O presente estudo teve como objetivo explorar a influência de diferentes resíduos orgânicos e seu manejo sobre o crescimento e a produtividade da batata. Três experimentos foram realizados, no inverno, de 2009 a 2012. O Exp.1 estudou o efeito de composto e de AV (crotalária) combinados com 4 concentrações de extrato de composto (EC) e comparados com adubo mineral ( $250 \text{ kg N ha}^{-1}$ ). Os tratamentos foram dispostos em esquema fatorial ( $2 \times 4 + 1$ ) empregando parcelas subdivididas em DBC com quatro repetições. O estado nitrogenado da batata foi avaliado pelo índice SPAD e teor de N-total após a aplicação de EC. A produção de matéria seca, a eficiência de recuperação de N e a sua distribuição entre folhagens e tubérculos foram quantificados antes da senescência. Além disso, a produção e a eficiência de utilização de N foram avaliadas. A possibilidade de predição da produtividade através de estado nutricional da planta foi testada. O Exp.2 testou a eficiência de parcelamento de N do AV. Dez tratamentos em esquema fatorial, ( $2 \times 4 + 2$ ), sendo que 8 tratamentos foram da crotalária como fonte de N compostos por a incorporação da dose completa (FI) ou parcelada (SD), combinados com quatro doses de N (75, 150, 225 e  $300 \text{ kg ha}^{-1}$ ). Mais dois tratamentos, NM recomendada ( $250 \text{ kg ha}^{-1}$ ) e N zero. Os tratamentos foram dispostos em parcelas subdivididas em RCBD com quatro repetições. O índice SPAD e os teores de N-total e teor de clorofila total foram avaliados aos 21 DAP. A produção de matéria seca, a eficiência de recuperação de N e a sua distribuição entre folhagens e tubérculos foram quantificados antes da senescência. Na colheita, a produtividade e a EUN foram avaliadas. A possibilidade dos índices nutricionais a serem utilizado no prognóstico de produção foi testada. O terceiro experimento (Exp.3) explorou os padrões de acumulação de matéria seca, nitrogênio e de tubérculos ao longo do tempo sob a AV. O experimento consistiu de três doses de N ( $100, 200 \text{ e } 400 \text{ kg ha}^{-1}$ ) do AV crotalária, NM ( $250 \text{ kg ha}^{-1}$ ) e N zero. Além disso, quatro amostragens foram incluídos,

de 35 DAP a 85 DAP, para determinar o acúmulo de matéria seca e de nitrogênio ao longo do tempo. Assim, o delieamento em parcelas-subdivididas em DBC, com 4 repetições foi adotado. O índice de colheita, a recuperação de N, índice de colheita de N e a recuperação aparente de N foram avaliados antes da senescência. A produtividade e a eficiência de uso de N foram avaliados. Os resultados de Exp.1 mostraram que a integração da EC com o composto e a crotalária confirmou-se como pratica sustentável. Os rendimentos obtidos foram semelhantes aos obtidos com NM. A crotalária aumentou a absorção uma vez que e a recuperação de N de forma mais eficaz que o composto. No entanto, o composto apresentou melhor eficiência no rendimento e na eficiência de utilização de N. O extrato de composto influenciou as variáveis avaliadas e mostrou-se um estímulo no crescimento e aumento na produtividade da batata. Os índices nutricionais, SPAD e N-total, apresentaram-se ser viáveis no diagnóstico de estado nitrogenado e na previsão da produtividade. Os resultados do Exp.2 demonstraram que o AV forneceu N adequadamente para a batata. A batata sob AV apresentou menores valores de índice SPAD e não foi afetado pelo parcelamento da dose. No entanto, os teores de CLT e N-total foram semelhantes à de N mineral. A produção de matéria seca foi maior do que do NM. Entretanto, a recuperação de N foi menor do que NM e não foi afetada pelo parcelamento de dose. Além disso, a eficiência de recuperação de N e a recuperação aparente de N foram superiores que NM. A produtividade foi semelhante ou superior do NM e foi influenciada pelo parcelamento de dose onde a produtividade comercial e total apresentaram um aumento de 7%. A produção de MS aumentou em 9,3% também. O AV crotalária aumentou a eficiência de utilização de N do que o NM. Além disso, o parcelamento de N do AV apresentou maior eficiência de N que a incorporação plena antes do plantio. Alem disso, Os índice empregados apresentaram uma possibilidade no prognostico da produtividade sob a adubação verde. Os resultados de Exp.3 demonstraram que o N do AV não foi um fator limitante durante o pico de crescimento da batata. O Av estimulou o acúmulo de matéria seca tubérculos no início de ciclo maior que o adubo mineral em que a proporção da produção (aos 80 DAP) superou 90% da colheita. A batata sob a adubação verde apresentou maior produtividade e eficiência de utilização de N. Assim, o rendimento econômico e a colheita precoce podem ser alcançados através da substituição de N-mineral pelos adubos verdes.

**Palavras-chave;** *Solanum tuberosum* L., adubação orgânica, nutrição nitrogenada, crescimento e produtividade.

## 1. GENERAL INTRODUCTION

Potato (*Solanum tuberosum* L.) is the fourth consumed staple food after cereals, and its importance is increasing as a vital food-security crop as well as a suitable alternative to costly imported cereals. Potato production worldwide mainly depends on the intensive use of synthetic nitrogen fertilizers in order to achieve economic yields and high tuber quality. Meanwhile, the crop is characterized by low recovery of applied nitrogen, from 40-60%, leading to environmental risks and economic losses (Vos, 2009). Attempts to optimize nitrogen supply in potato fields for sustainable production systems have developed diverse approaches to better match N supply with potato uptake, improve N use efficiency, optimize yield and overcome environmental threats (Goffart *et al.*, 2008).

In-season crop nitrogen status monitoring approach, on a plant scale, relies on the plant to indicate its N status whereas; plants are good Integrators of various factors such as soil N availability, weather conditions and crop management (Schröder *et al.*, 2000). Therefore, plant tissues analysis aims to monitor crop N requirement and to decide the necessity for topdressing N supply (Vos and MacKerron, 2000). This approach includes different methods, which have been developed and can be operated at different scales, such as Kjeldahl digestion, Dumas combustion, NIR spectroscopy analysis, nitrate specific electrodes and nitrate test strips, Chlorophyll meters, chlorophyll fluorescence and cropsan, (Goffart *et al.*, 2008). Moreover, critical thresholds for each employed method, particularly under conventionally produced potato, have been established and their feasibility to forecast yield have been reported.

Another management approach is the integration of organic residues, e.g. animal manures, compost and green manures, into the production systems. The use of organic residues can be the primary factor contributing to sustainable crop production by increasing the use of on-farm renewable resources and improving soil quality (Stark and Porter, 2005). Research attempts have reported the benefits of organic residues to improve soil properties as well as plant nutrient supply (Wolkowski, 2003). Furthermore, organic residues vary greatly in their mineralization potentials and capacity to provide crops with nutrients over time, particularly N, due to their biochemical composition. Thus, to successfully manage nutrient cycling from organic residues it is necessary to know their biochemical composition and decomposition pattern in order to predict N dynamics and its fate within the soil (Magdoff and Weil., 2004). In addition, the

use of organic amendments as a N source do not necessarily ensure an adequate synchronization of available N with crop demand whereas the balance of N mineralization-immobilization process is highly unpredictable, even when N mineralization indicators, such as C/N ratio, are used. Thus, N supply from organic amendments may be too small or too high (Pang and Letey, 2000).

From this context, attempts to optimize the use of organic residues to achieve economic crop yields in different cropping systems reported that combining organic residues, i.e. green manure, composts and animal manures, with mineral fertilizers may be an effective approach to enhance N supply and synchrony with crop demand (Sikora and Enkiri, 2000). Different research investigations have been conducted to test this approach in potato production. Berjón *et al.* (1997) evaluated the effect of three doses of compost (0, 18, 36 t ha<sup>-1</sup>) combined with three levels of N-mineral fertilizer (0, 125, 250 Kg N ha<sup>-1</sup>) for three years. Results showed that the combined application of compost and N-mineral stimulated plant development with respect to the non-treated controls. Additionally, compost application reduced the optimal levels of N<sub>mineral</sub> and increased its efficacy. Mkhabela and Warman, (2005) studied the effect of using municipal waste (MSW) compost in combinations with mineral fertilizers in potato and sweet corn production. They concluded that combining MSW compost with inorganic fertilizer produced higher yields than MSW compost alone. Additionally, they recommended this approach to enhance N availability to cultivated crops. Warman *et al.* (2011) compared different rates of MSW compost (21.7, 43.4, and 65.1 t ha<sup>-1</sup>) with an integrated treatment (50% compost+ 50% NPK) and full NPK treatment on potato yield and nutrients uptake for three years. The results showed that the integrated treatment produced higher yield than compost alone and was statistically equal to NPK treatment. They concluded that N-compost mineralization is slow to meet crop requirements, thus integrating other N sources that contain readily available N can enhance mineralization and meet potato requirement.

Another organic practice, which became a part of more sustainable production systems, is the green manuring. Green manures provide several benefits when grown as cover crops, i.e. protect soil from erosion and prevent nutrient loss over winter, and when incorporated preceding cash crops, improve soil quality and provide substantial nitrogen addition (Hirel *et al.*, 2011). Integrating green manures into potato rotations has been reported to increase N availability, improve tuber yield and quality as well as soil properties and suppress soil-borne diseases (Griffin and Hesterman, 1991). Numer-

ous investigations have reported the benefit of different green manures either legumes or non-legumes when preceded potato crop. Carter *et al.* (2003) found that potato tuber yield average was higher after Italian ryegrass than after red clover in a 2 year rotation during 11 years. Larkin, (2008) reported 9 to 12% potato yield increases after soybean-canola than after soybean-barly. Moreover, potato productivity in the rotation was higher than the continuous potato crop in a 3 year rotation. Sincik *et al.* (2008) compared the efficiency of three green manure species combined with mineral N fertilizer rates on potato yield. They found that potato yield response to N significantly varied according to green manure crops whereas potatoes following legumes produced approximately 36% to 38% higher tuber yield than following non-legumes in the absence of mineral N. Furthermore, yield was higher by 12.7 to 15% in the presence of mineral N fertilizer. In addition, incorporating legumes before potatoes reduced the economic optimum N rate by 30 kg ha<sup>-1</sup>. Campiglia *et al.* (2009) compared the efficiency of rapeseed (*Brassica napus* L.), Italian ryegrass (*Lolium multiflorum* L.), hairy vetch (*Vicia villosa* Roth.), snail medick (*Medicago scutellata* Mill.) and subclover (*Trifolium subterraneum* L.) on potato yield. The results showed that potato fresh marketable yield following subclover and hairy vetch was similar to that obtained by mineral nitrogen fertilization (48.5 t ha<sup>-1</sup>). These studies demonstrated the potential of green manures to provide potato with adequate nitrogen supply, resulting in yield equal to that of mineral nitrogen.

Another alternative to mineral N fertilizer which is increasingly used in sustainable production, particularly organic, is the compost extract. Applying compost extract was reported to enhance plant growth by providing soluble nutrients and growth promoters and improve soil quality by providing organic molecules (Ingham, 2005). Some research investigations reported the efficiency of compost extract as a nitrogen source in some horticultural crops. Welke, (2001) found that extracts from cattle manure composts substantially increased marketable number and weights of strawberries and broccoli heads. Hargreaves *et al.* (2009) reported the potential of compost extract to provide strawberries with adequate nutrients when considered as a sole nutrients source. Jontgae, (2010) reported similar onion yield following compost extract application to chemical and solid organic fertilizers. Pant *et al.* (2011) investigated the effects of vermicompost extract on yield and quality of pak choi (*Brassica rapa*) and reported yield increases as a result of extract application. However, investigations of compost extract efficiency on potato production either as nutrient source or bio-control agent

reported inconsistent results. El Nagar *et al.* (2001) studied the effect of compost extracts from on-farm, commercial compost and guano as a nutritional supplement. The compost was extracted in different water proportions (1:5 and 1:20) then raised to ten folds. Potato yield reflected a positive response to compost extracts application whereas extracts with high N content resulted in higher yields. Furthermore, compost extract application resulted in increased yield by 86 to 200%. However, Nikolic *et al.* (2003) found contradictory results to that reported by El Nagar *et al.* (2001) as the lower concentrations of compost extracts resulted in higher yield than concentrated ones when on-farm and commercial compost were extracted in water (1:5, 1:10 and 1:20 compost to water). Larkin, (2008) compared the efficiency of aerated compost extract (ACT), microbial inoculants and microhyza on the incidence of some tuber diseases and production of potato. He reported that Soil-applied aerated compost extract (ACT) and its combination with microbial inoculants reduced stem canker, black scurf, and common scab by 18–33% and increased yield by 20–23%. Nevertheless, the results were not persistent and depended on the preceding crop.

The literature has provided substantial information concerning organic residues incorporation benefits in enhancing agricultural systems sustainability. However, the more extensive use of organic residues as a nitrogen source in potato cropping systems still have a relative lack of quantitative information on the effect residues management practices on nitrogen supply, N use efficiency and economic returns. Furthermore, knowledge regarding potato growth patterns and performance adaptability to organic residues is still scarce. Therefore, the current study aimed to explore the influence of different organic residues as N source as well as diversified management practices on potato growth and productivity.

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# 1. CHAPTER 1

## Potato Performance under Integrated Organic Fertilization

### 2.1. ABSTRACT

Organic fertilizers have the potential to provide crops with adequate N and reduce economic and environmental risks of N losses. However, proper management strategies to synchronize N release with crop demand for sustained systems still a challenge. A field experiment was carried out in Viçosa MG, Brazil in 2010 to study the effect of integrating non-aerated aqueous compost extract with either the GM sunhemp (*Crotalaria juncea* L.) or organic compost on potato growth and productivity compared with the recommended mineral N rate. Nine treatments, (2×4+1) in a factorial scheme, were arranged in a split plot in RCBD with 4 replicates. Organic compost and the green manure sunhemp were incorporated before planting (188 kg N ha<sup>-1</sup>) combined with four compost extract (CE) concentrations (0, 30%, 60% and 90% CE to water V/V) which were supplied in the sub plots at 40, 50 and 60 days after planting. Potato nitrogen nutrition status was estimated employing SPAD index and leaf N-total content at 45, 55 and 65 DAP in the fourth expanded leaf from plant apex. Total dry matter production, N assimilation and partitioning between plant canopy and tubers, N recovery and N recovery efficiency were determined at 85 DAP. At harvest (103 DAP) fresh tuber yield and its components as well as N use efficiency were quantified. The obtained data were subjected to ANOVA and regression analysis (p<0.05). Furthermore, comparison between organic fertilizers combinations and MN control was implemented by Dunnett test (p<0.05). Finally, Pearson correlation between nutritional indices and total and marketable yield for organic fertilization treatments was tested.

SPAD index distinguished between sunhemp and compost at 55 and 65 DAP whereas great values were recorded under sunhemp. Meanwhile, leaf N-total content did not differentiate between compost and sunhemp over sampling. At 85 DAP, sunhemp and compost did not influence dry matter production, N partitioned in the canopy and tubers as well as N proportion within tubers. Meanwhile, sunhemp increased potato N recovery and N recovery efficiency by 13.3% and 7%, respectively. Inversely, compost increased tuber total and marketable yield. Dry matter content and production was not affected by either sunhemp or compost. Additionally, compost application presented superior NUE to sunhemp for total and marketable yield by.

SPAD index presented a strong quadratic response to CE concentrations ( $R^2=0.82$ ) at 55 DAP unlike the posterior evaluation which showed strong linear responses ( $R^2=0.87$ ). Meanwhile, leaf N-total content showed linear responses over sampling ( $R^2=0.98$ , 0.98 and 0.93 at 45, 55 and 65 DAP, respectively). N recovery, among the evaluated parameters at 85 DAP, was influenced by CE supply whereas a strong linear trend ( $R^2=0.98$ ) was obtained. Total and marketable yield was influenced by the interaction of CE with sunhemp and compost and increased linearly. On the hand, dry matter content and production were just influenced by CE concentrations and presented linear increases patterns.

Combinations of organic fertilizer presented lower SPAD values over sampling than MN control ( $p<0.05$ ). Meanwhile, leaf N-total content did not differ from the control in the early stages and presented lower values than MN control just at 65 DAP. Organic fertilization produced similar or higher dry matter and portioned less N within potato canopies and tubers than MN control but with no significant differences. Meanwhile, the N proportion within tubers to the total assimilated N was not affected by either organic or mineral fertilization. Inversely, organic fertilizers combinations presented inferior N recovery between 7.9 % and 31.9% to MN control. The majority of organic fertilizers treatments presented higher NRE as well as fresh tuber yield than MN control. However, dry matter content was not influenced by N sources despite the similar and/or higher dry matter production.

The tested correlations between SPAD and N-total and marketable yield recorded high and significant coefficients under green manure and compost with superiority under green manure. Under compost, SPAD index at 45 DAP presented low and insignificant correlation with total and marketable yield. Furthermore, leaf N-total content presented the same trend with marketable yield at 55 DAP. Generally, the tested nutritional indices presented a visible opportunity to predict yield at different growth stages through plant N status. Integrating non-aerated compost extract as a supplemental N source with sunhemp and compost sounds sustainable practice in potato production.

**Key words;** *Solanum tuberosum* L, organic fertilizers, compost extract, N efficiencies, productivity.

## 2.2. RESUMO

Os adubos orgânicos têm o potencial de suprir adequadamente as culturas com N, reduzindo custos de produção e os riscos ambientais. No entanto, as estratégias de manejo apropriadas para sincronizar a mineralização do N com a demanda das culturas ainda é um desafio. O experimento foi realizado em Viçosa, MG, Brasil, em 2010, e teve como objetivo estudar o efeito da aplicação conjunta de extrato de composto não-aerado com o adubo verde (*Crotalaria juncea* L.) ou composto orgânico, sobre o crescimento e produtividade de batata. O experimento consistiu de 9 tratamentos ( $2 \times 4 + 1$ ), em esquema de parcelas subdivididas em DBC com 4 repetições. O composto orgânico e a crotalária, incorporados antes do plantio em quantidades equivalentes a 188 kg de N ha<sup>-1</sup>, foram combinados com quatro concentrações do extrato de composto (EC) (0, 30%, 60% e 90% em água v/v), que foram aplicadas nas subparcelas aos 40, 50 e 60 dias após o plantio (DAP). O tratamento adicional foi a fertilização mineral de N (MN) na dose de 250 kg ha<sup>-1</sup>. O estado nutricional nitrogenado da batata foi estimado pelo índice SPAD e pelo teor de N-total na quarta folha expandida a partir do ápice aos 45, 55 e 65 DAP. A produção de matéria seca, assimilação de N e sua partição nos órgãos da planta, a recuperação de N e a eficiência de recuperação (ERN) foram determinadas aos 85 DAP, antes da senescência da parte aérea. Na colheita (103 DAP), a produtividade foi quantificada e foi determinada a eficiência de uso de N (EUN). Os dados obtidos foram submetidos à ANOVA e análise de regressão ( $p < 0,05$ ). Além disso, a comparação entre as combinações de adubos orgânicos e a testemunha (MN) foi executada pelo teste Dunnett ( $p < 0,05$ ). Finalmente, foi determinada a correlação de Pearson entre os índices nutricionais e a produção total e comercial.

Plantas que receberam massa de crotalária apresentaram maior índice SPAD aos 55 e 65 DAP do que as fertilizadas com composto. Entretanto, o teor de N-total foi similar nessas mesmas plantas. Aos 85 DAP, não houve influência da crotalária comparativamente ao composto sobre o acúmulo de matéria seca, a partição de N no dossel e tubérculos, bem como proporção N nos tubérculos.

Na colheita (103 DAP) a crotalária aumentou tanto a recuperação de N (13,3%) quanto a eficiência de recuperação de N (7%) pela batateira comparativamente à aplicação de composto. No entanto, o composto aumentou a produção total e comercial de tubérculos, O teor da matéria seca e a produção de matéria seca foram similares em plantas que receberam crotalária ou composto. Adicionalmente, a aplicação do compos-

to resultou em EUN superior à aplicação da crotalária quando considerada tanto a produção total quanto a comercial.

O índice SPAD apresentou uma resposta quadrática positiva em função de concentrações de EC ( $r^2 = 0,82$ ) aos 55 DAP, ao contrário das avaliações posteriores que mostraram respostas lineares ( $R^2 = 0,87$ ). Enquanto isso, o teor de N-total apresentou respostas lineares ao longo de amostragem ( $R^2 = 0,98, 0,98$  e  $0,93$  aos 45, 55 e 65 DAP, respectivamente). A recuperação de N, entre os parâmetros avaliados aos 85 DAP, foi influenciada pelo EC e aumentou linearmente ( $r^2 = 0,98$ ). A produção total e comercial foi influenciada pela interação da CE com a crotalária e o composto e aumentou linearmente sob ambos. O teor de matéria seca e sua produção aumentaram linearmente com somente as concentrações de EC.

As combinações de adubo orgânico apresentaram menores valores de SPAD do que a testemunha MN ( $p < 0,05$ ) nas datas de amostragem. Enquanto isso, o teor de N-total não diferiu do MN nos estágios iniciais e apresentou valores inferiores a MN apenas aos 65 DAP. Também comparativamente à aplicação de MN, as combinações de adubos orgânicos resultaram em resultados similares de produtividade de matéria seca e menor acúmulo de N nos dosséis e nos tubérculos. Inversamente, as combinações dos adubos orgânicos resultaram em menores recuperações de N entre 7,9% a 31,9%. A maioria dos tratamentos de adubos orgânicos resultou em maior ERN e produtividade de tubérculos do que MN. No entanto, o teor da matéria seca não foi influenciado pelas fontes de N apesar de semelhança ou superioridade da produção de matéria seca.

As correlações entre a produtividade total e comercial com o índice SPAD e os teores de N-total registraram coeficientes significativos sob adubo verde e a composto com superioridade sob adubo verde. O índice SPAD apresentou correlação baixa e insignificante com produção total e comercial aos 45 DAP. Além disso, o teor de N-total apresentou a mesma tendência com produção comercial aos 55 DAP. Geralmente, os índices testados podem ser utilizados para prever o rendimento em diferentes estágios de desenvolvimento. A integração de extrato de composto com a crotalária e o composto é uma prática que pode consistir em fonte de N para produção da batata.

**Palavras-chave;** *Solanum tuberosum* L, adubos orgânicos, extrato de composto, eficiência de N, produtividade.

### 2.3. INTRODUCTION

Potato crop (*Solanum tuberosum* L.) is highly responsive to N fertilizer. Therefore, adequate N supply is necessary for plant growth and development as well as for economic yields. It is well known that fertilizer-N over supply generates excessive vegetative growth, lower tuber quality, delay maturity, decline dry matter content and N use efficiency. On the contrary, N deficiency substantially reduces growth development, and limits yield (Alva, 2004). Studies on N uptake characteristics of potato have shown that applied N fertilizers usually exceed the optimal rate for maximum yield thus economic losses and environmental risks are common threats where potato production dominates (Munoz *et al.*, 2005). In addition, potato crop is known to have relatively low N recovery efficiency, up to 50 %, due to its naturally shallow and poorly developed root system (Tyler *et al.*, 1983).

Potato production worldwide mainly depends on the intensive use of chemical N fertilizers whereas recommendations for economic yields vary widely and reach up to 320 kg ha<sup>-1</sup> in Europe and the US and 250 kg ha<sup>-1</sup> in Canada and Brazil. The intensive use of chemical N fertilizers led to negative environmental impacts such as decreased soil organic matter and physical properties degradation due to increased mineralization (Fox, 2004), contamination of ground water by NO<sub>3</sub> leaching (Kraft and Stites, 2003) and reduced N-recovery by crops (Cambouris *et al.*, 2008). Sustainable production aims to create economic and environmental-friendly agriculture, with the emphasis on a self-sustaining biological system by further dependence on biological inputs. Therefore, the association of nutrient cycling with living organisms can fit well with the aim of sustaining soil fertility by employing natural processes rather than external inputs (Shepherd *et al.*, 2000).

Considerable research demonstrated the benefits of using organic fertilizers, i.e. composts, animal and green manures, to improve soil physical (water-holding capacity, porosity, and bulk density), chemical (pH, electrical conductivity, and nutrient content), and biological properties as well as plant growth (Wolkowski, 2003). However, organic fertilizers and residues vary greatly in their capacity to release nutrients, particularly N, to succeeding crops due to their chemical composition. Generally, soil N supply from organic fertilizers depends on both the initial availability of inorganic N in the amendments and also the long-term rate of mineralization and subsequent immobilization. Therefore, organic fertilizers can either be a source of plant available N or compete with plants for it. Thus, to successfully manage nutrient cycling from organic amend-

ments, it is necessary to know their biochemical composition and decomposition rate in order to predict N processes and its fate within the soil. In addition, the use of organic fertilizers as a N source do not necessarily ensure an adequate supply of available N and meet crop demand whereas the balance of N mineralization-immobilization process is highly unpredictable, even when N mineralization indicators, such as C/N ratio, are used. Accordingly, N supply from organic amendments may be too small or too high (Pang and Letey, 2000).

Research attempts to optimize the use of different organic sources and to achieve maximum crop yields demonstrated that combining organic sources, green manure residues and animal manures, with mineral fertilizers may be an effective approach to enhance N supply and synchrony with crop demand (Sikora and Enkiri, 2000). In a particular case of potato, different investigations have reported yield increases by integrating green manures with mineral N fertilizers. Li *et al.* (1999) obtained optimal potato production using only 70 kg N ha<sup>-1</sup> following plowing under grasslands. Riley, (2000) reported 80 kg N ha<sup>-1</sup> for a yield of 15 t ha<sup>-1</sup> as optimum level and up to 120 kg N ha<sup>-1</sup> for a yield of 40 t ha<sup>-1</sup>. Nyiraneza and Snapp, (2007) examined potato response to fallow and/or rye cover crop with 0N and 5.6 Mg ha<sup>-1</sup> poultry manure for 4 years in a field and pot experiments. Organic N source (manure, cover crop residues, or both) combined with mineral N availability was used to adjust fertilizer rate downward to provide an estimated 224 kg N ha<sup>-1</sup> for all treatments. They found that the integrated treatment (179 kg N ha<sup>-1</sup> fertilizer+ manure) consistently increased tuber yield and N uptake efficiency by 20% compared with the unamended conventional management (224 kg N ha<sup>-1</sup> fertilizer). Similarly, tuber yield and N uptake in the integrated treatment in the pot experiment was 14 to 33% higher than fertilized and unamended treatment. They concluded that integrating organic and inorganic N sources sounds an environmental and economic management strategy. Sincik *et al.* (2008) compared three green manure species combined with mineral N fertilizer (0, 75, 150 and 225 kg ha<sup>-1</sup>). They found that potato yield response to N varied significantly according to green manure species whereas potatoes following legumes produced approximately 36% to 38% higher tuber yields than following winter wheat in the absence of mineral N. Else more, yield was higher by 12.7 to 15% in the presence of mineral N fertilizer. In addition, incorporating legumes before potatoes reduced the economic optimum N rate by 30 kg ha<sup>-1</sup>.

Compost is another organic amendment which is gaining massive popularity because of environmental concerns associated with its disposal in landfills. Compost application at reasonable rates was reported to improve plant growth, soil physical properties and increase the available soil nutrient levels (Moldes *et al.*, 2007). Furthermore, compost application to soil has several benefits to crop yield and soil fertility by improving soil organic matter, water-holding capacity, nutrient retention and content, soil aggregation, and microbial activity (Griffin and Porter, 2004). However, some reports demonstrated the low potential of compost, as N source, to release N when incorporated prior to crops. Jimenez and Alvarez, (1993) reported that 16–21% of the total N in compost were available 6 months after incorporation. Hadas and Portnoy, (1997) reported 22% N recovery of compost-N, dependent on the soil type. Zhang *et al.* (2006) reported that the released N from compost was 10% in the first year after application with some reports of N release in the second year after application. Compost is often reported to be less effective in supplying available N when used as N source in the first year of application to the soil–plant system. In addition, compost is considered as a soil conditioner rather than as a nutrient source and the non-nutritional benefits to soil physical, chemical and biological properties are primarily responsible for the crop yield increases (Foley and Cooperband, 2002).

Previous investigations reported potatoes responses to combined compost and mineral N fertilizers. Berjón *et al.* (1997) tested the effect of three levels of compost incorporation (0, 18, 36 t ha<sup>-1</sup>) combined with three levels of mineral-N fertilizer (0, 125, 250 Kg N ha<sup>-1</sup>) for three years. Results showed that the combined application of compost and N-mineral stimulated plant development with respect to the non-treated control. Potato productivity indicated that this crop reacted strongly and positively to the N-mineral fertilizer application, but slightly to compost levels. In addition, compost application reduced the optimal N levels and increased the efficacy of mineral-N fertilizer. Mkhabela and Warman, (2005) studied the effect of using municipal waste compost in combinations with mineral fertilizers in potato production and sweet corn. They concluded that inorganic fertilizer (NPK) and the mixture its mixture with MSW compost produced higher yields than MSW compost alone. Moreover, MSW compost increased soil pH compared to inorganic fertilizer. However, due to lower compost-N availability (especially during the first year), application of MSW compost together with inorganic N is recommended to enhance N availability and afford N supply to the cultivated crops. Warman *et al.* (2011) compared different rates of MSW compost

(21.7, 43.4, and 65.1 t ha<sup>-1</sup>) with an integrated treatment (50% compost+ 50% NPK) and full NPK treatment on potato yield and nutrient uptake for three years. The obtained results showed that the integrated treatment produced higher yield than compost alone and was similar to NPK treatment. They concluded that N-compost mineralization is slow to meet crop requirements. Therefore, integrating other N sources that contain readily available N can enhance mineralization and meet potato nitrogen demand as well.

Another alternative to mineral N fertilizer which is broadly used in sustainable production systems, particularly organic, is the compost extract. Compost extract was a part of farming techniques centuries ago, when animal manures were steeped in water and the resulting liquid was poured around plants (Robson, 2000). Compost extract was defined by Bess, (2000) as the liquid obtained by many preparations using compost as a starting material. Alternatively, it is described as the liquid version of the original compost. Ingham, (2005) defined compost extract as the water extract of compost which is actually brewed and contains soluble nutrients and a diversity of bacteria, fungi and protozoa. In addition, compost extract produced by purposely adding water to compost and collecting the water that passed through the pile. This water contains soluble nutrients but very few organisms. By cycling water, number of times through the pile, nutrients concentration will increase, and organisms may grow reaching an adequate number to protect leaf and root surface.

The interest in organic extracts for use in agriculture and horticulture has grown rapidly in the recent past. Grobe, (1997) indicated that compost extract is a key element in the transition from conventional to biological agriculture. Applying compost extract through drip or flood irrigation enhances root growth by providing nutrients and growth promoters. In orchards and vineyards, it can insulate against cold. In potato farms, when compost tea is applied, it can prevent plants from blight incidence. Welke, (2001) studied the effect of using aqueous extracts of two composts, cattle and chicken manure, on fresh market crops (strawberries, lettuce, broccoli, and leeks). Cattle manure compost extract was effective in increasing marketable number and weight of strawberries and broccoli heads. Hargreaves *et al.* (2009) compared the effect of two different composts and their liquid extracts in association with mineral fertilizer on strawberries leaf N content, soil mineral N and other nutrients. Liquid extracts were supplied weekly until blooming. The results of the two seasons did not show persistent modifications of plant tissues and soil mineral N content. Moreover, compost extract was reported to

supply strawberries with sufficient nutrients when considered as a sole source. Jontgae, (2010) evaluated the efficiency of liquid organic fertilizer, six applications, at the rate of  $133.2 \text{ t ha}^{-1}$  ( $1154 \text{ mg N kg}^{-1}$ ) in relation to mineral and solid organic fertilizers using the same N dose on onion production. Onion presented inferior N uptake following liquid and solid organic fertilizers ( $65 \text{ kg ha}^{-1}$ ) to mineral ones ( $76.5 \text{ kg ha}^{-1}$ ). Inversely, yield following liquid manure application ( $46.9 \text{ ton ha}^{-1}$ ) was superior to that following chemical fertilizer ( $45.9 \text{ t ha}^{-1}$ ) with no significant differences. Pant *et al.* (2011) investigated the effect of vermicompost extract on yield and quality of pak choi (*Brassica rapa*). Extracts were combined with compost and synthetic fertilizer. Compost was extracted in water (1:10 v/v) and applied weekly at the rate of  $200 \text{ mL plant}^{-1}$  for four weeks. Results showed production increases following extract application. Moreover, the effect was most prominent when the extract was combined with compost.

Efficiency of compost extracts supply on potato production was investigated as well. El Nagar *et al.* (2001) studied the effect of different compost extracts, as a nutritional supplement with  $1 \text{ t ha}^{-1}$  of compost and guano in soil bed preparation, that derived from different composts; on-farm, commercial compost and guano. Composts were extracted in different water proportions (1:5 and 1:20) then extracts were raised to ten folds. The obtained extracts presented different chemical properties, which were attributed to dilution and compost feedstock composition. Potato yield presented positive responses to compost extract's application whereas extracts with high N content, increased tuber yield. Yield increases as a result of extract application ranged between 86 to 200% compared to control treatment. As well as N content of plant leaves, stems and tubers was increased following concentrated extracts application. In another trial by Nikolic *et al.* (2003), they compared two different composts (on-farm and commercial) which were extracted in three water proportions (1:5, 1:10 and 1:20 compost to water) for eight days. Results showed a gradual increase of total N and ammonia content by decreasing water proportion to compost across extraction. The obtained yield results were contradictory to that of El Nagar *et al.* (2001) as the low concentrations of compost extracts produced greater yield than concentrated ones.

Besides compost extract nutritive effects, some investigations reported its efficacy to control potato foliage diseases. Ghorbani *et al.* (2005) reported the role of compost extract to control potato late blight compared with agrochemicals. They indicated that extracts derived from different compost feedstocks of different ages suppressed leaflet blight infection. However, results were very limited and inconsistent. Moreover,

the effects were much smaller than copper oxychloride. Al-Mughrabi, (2007) compared the efficiency of compost extract with chemical fungicides to reduce potato late blight (*Phytophthora infestans*) incidence and severity. Results showed that extract application reduced late blight severity by 29%. He concluded that extract application is an environmentally safe tool for potato late blight control. Furthermore, such new tools are essential for fungicide resistance management. Larkin, (2008) compared the efficiency of aerated compost tea (ACT), microbial inoculants and mycorrhiza on the incidence of potato tuber diseases and production. He reported that soil-applied ACT and its combination with microbial inoculants reduced stem canker, black scurf, and common scab by 18–33% as well as increased yield by 20–23%. However, results were not persistent and the efficacy correlated to the preceding crop.

The literature suggests that a unique organic fertilization technique may not provide potato crop with an adequate nitrogen supply and additional N is probably required to satisfy crop requirements. The current study is a research approach to enhance the performance of organic fertilizers, as N sources to assure potato N demand and improve its productivity, by integrating various organic fertilizers. Additionally, estimate the efficiency of legume green manure and compost with compost extract on potato growth and productivity. Furthermore, investigate the usefulness of SPAD index and leaf N-total content in the potato N status diagnoses and yield prognoses.

## 2.4. MATERIALS AND METHODS

### 2.4.1. Experimental site, meteorological data and soil properties

A field experiment was conducted from June 18<sup>th</sup> to October 4<sup>th</sup>, 2010 in the Horta Velha Research facility of Universidade Federal de Viçosa MG, Brazil. The experimental farm is located at 693 m altitude, latitude 20°45' S and 42°51' W. The region is characterized by dry moderate to cold winter and rainy hot summer. Meteorological data across the experiment (Table 1) were obtained from a local weather station nearby the facility.

**Table 1.** Mean daily air and night temperatures and precipitation rate from June to October during potato plantation, 2010.

Variable	June	July	August	September	October
Mean daily temperature (°C)	15.1	17.0	16.1	18.8	20.3
Mean night temperature (°C)	13.7	15.4	14.5	17.4	19.3
Precipitation (mm)	0.06	0	0	6.6	63.6

Before installing the experiment, representative soil samples were collected by a probe to the depth of 30 cm and mixed in a composite sample. A sub-sample was collected and submitted to routine chemical analysis. Initial soil properties are shown in Table 2.

**Table 2.** Initial chemical characteristics of the experimental soil before sowing the green manure and amending fertilizers

Characteristics	Unit	Value	Method
OM	dag kg <sup>-1</sup>	3.4	Walkely-Black =Org C×1.724
pH (in water1:2.5)		5.8	
P	mg dm <sup>-3</sup>	19.8	Mehlich-1 extractor
K	mg dm <sup>-3</sup>	92	Mehlich-1 extractor
Ca <sup>2+</sup>	cmol <sub>c</sub> dm <sup>-3</sup>	2.1	KCL- 1mol L <sup>-1</sup> extractor
Mg <sup>2+</sup>	cmol <sub>c</sub> dm <sup>-3</sup>	0.6	KCL- 1mol L <sup>-1</sup> extractor
Al <sup>3+</sup>	cmol <sub>c</sub> dm <sup>-3</sup>	0	KCL- 1mol L <sup>-1</sup> extractor
Effective CTC	cmol <sub>c</sub> dm <sup>-3</sup>	2.94	
CTC in pH 7.0	cmol <sub>c</sub> dm <sup>-3</sup>	5.09	
V	%	58	
Rem-P	mg L <sup>-1</sup>	27.9	

#### 2.4.2. Treatments and experimental layout

The experiment consisted of 9 treatments organized in a factorial scheme (2×4 +1) and arranged in a split-plot design in a complete randomized block with four replicates. Two organic N sources, the green manure (GM) sunhemp (*Crotalaria juncea* L.) and organic compost (C), occupied the main plots plus the control treatment, the recommended mineral N dose (250 kg ha<sup>-1</sup>) according to Fontes, (2005). The GM and C were combined with four compost extract concentrations which comprised the sub plots. The experimental plot had an area of 7.5 m<sup>2</sup> with dimensions of 3.0 m width and 2.5 m length. Additionally, a distance of 0.5 m between plots and 1.0 meter between blocks were left as border to avoid contamination.

#### 2.4.3. Sunhemp production, characterization and incorporation

The sunhemp was sown as pre-crop, in the destined plots for GM, by January 15<sup>th</sup> (2010). Sunhemp seeds were sown on a 40 seed/m at 0.5 m apart lines after soil plowing by moldboard and hoeing with a rotary tiller. The GM was grown for eighty five days until full blooming. Thereafter, the GM was harvested, uniformly distributed and incorporated using a rotary tiller. To estimate the N amount present on the green manure biomass, two random linear meters, before harvest, from each block were harvested and weighed. Then sub samples were collected for dry matter, total N and total

carbon content determination. Thereafter, the remaining biomass was returned to plots. Dry samples were ground to pass through a 2 mm sieve and subjected to total N content determination by micro-Kjledahl (Miyazawa *et al.*, 2009). Total carbon content (C %) was determined by Walkely-Black.

The GM biomass presented 23% dry matter, 1.7% N-total content, 28% total carbon and 16.5 C/N ratio. The estimated N amount of the green manure biomass was 188 kg ha<sup>-1</sup>. It was projected to incorporate the green manure fifteen days before planting potato. However, the delayed potato seed delivery had extended the interval between incorporation and planting for more two weeks than was anticipated.

#### 2.4.4. Compost preparation and application

On farm compost was previously prepared in the Agroecologia Facility on UFV campus. For compost preparation, 8.0 m<sup>3</sup> of *Pennisetum purpureum*, 2.0 m<sup>3</sup> of chicken manure and 2.5 m<sup>3</sup> of coffee berry bark were mixed and composted for 4 months until maturity as the volume decreased, temperature stabilized at low values and color changed to dark. Mature compost was incorporated in the base dressing, as N source, as well as in compost extract preparation.

Before application, the pile was homogenized and a representative sample was collected for chemical composition determination. The sample was oven dried at 60 °C until constant weight then ground and subjected to chemical analysis. Compost chemical characteristics are shown in Table 3.

**Table 3.** Organic compost chemical characteristics

Characteristics	Unit	Value	Method
pH		7.25	in H <sub>2</sub> O (1:2,5), KCL and CaCL <sub>2</sub>
DM	%	56	
OM	%	28.4	Walkely-Black; OM=org C×1.724
N	%	1.66	Kjledahl
P	%	1.67	Mehlich-1 extractor
K	%	1.36	Mehlich-1 extractor
Ca	%	5.77	KCL- 1mol L <sup>-1</sup> extractor
Mg	%	0.66	KCL- 1mol L <sup>-1</sup> extractor
Zn	ppm	334	Mehlich-1 extractor
Fe	ppm	16695	Mehlich-1 extractor
Mn	ppm	488	Mehlich-1 extractor
Cu	ppm	47	Mehlich-1 extractor
B	ppm	31.3	Hot water extraction
C/N		9.94	

Compost was applied immediately in the furrows before planting. The N amount added by compost was the same of the amount provided by the sunhemp biomass ( $188 \text{ kg ha}^{-1} \text{ N}$ ) and was equivalent to  $20.24 \text{ t ha}^{-1}$  of fresh compost.

#### **2.4.5. Soil preparation and potato planting**

Soil was disc harrowed and tilled by a rotary tiller to incorporate the green manure then furrowed (0.75m apart). Before planting, macro-nutrients rather than N, phosphorus ( $420 \text{ kg ha}^{-1}$ ), potassium ( $220 \text{ kg ha}^{-1}$ ), magnesium ( $200 \text{ kg ha}^{-1}$ ) and micro-nutrients; boron, copper, zinc ( $10 \text{ kg ha}^{-1}$  of each) and molybdenum ( $250 \text{ g ha}^{-1}$ ) were supplied to all plots. Mineral nutrients were obtained from simple superphosphate (18%  $\text{P}_2\text{O}_5$ ), potassium chloride (60% de  $\text{K}_2\text{O}$ ), magnesium sulfate (9 %  $\text{MgO}$ ), borax (11% B), copper sulfate (24% Cu), zinc sulfate (22% Zn) and sodium molybdate (39% Mo), respectively. Fertilizers were blended and identically broadcasted inside the furrows.

Fertilizer-N, in the control treatment (MN), was supplied as ammonium sulfate (20 % N) and was split into two applications as  $150 \text{ kg ha}^{-1}$  in the base dressing inside the furrows and  $100 \text{ kg ha}^{-1}$  was as a side dressing at 21 days after emergence (Fontes, 2005). 'Ágata' homogeneous seeds, 35-50 mm diameter, were pre-germinated in room temperature and indirect sunlight. When sprouts reached around 3 cm length, tubers were manually distributed on furrows and covered with approximately 10 cm of soil. Ridges were created by hoeing the soil up at 22 days after emergence. Furthermore, agronomic practices such as irrigation and sanitation were implemented following recommendation for potato plantation in the region.

#### **2.4.6. Compost extracts preparation and application**

Non-aerated compost extract was prepared using the above mentioned compost. The Bucket fermentation method (Divers *et al.*, 2007) was adopted. Compost was extracted in water (1:5 weight/volume). Compost was weighed then placed into plastic permeable bags and immersed in water for five days to allow nutrient release into the solution. Thereafter, the sludge was removed and volumetric dilutions using different proportions of extract to water were implemented (Table 4).

Compost extracts were applied over potato plants using manual sprayer, with the rate of 1 L plant<sup>-1</sup> in each application, at 40, 50 and 60 days after planting which corresponded to 23, 33 and 43 days after emergence. Samples of diluted extract as well as water were collected and kept in 4 °C until running chemical analysis. Samples were filtered and electrical conductivity (EC) and pH were measured at room temperature using EC meter and glass electrode pH meter. Mineral N,  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , was deter-

mined by steam distillation using 25 mL of the filtered extract with devarda alloy and magnesium oxide (MgO), respectively (Bremner and Mulvaney, 1982). Thereafter, total N was determined following the procedure described by Cabrera and Beare, (1993). Twenty-five mL of the extract were acidified with 5 mL of H<sub>2</sub>SO<sub>4</sub> and heated (120 °C) until volume reduction to 10 mL. After cooling, the mixture was transferred to 100 ml tubes and N-total content was determined by micro- Kjeldhal method (Miyazawa *et al.*, 2009). The characteristics of compost extract concentrations are shown in Table 5.

**Table 4.** Description of compost extracts preparation and application

Main plots	Formula (CE :W)	No. of applications
Green manure	00 : 100	3
	30 : 70	3
	60 : 40	3
	90 :10	3
Compost	00 : 100	3
	30 : 70	3
	60 : 40	3
	90 :10	3
Control	0	0

**Table 5.** Chemical properties of the filtered compost extracts before application

CE (% , CE/W)	EC (ms cm <sup>-1</sup> )	pH	N-NH <sub>4</sub> <sup>+</sup> (mg L <sup>-1</sup> )	N-NO <sub>3</sub> <sup>-</sup> (mg L <sup>-1</sup> )	N-total (mg L <sup>-1</sup> )	N supplied* (kg ha <sup>-1</sup> )
0	0.032	6.10	2.32	2.37	5.9	0.885
30	0.550	7.23	3.64	43.13	65.00	9.75
60	1.470	7.28	4.52	88.40	112.5	16.88
90	1.870	7.40	6.17	104.3	165.0	24.75

Values represent means of three analyzed samples.

\*N supplied represents the sum of three applications.

#### 2.4.7. Potato N nutrition status

Measurements of plant N status were implemented employing two methods, non-destructive or indirect chlorophyll determination using the portable SPAD 502, Soil and Plant Analysis System, and N-total content in the fourth expanded leaf from plant apex. The standpoint of data collection was at 45 DAP, as 5 days after the first application of compost extract. Additionally, two sequential readings were conducted at 55 and 65 DAP.

SPAD readings were collected from five representative plants of the two central rows in each plot. Readings were collected from the leaflet between leaf margin and

mid rib in the morning between 8 to 11 o'clock. The mean value of five readings represented one plant and the mean value of five plants represented 1 plot.

After SPAD reading collection, the same leaves were detached, gathered and submitted to N-total content determination. The leaves were cleaned and dried at 60 °C until constant weight then ground to pass through a 2 mm sieve. N-total content was determined by micro-Kjeldhal (Miyazawa *et al.*, 2009).

#### **2.4.8. Dry matter production, N recovery (NR) and N recovery efficiency (NRE) quantification**

Total dry matter production and nitrogen partitioning between plant foliage and tubers were determined at 85 DAP, before senescence, following the method described by Sullivan *et al.* (2008). Three sequential plants from each plot were harvested, excluding roots, and segmented into foliage and tubers. The foliage was cleaned and dried in air forced oven at 60 °C until constant weight. Dry weights were recorded, then samples were ground to pass through a 2 mm sieve and the total nitrogen content (%) was determined thereafter represented in content (g kg<sup>-1</sup>). Tubers were weighed and a sample of five tubers was collected, washed by tap water followed by distilled water and left to dry. Tubers were cut into cubes of approximately 1 cm<sup>3</sup> and mixed. A sub sample of 200 g was dried at 60 °C until constant weight. Samples were ground to pass through a 2mm sieve and N-total content (%) was determined. N content was multiplied by dry matter weight to represent total nitrogen content (kg ha<sup>-1</sup>). Sum of foliage and tubers dry matter weights were expressed in kg per hectare basis (50,000.00 plants ha<sup>-1</sup>) to represent total dry matter per hectare. Furthermore, nitrogen recovery (kg ha<sup>-1</sup>) was obtained by summing foliage and tubers N-content to represent N-total per plant and then expressed in a per hectare basis.

N Recovery Efficiency (NRE %) was calculated by the following equation:

$$\text{NRE (\%)} = \frac{\text{N recovery for treatment (kg ha}^{-1}\text{)}}{\text{N applied in the same treatment (kg ha}^{-1}\text{)}} \times 100$$

Where N recovered was calculated as mentioned above and N applied was known as the N given for each treatment.

#### **2.4.9. Yield components determination**

By October 4<sup>th</sup> (103 DAP), 17 plants of the two central rows were harvested. Tubers were left two hours for curing then graded according to the standards of Ministério da Agricultura, Agropecuária e Abastecimento (Brazilian Ministry of Agriculture, 1995). Yield grades were as follow; class 1 (diameter > 8,5 cm), class 2 (8,5 ≤ tubers diameter < 4,5 cm), class 3 (4,5 ≤ tubers diameter < 3,3 cm) and class 4 included

tubers with a diameter less than 3,3 cm and blemish tubers, with any commercial disorders such as greenish, rotten and infected by insects or diseases. The sum of the classes 1, 2 and 3 represented commercial yield and, the sum of all classes represented total yield.

Dry matter content (%) was determined by collecting 5 tubers from the commercial classes. Tubers were washed by running water followed by distilled water and cut into cubes of 1 cm<sup>3</sup> and mixed. A composite sub sample of 200 g was dried in 60 °C until constant weight. Dry matter (%) content was multiplied by total yield to calculate dry matter production.

Nitrogen use efficiency (NUE, kg tuber kg<sup>-1</sup> N) for total and commercial yield was calculated by dividing total and marketable yield by the applied nitrogen.

#### **2.4.10. Statistical analysis**

The obtained data were subjected to analysis of variance ( $p < 0.05$ ) using SAEG software (V, 9.1, 2007). ANOVA was performed in sequential steps. First of all, general ANOVA was performed including the control (MN) considering the experimental design as a complete randomized block design with nine treatments. Moreover, comparison between the MN control and organic fertilizers combinations was implemented by Dunnett test ( $p \leq 0.05$ ). Thereafter, the control treatment was excluded and organic fertilizers treatments were subjected split plot analysis. During this step, regression analysis of compost extract efficiency was implemented. Regression models were selected according to determination coefficient and biological behavior of each variable. Additionally, data of SPAD index and N-total content was analyzed separately within each sampling event. Moreover, correlations between SPAD index, N-total, total and marketable yield for compost and sunhemp were tested, using 16 data sets. Based on coefficient significance, linear models between variables were adjusted. Finally, the two to separate ANOVA were merged to include the contrast of control versus factorial treatments. Then, sum square of error (a) and degree of freedom were corrected.

## 2.5. RESULTS

### 2.5.1. Potato N nutrition status

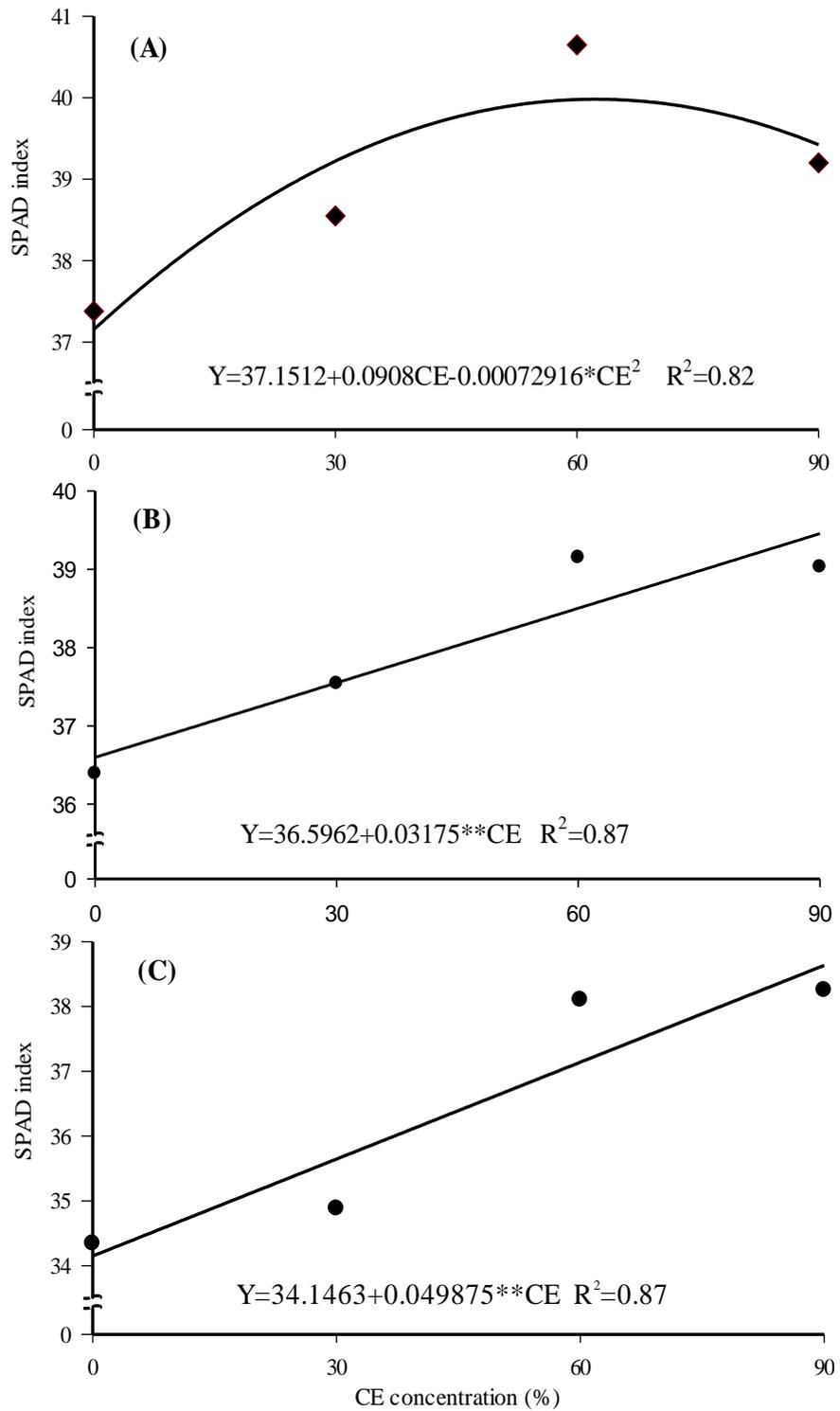
Analysis of variance showed that GM and compost incorporation in the pre-planting did not influence the SPAD reading after the first application of compost extract at 45 DAP (Table 6). However, from 55 DAP on SPAD index presented higher and significant values under GM than under compost treatments ( $p < 0.05$ ). Meanwhile, despite SPAD values remained higher under GM than compost, a decline in SPAD values was observed under both green manure and compost at 65 DAP.

At 45 DAP, the interaction between compost extract and organic N sources was significant. However, no regression model was adjusted under GM. On the other hand, SPAD index presented a quadratic response to compost extract concentrations under compost (Figure 1 A). Maximum SPAD value was 39.98 and corresponded to 62.3 % CE concentration. Unlikely, at 55 and 65 DAP, the interaction between organic N sources and compost extract was not significant. Furthermore, SPAD index was influenced by compost extract concentrations and presented linear patterns to compost extract concentrations (Figure 1 B and C) with high determination coefficients ( $R^2 = 0.87$ ) for both evaluations

**Table 6.** SPAD index readings as affected by sunhemp (GM) and organic compost (C) at 45, 55 and 65 days after planting

Treatment	SPAD index readings		
	45 DAP	55 DAP	65 DAP
GM	38.73 <sup>a</sup>	38.36 <sup>a</sup>	36.78 <sup>a</sup>
C	38.94 <sup>a</sup>	37.70 <sup>b</sup>	36.00 <sup>b</sup>
CV (%)	4.75	1.58	3.12

Means followed by the same letter in the column are not significantly different by F test ( $p \geq 0.05$ ).



**Figure 1.** SPAD index readings on potato leaf as affected by compost extract concentrations (CE) at 45 (A), 55 (B) and 65 (C) DAP.

Comparison between MN control and organic fertilizers combinations (CE with GM and C) for the three sampling events (Table 7) showed lower SPAD values under organic fertilizers than the MN control regardless of measurement time. Furthermore, SPAD values under MN control remained high until the last evaluation event.

**Table 7.** Comparison of the fourth leaf SPAD index between MN control and organic fertilizers combinations at 45, 55 and 65 days after planting (DAP)

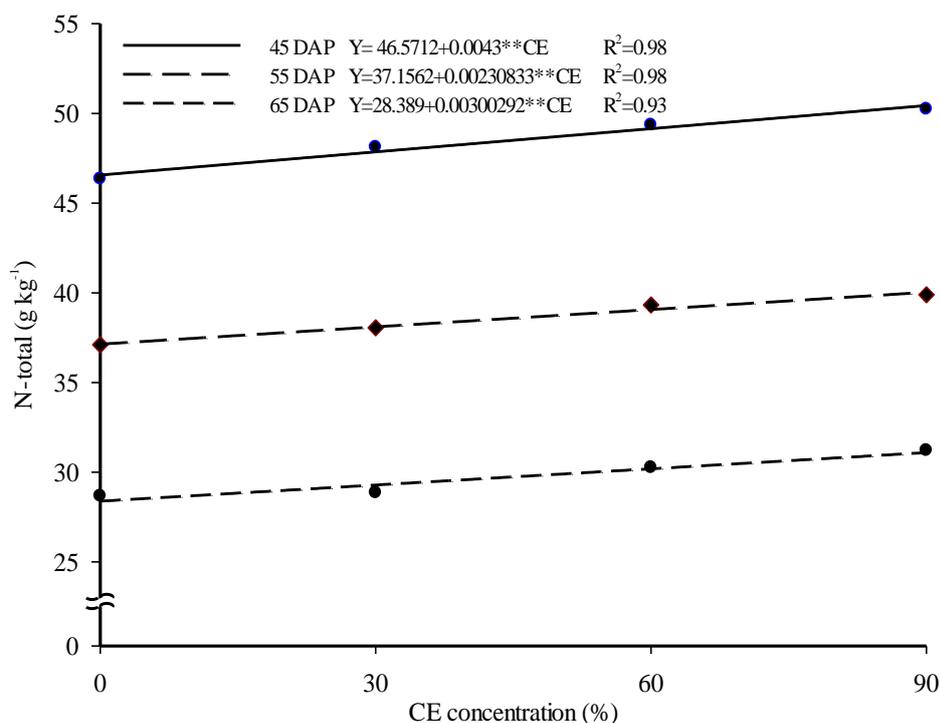
Treatment	SPAD		
	45 DAP	55 DAP	65 DAP
MN	43.48	44.25	45.95
GM0	38.75 *	36.78 *	34.68 *
GM30	39.13 *	38.38 *	35.35 *
GM60	38.55 *	39.28 *	38.63 *
GM90	38.50 *	39.00 *	38.48 *
C0	37.38 *	36.00 *	34.00 *
C30	38.55 *	36.70 *	34.40 *
C60	40.65 *	39.03 *	37.60 *
C90	39.20 *	39.05 *	38.03 *
LSD	2.02	1.13	1.52
CV (%)	2.87	1.62	2.27

Means followed by \* in the column are significantly different from MN control by Dunnett test ( $p < 0.05$ ). GM; green manure, C; compost and numerical values adjacent refer to CE concentration.

Analyses of variance of the green manure and compost effect on the fourth leaf N-total content at sampling events did not show significant differences by F test ( $p \geq 0.05$ ). At 45 DAP, leaf N-total content presented approximately  $55 \text{ g kg}^{-1}$  and declined to reach  $48.5 \text{ g kg}^{-1}$  at 55 DAP. Furthermore, the decline was more drastic after 55 days whereas it reached  $29.7 \text{ g kg}^{-1}$  65 DAP for compost and green manure.

The interaction between organic fertilizers and CE concentrations was not significant over the three sampling events ( $p \geq 0.05$ ). Meanwhile, leaf N-total content was influenced by CE concentrations and increased linearly by increasing concentration at all sampling dates (Figure 2).

Comparison between MN control and organic fertilizers combinations (green manure, compost and compost extract) presented no significant differences by Dunnett test ( $p \geq 0.05$ ) at 45 and 55 DAP (Table 8). Additionally, treatments CE90 at 45 DAP and GM60 and CE90 at 55 DAP were statistically greater than MN control. However, organic fertilizers combinations resulted in statistically lower N-total content than MN control at 65 DAP ( $p < 0.05$ ).



**Figure 2.** Effect of compost extract concentrations (CE %) on potato leaf N-total content at different sampling times.

**Table 8.** Comparisons of leaf N-total content between the MN control and organic fertilizers combinations at 45, 55 and 65 days after planting (DAP)

Treatment	N-total (g kg <sup>-1</sup> DM)		
	45 DAP	55 DAP	65 DAP
MN	47.90	38.00	36.00
GM0	46.10	37.50	28.80 *
GM30	48.70	38.80	29.30 *
GM60	49.50	41.10 *	29.70 *
GM90	49.70	39.60	31.10 *
C0	46.70	36.70	28.60 *
C30	47.60	37.40	28.40 *
C60	49.20	38.60	30.80 *
C90	50.70 *	40.30 *	31.30 *
LSD	2.13	1.98	2.32
CV (%)	2.46	2.78	4.26

Means followed by \* in the column are significantly different from MN control by Dunnett test ( $p < 0.05$ ). GM; green manure, C; compost and numerical values adjacent refer to CE concentration.

### 2.5.2. Whole plant dry matter production, N recovery and N recovery efficiency before senescence of potato plants

Analysis of variance for GM and compost influence on dry matter production at 85 DAP (Table 9) did not present significant difference ( $p \geq 0.05$ ) despite the greater production under GM than compost. Potato aboveground N content was not influenced

by the organic N source whereas the difference between GM and compost was not significant ( $p \geq 0.05$ ). Meanwhile, green manure incorporation resulted in greater N accumulation within potato tubers than compost. However, the difference was not significant by F test ( $p \geq 0.05$ ). Accumulated N proportion within potato tubers to total N (TNP) was not affected by organic fertilizer ( $p \geq 0.05$ ) whereas as N proportion presented the same ratio under both green manure and compost.

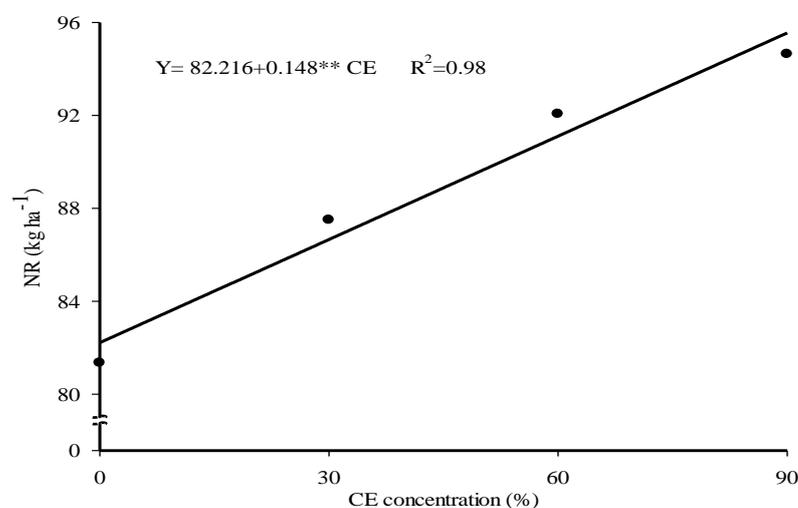
Analysis of variance for potato N recovery showed significant difference between green manure and compost ( $p < 0.05$ ). The GM sunhemp increased potato N recovery by approximately 16% more than compost (Table 9). Furthermore, N recovery efficiency was higher after sunhemp incorporation by approximately 7% than after compost as well.

**Table 9.** Whole plant dry matter production (DM), aboveground N (FN) and, tubers N (TN) content, tubers` N proportion (TNP), N recovery (NR) and N recovery efficiency (NRE) as affected by green manure (GM) and compost (C) at 85 DAP

Treatment	DM (t ha <sup>-1</sup> )	FN (Kg ha <sup>-1</sup> )	TN (Kg ha <sup>-1</sup> )	TNP	NR (kg ha <sup>-1</sup> )	NRE (%)
GM	8185.19 <sup>a</sup>	14.26 <sup>a</sup>	80.22 <sup>a</sup>	0.83 <sup>a</sup>	95.73 <sup>a</sup>	47.62
C	7930.38 <sup>a</sup>	13.82 <sup>a</sup>	67.70 <sup>a</sup>	0.83 <sup>a</sup>	82.04 <sup>b</sup>	40.77
CV (%)	29.01	37.90	25.67	26.22	6.06	-

Means followed by the same letter in the column are not significantly different by F( $p \geq 0.05$ )

Dry matter production, accumulated N within the potato foliage and tubers and tubers N proportion were not influenced by both compost extract and by the interaction between CE and organic fertilizers by F test ( $p \geq 0.05$ ). Meanwhile, N recovery was influenced by CE concentrations alone. Increasing compost extract concentration linearly increased N recovery with a high determination coefficient (Figure 3).



**Figure 3.** Potato N Recovery (kg ha<sup>-1</sup>) as influenced by compost extract (CE) concentrations at 85 DAP.

Comparisons between MN control and organic fertilizers combinations by Dunnett test (Table 10) showed no significant differences in total dry matter production ( $p \geq 0.05$ ) despite the greater production under some combination. Meanwhile, potato foliage and tubers presented lower accumulated N under organic fertilizers than MN control but with no statistical difference ( $p \geq 0.05$ ). As well as tubers N proportion presented similar or higher ratios than MN control but with no significant difference.

**Table 10.** Comparison of dry matter production (DM), foliage N (FN) and tubers N (TN) content, N recovery (NR) and N use efficiency (NRE) between MN control and organic fertilizers combination treatments

Treatment	DM (kg ha <sup>-1</sup> )	FN (kg ha <sup>-1</sup> )	TN (kg ha <sup>-1</sup> )	TNP	NR (kg ha <sup>-1</sup> )	NRE (%)
MN	7910.75 ns	20.86 ns	88.61 ns	0.813 ns	109.4	43.76
GM 0	8227.50	16.29	74.83	0.823	88.14 *	46.66
GM30	8164.75	14.68	81.52	0.848	96.19 *	48.71
GM60	8459.25	15.10	82.73	0.838	97.83 *	47.75
GM90	7889.25	18.95	81.79	0.811	100.74	47.35
C 0	7669.50	12.03	62.52	0.839	74.59 *	39.47
C30	7750.38	15.51	63.27	0.804	78.78 *	39.90
C60	7822.88	14.00	72.28	0.835	86.28 *	42.11
C90	8478.75	15.85	72.69	0.824	88.53 *	41.61
LSD	3055.3	9.41	31.82	0.28	10.79	-
CV (%)	21.15	30.31	21.61	2.46	9.11	-

Means followed \* in the column are significantly different from the MN control by Dunnett test ( $p < 0.05$ ). GM; green manure, C; compost and numerical values adjacent refer to CE concentration.

Organic fertilizers combinations presented statistically lower N recovery than MN control with the exception to GM90 which was similar to MN control despite the low numerical mean.

Nitrogen recovery efficiency (NRE %) by potato plants presented different means and depended on the organic nitrogen source. Combinations of compost extract with green manure presented greater recovery efficiency than MN control as well as combinations with compost. Additionally, gradual increase in N recovery by increasing CE concentration was observed under both green manure and compost.

### 2.5.3. Tuber yield components

Analysis of variance for tuber yield components showed significant influences of the GM and compost (Table 11). Decay yield (Class 4) was higher under compost than under the GM ( $p < 0.05$ ) by approximately 15%. Meanwhile, tuber (class 3) was

higher by approximately 7%. On the other hand, despite the greater production of class 2 under compost, the difference was insignificant ( $p \geq 0.05$ ).

Fresh marketable yield was greater for compost than green but the difference was not significant ( $p > 0.05$ ). However, total yield was higher under compost than under the GM sunhemp. Total yield increment by compost represented around 3%.

**Table 11.** Comparison of tuber yield components as affected by green manure (GM) and compost (C)

Treatment	Class 4 (kg ha <sup>-1</sup> )	Class 3 (kg ha <sup>-1</sup> )	Class 2 (kg ha <sup>-1</sup> )	MY (kg ha <sup>-1</sup> )	TY (kg ha <sup>-1</sup> )
GM	1369.98 <sup>b</sup>	6168.14 <sup>b</sup>	36454.21 <sup>a</sup>	42622.35 <sup>a</sup>	43992.32 <sup>b</sup>
C	1581.45 <sup>a</sup>	6601.63 <sup>a</sup>	37067.75 <sup>a</sup>	43669.38 <sup>a</sup>	45250.83 <sup>a</sup>
CV (%)	6.37	1.23	3.06	2.75	2.78

Means followed by the same letter in the column are not significantly different by F test ( $p \geq 0.05$ ). Class 4; (diameter <3.3 cm plus blemish), Class 2; ( $8.5 \leq$  diameter  $\geq 4.5$  cm), Class 3; ( $4.5 \leq$  diameter  $\geq 3.3$  cm), MY, marketable yield and TY, total yield.

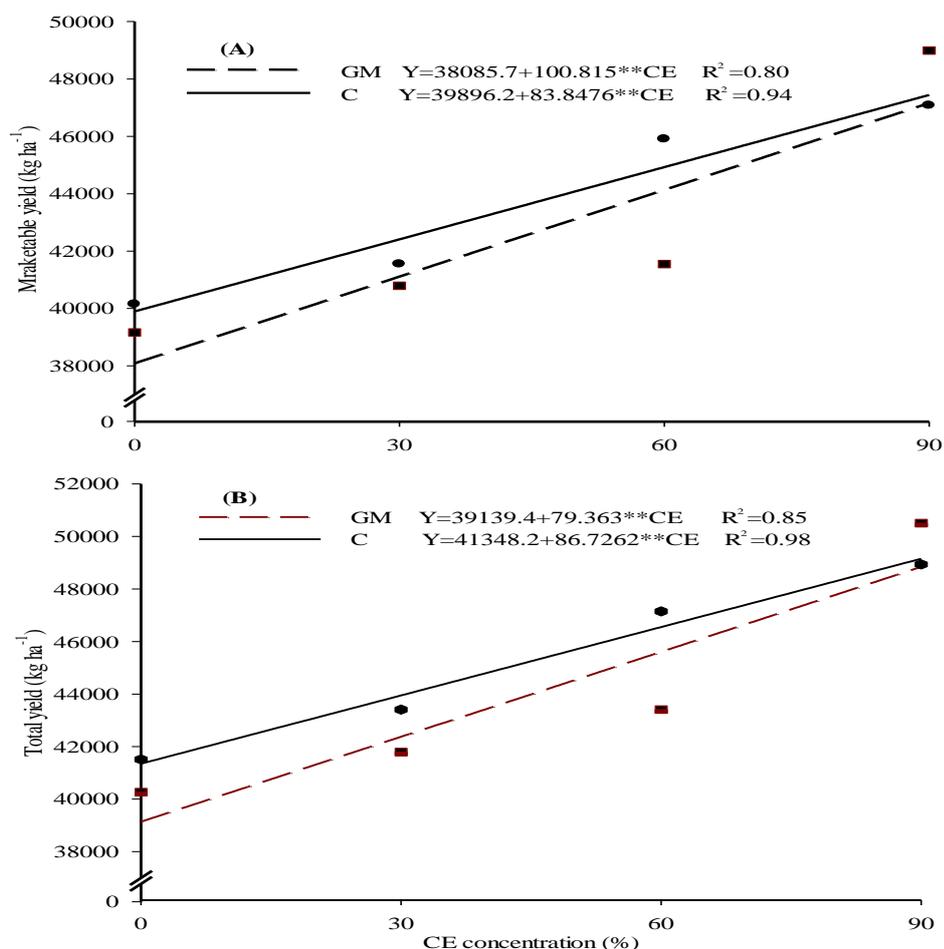
The interaction effect between organic fertilizers (GM and C) and compost extract concentrations showed significant influence on fresh tubers` classes ( $p < 0.05$ ). However, tuber classes (4, 3 and 2) increases as influenced by compost extract, under green manure and compost, were fluctuating. Thus, no regression models were adjusted. Additionally, means comparison was implemented (Table 12) by Tukey test ( $p \leq 0.05$ ).

**Table 12.** Fresh tuber yield classes as influenced by compost extract concentrations (CE) under the green manure (GM) and organic compost (C)

CE (%)	Class 4 (kg ha <sup>-1</sup> )		Class 3 (kg ha <sup>-1</sup> )		Class 2 (kg ha <sup>-1</sup> )	
	GM	C	GM	C	GM	C
0	1369.98 <sup>b</sup>	1581.45 <sup>a</sup>	6168.14 <sup>b</sup>	6601.63 <sup>a</sup>	36454.21 <sup>a</sup>	37067.75 <sup>a</sup>
30	999.500 <sup>b</sup>	1862.82 <sup>a</sup>	6081.23 <sup>b</sup>	8323.09 <sup>a</sup>	34707.56 <sup>a</sup>	33223.86 <sup>a</sup>
60	1862.32 <sup>a</sup>	1244.14 <sup>b</sup>	8323.09 <sup>a</sup>	5770.19 <sup>b</sup>	33223.86 <sup>b</sup>	40134.23 <sup>a</sup>
90	1516.50 <sup>b</sup>	1856.50 <sup>a</sup>	5561.02 <sup>b</sup>	6806.33 <sup>a</sup>	43430.30 <sup>a</sup>	40272.88 <sup>b</sup>
CV (%)	2.62		3.52		4.52	

Means followed by the same letter within rows under the same class are not statistically different by Tukey test ( $p \geq 0.05$ ). Class 4; diameter <3,3 cm plus blemish, Class 2; ( $8.5 \leq$  diameter  $\geq 4.5$  cm), Class 3; ( $4.5 \leq$  diameter  $\geq 3.3$  cm), MY; marketable yield and TY, total yield.

Fresh tuber marketable and total yield were significantly influenced by the interaction between organic N sources and CE concentrations ( $p < 0.05$ ). Both marketable and total yield presented linear increase patterns to CE concentrations under both GM and compost (figure 4 A and B). The determination coefficient for the adjusted regression models under compost was higher than under GM.



**Figure 4.** Effect of CE concentrations on marketable yield (A) and total yield (B) under green manure (GM) and organic compost (C) as N sources

Comparison of combining CE with green manure and compost by Tukey test (Table 13) showed that CE application resulted in similar yield under compost and sunhemp. However, two exceptions were observed whereas, CE 60% under compost resulted in higher and significant marketable yield than under green manure. On the other hand, CE 90% resulted in higher total yield under GM than under compost.

**Table 13.** Comparison of marketable yield (MY) and total yield (TY) as influenced by compost extract concentrations (CE) under green manure (GM) and organic compost (C) as N organic sources

CE (%)	MY (kg ha <sup>-1</sup> )		TY (kg ha <sup>-1</sup> )	
	GM	C	GM	C
0	42622.35 <sup>a</sup>	43669.38 <sup>a</sup>	43992.32 <sup>a</sup>	45250.84 <sup>a</sup>
30	40788.80 <sup>a</sup>	41546.95 <sup>a</sup>	41788.29 <sup>a</sup>	43409.78 <sup>a</sup>
60	41546.95 <sup>b</sup>	45904.42 <sup>a</sup>	43409.28 <sup>b</sup>	47148.56 <sup>a</sup>
90	48991.21 <sup>a</sup>	47079.21 <sup>a</sup>	50507.68 <sup>a</sup>	48935.67 <sup>a</sup>
CV (%)	4.02		3.88	

Means followed by the same letter in the row are not statistically different by Tukey test ( $p \geq 0.05$ )

Comparison of fresh tuber yield classes, total and marketable yield between organic fertilizers combinations and MN control by Dunnett test is shown in Table 14. CE applied with compost produced a greater proportion of decay tubers (class 4) than MN control. Meanwhile, the high concentrations of CE (60 and 90%) combined with sunhemp produced more class 4 tubers than MN.

**Table 14.** Comparison of fresh tuber yield components between MN control and organic fertilizers combination treatments

Treatment	Class 4 (kg ha <sup>-1</sup> )	Class 3 (kg ha <sup>-1</sup> )	Class 2 (kg ha <sup>-1</sup> )	TY (kg ha <sup>-1</sup> )	MY (kg ha <sup>-1</sup> )
MN	1049.68	4194.60	36,640.67	41,884.94	40,835.27
GM0	1101.63	4707.20 *	34,455.23	40,264.05	39,162.43
GM30	999.500	6081.24 *	34,707.56	41,788.29	40,788.80
GM60	1862.32 *	8323.10 *	33,223.86 *	43,409.28	41,546.96
GM90	1516.46 *	5561.03 *	43,430.19 *	50,507.68 *	48,991.21 *
C0	1462.42 *	5506.91 *	34,640.02	41,509.32	40,146.94
C30	1862.82 *	8323.09 *	33,223.86 *	43,409.78	41,546.96
C60	1244.14 *	5770.19 *	40,134.23 *	47,148.56 *	45,904.42 *
C90	1856.47 *	6806.33 *	40,272.88 *	48,935.67 *	47,079.21 *
LSD	99.87	341.99	2773.75	2882.69	2871.68
CV (%)	3.91	3.11	4.22	3.64	3.74

Means followed by \* in the column are significantly different from MN control by Dunnett test ( $p < 0.05$ ). GM; green manure, C; compost and numerical values adjacent refer to CE concentration.

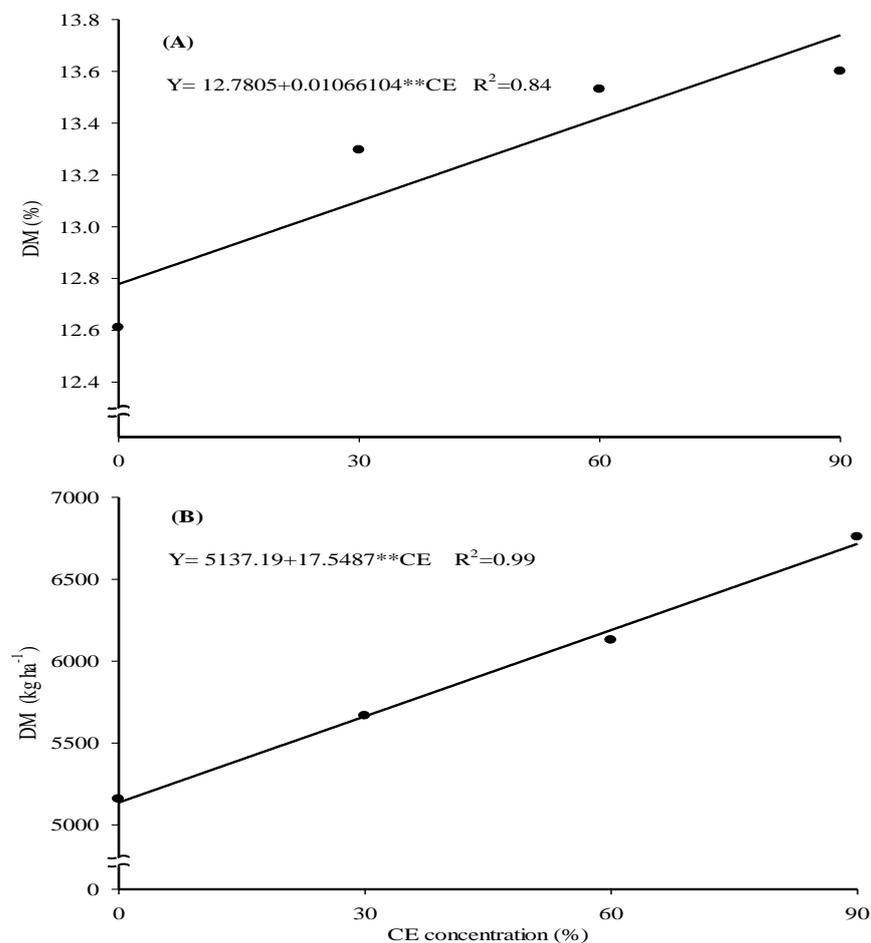
Likely, class 3 followed the same trend of decay tubers. Meanwhile, compost extract concentration (90%), combined with green manure, produced greater yield of class 2 than MN. However, all CE concentrations with compost produced similar or higher yield of class 2 than MN, except the concentration (30%) which was statistically similar to MN control.

Organic fertilizers combinations which produced higher total and marketable yield than MN control were CE 90% with GM and CE 60 and 90 % with compost. The concentrated CE (90%) with GM increased total and marketable yield by approximately 20.6% and 20% when compared with MN, respectively. Furthermore, CE (60%) with compost increased total and marketable yield by 12.6% and 12.4% compared to MN control, respectively. Meanwhile, CE (90%) with compost resulted in total yield increment by 16.8 % and marketable yield by 15.3% greater than MN control.

Analysis of variance for potato tubers dry matter content (%) and production presented no significant differences ( $p \geq 0.05$ ) between green manure and compost. Dry matter content and production were 13.22%, 13.3 %, 5823.9 and 6029 kg ha<sup>-1</sup>, respectively.

Nitrogen use efficiency (NUE) for total and marketable yield presented higher nitrogen productivity of compost than green manure despite the inferior N recovery. Mean values for nitrogen production was 224.92 and 218.5 (kg tuber kg<sup>-1</sup> N) for total yield and 217.05 and 211.72 (kg tuber kg<sup>-1</sup> N) for marketable yield for compost and green manure, respectively. Nitrogen use efficiency following compost was increased by 2.94% and 2.52 % more than sunhemp for total and marketable yield, respectively.

The interaction between sunhemp, compost and CE did not influence tuber dry matter content and production whereas they were influenced by just compost extract concentrations. The increment followed a linear pattern (figure 5 A) with a high determination coefficient ( $R^2=0.84$ ). As well as, tuber dry matter production followed the same linear trend of dry matter content (figure 5 B) whereas increasing CE concentrations resulted in a linear increase ( $R^2=0.99$ ) of dry matter yield.



**Figure 5.** Responses patterns of tubers dry matter content (A) and production (B) to compost extract concentrations

Comparison of dry matter content (%) between organic fertilizers combinations and MN control (Table 15) did not show significant differences by Dunnett test

( $p \geq 0.05$ ). Furthermore, the tuber dry matter yield was similar too, except for the treatments GM90, C60 and C90, which presented greater tuber dry matter production than MN control

Nitrogen use efficiency (NUE) followed the same trend for both total and marketable yield whereas organic fertilizers combinations showed higher efficiencies of the applied N than MN control. Organic fertilizer combinations increased N use efficiency, in relation to MN, by 22.3 to 37.35% and from 24.44 to 41% of total and marketable yield, respectively.

**Table 15.** Comparison of tuber dry matter content and production and N use efficiency (NUE) between MN control and organic fertilizers combinations.

Treatment	DM (%)	DM (kg ha <sup>-1</sup> )	NUE TY (kg tuber kg <sup>-1</sup> N)	NUE MY (kg tuber kg <sup>-1</sup> N)
MN	13.44 ns	5628.13	167.54	163.34
GM0	12.64	5088.93	213.15	207.32
GM30	13.25	5540.74	211.59	206.53
GM60	13.39	5812.16	211.86	202.77
GM90	13.58	6853.70 *	237.40	230.28
C0	12.59	5224.27	219.74	212.53
C30	13.34	5790.52	219.80	210.36
C60	13.67	6443.66 *	230.11	224.03
C90	13.61	6661.06 *	230.02	221.29
LSD	0.93	617.39	-	-
CV (%)	4.11	5.86	-	-

Means followed by \* in the column are statistically different from MN control by Dunnett test ( $p < 0.05$ ). GM; green manure, C; compost and numerical values adjacent refer to CE concentration.

#### 2.5.4. Correlation between N nutrition indices and yield under organic fertilizers combinations

The employed nutritional indices presented correlations with total and marketable yield with higher coefficients under green manure than under compost. SPAD index and N-total content at all sampling events presented significant correlations with total and marketable yield and enabled to adjust equations (Table 16) for yield prediction. Under compost however, the SPAD index at 45 DAP presented low and insignificant correlation coefficients with total and marketable yield. Meanwhile, the two following determinations at 55 and 65 DAP showed an increase in coefficient values for both total and marketable yield therefore linear equations which can be employed to predict yields were adjusted. On the other hand, the correlation between leaf N-total showed a different trend whereas strong and highly significant correlation coefficients with total

and marketable yield were obtained at 45 and 65 DAP. However, coefficients at 55 DAP were low but were significant.

**Table 16.** Pearson correlation coefficients and estimated linear equations between SPAD index, leaf N-total content with total and marketable yield under organic Fertilizers (green manure, compost and compost extract) combinations

Variable	Compost		Green manure	
	r <sup>2</sup>	Estimated equation	r <sup>2</sup>	Estimated equation
Total yield				
SAPD 45	-	-	0.672**	$\hat{Y} = -13503.803 + 1508.705^{**} \text{SPAD}$
SPAD 55	0.586*	$\hat{Y} = -50060.39 + 2452.083^{**} \text{SPAD}$	0.828**	$\hat{Y} = -18618.806 + 1694.436^{**} \text{SPAD}$
SPAD 65	0.581*	$\hat{Y} = -6024.158 + 1359.836^{*} \text{SPAD}$	0.90**	$\hat{Y} = -6223.922 + 1429.854^{**} \text{SPAD}$
N-total 45	0.596*	$\hat{Y} = -37382.417 + 1677.83^{*} \text{N-total}$	0.848**	$\hat{Y} = -25976.357 + 1468.223^{**} \text{N-total}$
N-total 55	0.226ns	-	0.614*	$\hat{Y} = 5842.252 + 1030.626^{*} \text{N-total}$
N-total 65	0.783**	$\hat{Y} = -36348.327 + 2703.334^{**} \text{N-total}$	0.717**	$\hat{Y} = 5586.299 + 1332.450^{**} \text{N-total}$
Marketable yield				
SAPD 45	-	-	0.698**	$\hat{Y} = -16549.011 + 1546.291^{**} \text{SPAD}$
SPAD 55	0.549*	$\hat{Y} = -42701.548 + 2224.511^{*} \text{SPAD}$	0.838**	$\hat{Y} = -20180.988 + 1693.924^{**} \text{SPAD}$
SPAD 65	0.530*	$\hat{Y} = -1534.514 + 1200.526^{*} \text{SPAD}$	0.911**	$\hat{Y} = -7791.598 + 1429.472^{**} \text{N-total}$
N-total 45	0.567*	$\hat{Y} = -32234.68 + 1543.44^{*} \text{N-total}$	0.835**	$\hat{Y} = -25566.415 + 1427.174^{**} \text{N-total}$
N-total 55	0.186ns	-	0.599*	$\hat{Y} = 5710.446 + 992.715^{*} \text{N-total}$
N-total 65	0.781**	$\hat{Y} = -34968.882 + 2611.400^{**} \text{N-total}$	0.727**	$\hat{Y} = 3968.908 + 1333.657^{**} \text{N-total}$

Correlation coefficients were fitted between 16 pairs of data.

\*\* , \* , significant at 1% , 5% probability and ns; is not significant, respectively.

## 2.6. DISCUSSION

### 2.6.1. Potato N nutrition status

Nitrogen is essential for plant growth as a constituent of plant cell components, including amino acids, nucleic acids and chlorophyll as well. Chlorophyll consists of tetrapyrrol which is derived from prothobilinogen, the precursor of tetrapyrroles synthesis, and formed by the condensation of  $\delta$ -amino levulinate which is produced by glutamate reduction. Glutamate is the major portion of reduced N in plant mesophyll. Therefore under normal conditions, increased N availability for plant acquisition enhances biosynthesis of glutamate,  $\delta$ -amino levulinate and ultimately chlorophyll content (Heldt, 2005). Using leaf chlorophyll content as an indicator of plant N status was reported in numerous studies for different crops, potato is among them by Botha *et al.*, (2006). They found increment in chlorophyll concentration by increasing N supply. Besides, the SPAD meter measures the concentration of green color which is related to chlorophyll concentration and its values are relevant to plant N status indirectly as shown by Fontes, (2001) and Rodrigues, (2004).

In the present study, leaf N-total content did not reflect significant differences between plants submitted to sunhemp and compost until 65 DAP. Thus, it can be speculated that N release from both sunhemp and compost was similar. Meanwhile, SPAD index detected differences apart from 55 DAP as higher values with small differences were observed under sunhemp. In fact, no references were found to support our results. However, these results could be attributed to the similar N dose form sunhemp and compost in the pre-planting which did reflect differences in N supply for potato until 65 DAP. This speculation is in accordance with that reported by Gianquinto and Bona, (2006). They reported that the clear separation of treatments receiving medium rates of N fertilizer is detectable quite late in the season using SPAD and leaf N-content therefore early sampling may not accurately distinguish between moderate N doses. In addition, the difference between sunhemp and compost which was detected by SPAD can be attributed to other factors rather than N, particularly soil water retention. Magdoff and Weil, (2004) reported that compost application has a higher contribution to soil organic matter than GM thus improved water retention. Haverkort and Mackerron, (2006) reported a decline in the SPAD index as a result of increased soil water retention.

SPAD values and leaf N-total content lowered with the progress in plant age and tuber bulking. This decline is regarded to the translocation of N compounds from plant canopy to tubers. These results are consistent with that obtained by Minotti *et al.* (1994); Rodrigues, (2004); Moreira, (2008) and Souza, (2009).

Compost extract concentrations influenced SPAD index whereas SPAD values presented quadratic response at 45 DAP. This result can be attributed to the direct effect of compost extract mineral N content in chlorophyll biosynthesis. This result is in accordance with Rangel *et al.* (2011) in greenhouse produced tomato. They compared compost extracts of different feedstocks with standard NPK solution. Extracts application resulted in different leaf-N content and SPAD values. Furthermore, the leaf - N content was relevant to extract N content. Another attribute to this linear response can be regarded as the contribution of extract N content in stimulating N mineralization and release from sunhemp and compost. Magdoff and Weil, (2004) reported that the addition of either organic fertilizers or mineral N that contain labile N, enhances N release from soil organic matter, is so called priming effect, therefore adequate nitrogen for plant uptake can be afforded. In fact, no reference using organic fertilizers, particularly compost extract, was found to support this speculation.

Leaf nutritional indices within the fourth leaf as influenced by mineral N doses for the same potato variety and in the same region were reported to be curve linear (Gil *et al.*, 2002; Coelho *et al.*, 2010; and Silva *et al.*, 2011) thus the obtained pattern in this experiment is in accordance with these reports with inferior SPAD values. Meanwhile, SPAD index showed linear responses to CE concentrations at 55 and 65 DAP. These results are consistent with that obtained by Souza, (2009) whereas SPAD index showed linear responses to different N concentrations in the nutritive solution within potato grown in hydroponics. Moreover, SPAD index declined when plant aged. This observation also was documented by Rodrigues, (2004) and Moreira, (2008).

Leaf N-total content presented linear responses to CE concentrations across evaluations accompanied by a decline with plant age progress. These linear responses can be attributed to the low applied N rates by organic fertilizers whereas supplied N ranged between 189 and 213.75 kg ha<sup>-1</sup>. Thus, it could be seen less than the reported critical doses (~ 200 kg ha<sup>-1</sup>) from mineral N by Coelho *et al.* (2010) and Silva *et al.* (2011) since, not all add and mineralized N by organic fertilizers will be readily available for plant acquisition as in mineral N fertilizer. Furthermore, linear response of leaf N-total content at 42 DAP to mineral N doses was reported by Coelho *et al.* (2012). These increases in leaf N-total content by increasing CE concentration are consistent with that obtained by Rangel *et al.* (2011) in hydroponically produced tomato. They found that application of CE extracts with high N concentrations increased leaf N content. Moreover, compost extracts were much less efficient in increasing leaf N content than the standard Steiner nutritive solution.

Comparison of SPAD index under organic fertilizers combinations with the MN control showed lower values over sampling regardless of N dose. This inferiority can be attributed to other factors rather than supplied N such as soil water content as explained by Haverkort and Mackerron, (2006). They demonstrated that incorporating organic fertilizers increase soil organic matter leading to improved soil water retention and supply and decreased SPAD values. In addition, compost extract application increased water supply within organic fertilizers combinations which affirm this explanation. These results are also in accordance with that obtained by Rodrigues *et al.* (2006) and Shahnazari *et al.* (2008) whereas, inferior nutritional indices, particularly SPAD, under organic N fertilizers to mineral ones in sweet corn and potatoes were reported, respectively.

The similar content of leaf N-total of under organic fertilization to MN control at 45 and 55 DAP is not clear and could be regarded as physiological adaptation under N release dynamics of organic fertilizers to maximize radiation utilization and productivity. Meanwhile, the drastic reduction of leaf N-total content under organic fertilizers can be attributed to the early and fast translocation of N compounds from foliage to tubers as a result of reduced N rates leading accelerated senescence (Goffart *et al.*, 2008).

#### **2.6.2. Whole plant dry matter production, N recovery and N recovery efficiency before senescence.**

Dry matter production and N accumulation within the potato canopy and tubers before natural defoliation showed superiority under sunhemp to compost despite the insignificant differences. This superiority can be attributed to the fast N mineralization from sunhemp which was obvious in N recovery and N recovery efficiency. The fast decomposition and N mineralization rate of sunhemp was reported by Perin *et al.* (2006); Odhiambo, (2010) and Araujo, (2012) whereas more than 50% of the residue initial N content was mineralized during the first two months after incorporation. In addition, potato N recovery following green manures was 34 %, using the <sup>15</sup>N enriched technique, as reported by Bundy and Andraski, (2005) after winter rye (*Secale cereale L.*) and 29% when mustard (*Brassica hirta*) preceded potato (Collins *et al.*, 2007). Meanwhile, N recovery by potato following compost incorporation was reported to be low. Lynch *et al.* (2008) reported a broad variation of potato N-recovery, between 2.1 to 32 % of compost N and a mean recovery of approximately 18%, following compost application. Additionally, Seister and Horwath, (2004) demonstrated that nutrients in compost, particularly N, are usually less available than in fresh residues and manures due to stability by microbial assimilation and humification during the composting process.

Dry matter production, accumulated N within the potato canopy and tubers and tubers N proportion was not affected by compost extract application. Meanwhile, N recovery showed a linear response to CE concentrations. This result indicates the high efficacy of compost extract mineral N content whereas nitrate represented the major proportion and may have been assimilated by potatoes. In fact, no references were found to support this result with the exception of that work by El Nagar *et al.* (2001). They indicated an increase of accumulated N in tuber by increasing extract concentration at harvest time.

Comparison between organic fertilizers combinations with the MN revealed the efficiency of organic fertilizers to increase dry matter production. N accumulation within the potato canopy and tubers presented lower values than MN control with no significant differences as well. In addition, the tubers N content proportion of the total accumulated N was not affected by organic fertilizers. Numerical ranges under organic fertilizer combination are higher than that that of MN control but are in the range reported by Li *et al.* (2003) and Zebarth *et al.* (2004) whereas N present in tubers ranged from 0.7 to 0.85 of the total assimilated nitrogen with lower values occurring at high fertilizer rates or in immature crops.

Potato presented lower N recovery under organic fertilization treatments than MN. Meanwhile, combined organic fertilizers resulted in equal or higher N recovery efficiency, particularly under sunhemp than under compost. The low N recovery can be attributed to the lower N doses of organic fertilizers as well as the slower N release pattern than mineral N fertilizer. The obtained results are consistent with that obtained by Jontgae, (2010). He reported N recovery decline by 17.8 % under solid and liquid organic fertilizers by onions when compared with mineral ones despite the same N dose. Generally, N recovery and N recovery efficiency of crops for organic fertilizers were reported to be lower than mineral fertilizers. Rodrigues *et al.* (2006) reported 40% decline in N recovery by triticle and maize after amending soil with vegetumus when compared to ammonium nitrate during three years. However, the high N recovery efficiency in this experiment as a result of combing organic fertilizers can attributed to the effect of compost extracts in stimulating sunhemp and compost N mineralization which was synchronized with potato N uptake. These results are in accordance with that obtained by Mkhabela and Warman, (2005); Nyiraneza and snapp, (2007); and Warman *et al.* (2011) as they reported increased potato N recovery when combining either compost or green manure with mineral N more than the sole application of organic fertilizer.

### **2.6.3. Tuber yield components**

Fresh tuber yield components as well as N use efficiency were higher under compost than under sunhemp despite the low N recovery. These results cannot be attributed to N fertilizer because of the obvious inverse trends of N recovery and yield. In general, yield increases after organic fertilizers, compost, green manures and fresh manures, incorporation was attributed to other factors rather than N such as improved soil properties (water retention, cation exchange capacity and water infiltration), augmented microbial biomass and activity and reduced crop pathogens. This efficacy provides bet-

ter circumstances for crop growth and higher yields (Bath, (2000); Rodrigues *et al.*, (2006); Carter *et al.*, (2003); Sincik *et al.*, (2008) and Campilglia *et al.*, (2009). So, it can be speculated that compost performed better than sunhemp in improving soil conditions and potato growth, particularly soil water retention and supply, consequently improved yield.

Yield increase by increasing CE concentration is in accordance with the results obtained by El Nagar *et al.* (2001). Fresh tuber yield raised from 30.7 to 49.8 t ha<sup>-1</sup> by decreasing water proportion of 75% to 25 %. In addition, integrating compost extract with compost and guano resulted in higher yield than compost and guano alone (16.44 t ha<sup>-1</sup>) in the bed preparation. However, Nikolic *et al.* (2003) observed an inverse trend of yield response to CE concentrations whereas fresh tuber yield was declined from 21 to 16 t ha<sup>-1</sup> by increasing CE concentration. Moreover, yield within the control was higher in the absence of CE. In other crops rather than potato, Hargreaves *et al.* (2009) reported that compost extract provided strawberries with adequate nutrients and produced yields equal to solid organic fertilizers and chemical ones. Jontgae, (2010) reported that yield obtained by liquid manure supply (46.9 t ha<sup>-1</sup>) in onion production was higher than that obtained by chemical fertilizer (45.9 ton ha<sup>-1</sup>) with no statistical difference.

Dry matter content and production showed linear responses to compost extract concentrations. Generally, potato dry matter production follows a quadratic response to mineral N doses whereas excessive N supply leads to dry matter accumulation into different plant organs rather than tubers (Goffart, 2008). Fontes *et al.* (2010) reported a quadratic pattern of dry matter production to N doses using the same potato variety in the same region. Moreover, they mentioned 181.1 kg N ha<sup>-1</sup> as an optimum dose for maximum dry matter production (6027.1 kg ha<sup>-1</sup>). Therefore, it can be speculated that N supplied within this experiment did not exceed the optimum N rate for maximum dry matter production.

The dry matter content of potato was not influenced by organic fertilizers combinations despite the discrepancy from the MN control since no significant differences were found. Meanwhile, the dry matter production pattern deviated from the dry matter content pattern whereas some organic fertilization treatments showed higher production than MN control. It can be speculated that N was not a limiting factor under organic fertilization as well as N supply did not exceed the optimal rate for dry matter production. These results are in accordance with that obtained by Zebarth *et al.* (2012). They

reported that tuber quality parameters such specific gravity, which is an indicator of dry matter content, can be influenced by N rate when excessive N rates are supplied. However, increasing N at a lower rate can increase tuber quality parameters. In addition, the increased dry matter production under organic fertilization could be regarded as a potential for tuber bulking and dry matter accumulation under organic management (Moore *et al.*, 2011).

N productivity (NUE) of organic fertilizers combinations presented higher N use efficiency than MN control. This high efficiency can be attributed to the non-N effects of organic fertilizers incorporation in enhancing soil properties, particularly water holding capacity, as mentioned by Bath, (2000); Lynch *et al.* (2008) and Campiglia *et al.* (2009). Correlating the high NUE to non-N effect, particularly improved soil water holding capacity, under organic fertilizers and CE supply is in accordance with the results obtained by Irena *et al.* (2011) and Badr *et al.* (2012). They reported that the soil water status greatly affects potato N uptake and efficiency whereas, stable water supply (100% of evapotranspiration) combined with 50% of N dose resulted in similar yields to high N dose under low water supply leading to better NUE and decreased N losses.

#### **2.6.4. Correlation between N nutritional indices and yield**

The obtained correlation coefficients are similar to those obtained under mineral N fertilization and mentioned by Minotti *et al.* (1994). He mentioned coefficients between SPAD and yield from 0.61 to 0.87; Majic *et al.* (2008) 0.48 to 0.61 between SPAD and yield; and Coelho *et al.* (2010) who obtained correlation coefficients ( $r^2$ ) of 0.63 and 0.64 for total yield and 0.59 and 0.6 for marketable yield with SPAD index and N-total content, respectively. From these results, SPAD index and total N-total content of the fourth leaf can be valid to estimate potato N status and to predict yield under combined organic fertilizers with a particular attention to growth stage and undertaken method.

### **2.7. CONCLUSIONS**

Integration of liquid and solid organic fertilizers improved their performance to provide potato with adequate nitrogen supply and presented a notable opportunity to improve potato productivity as well as seemed to be more sustainable than mineral fertilization.

The GM sunhemp presented superior efficiency in enhancing potato N uptake and recovery over the growing season to the compost. Inversely, compost showed better efficiency in yield augmentation as well as N use efficiency.

Non-aerated compost extract in this experiment influenced almost all the tested variables and proved to stimulate potato growth and productivity.

The tested nutritional indices, SPAD and leaf N-total, are useful to diagnose potato nitrogen status and predict yield.

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## 2. CHAPTER 2

### Effects of Sunhemp Residue Incorporation Timing and Doses on Potato N Status, N Recovery and Productivity

#### 3.1. ABSTRACT

The contribution of legume green manures to nitrogen credit and improvement of potato yield in different production systems has been quantified. However, the efficiency of legume green manures incorporation timing and nitrogen doses as a unique N source on potato growth and yield has not been studied. The current study aimed to investigate the influence of incorporation timing and N doses of sunhemp (*Crotalaria juncea* L) on potato growth and productivity. The experiment was conducted in Viçosa MG, Brazil in 2011. Eight treatments (2×4) from sunhemp residue as full dose incorporation (FI) at 15 days before planting and split incorporation (SD) as 50% before planting and 50% at 15 days after planting combined with 4 N doses (75, 150, 225 and 300 kg ha<sup>-1</sup>). In addition, 2 extra controls were included whereas, MN (250 kg ha<sup>-1</sup> recommendation) and N0. Treatments were arranged in a split-plot in a complete randomized block design with 4 replicates. Potato N nutrition status was determined in the fourth expanded leaf from plant apex employing SPAD, CHLT and N-total content at 38 DAP. Dry matter production, N accumulation and partitioning between foliage and tubers, N recovery (NR) and N recovery efficiency (NRE) as well as apparent N recovery (ANP) was measured before senescence at 85 DAP. At harvest, 103 DAP, fresh yield and its components, dry matter content (%) and production (kg ha<sup>-1</sup>) as well as nitrogen use efficiency (NUE) was evaluated. In addition, sunhemp residue N mineralization and dry matter breakdown within each application time were accompanied using litter bags. Data were subjected to ANOVA and regression analysis (p<0.05). Comparison between sunhemp treatments and MN control was carried out by Dunnett test (p<0.05). In addition, Pearson correlation between N nutritional indices and marketable and total yield for sunhemp treatments was tested (p<0.05).

Sunhemp residue N mineralization in both incorporation timing presented fast patterns, particularly during the first few weeks after incorporation. The estimated N mineralization rates were 0.0168 and 0.0140 g g<sup>-1</sup> day<sup>-1</sup> and dry matter breakdown rates were 0.0094 and 0.0075 g g<sup>-1</sup> day<sup>-1</sup> for FI and SD, respectively. In addition, mineralized N from sunhemp initial content until potato harvest represented 76% and 68.18 %

which accounted for 57, 114, 171 and 228 kg ha<sup>-1</sup> and 54, 108, 162 and 216 kg ha<sup>-1</sup> from the supplied doses 75, 150, 225 and 300 kg ha<sup>-1</sup> for FI and SD, respectively.

Potato results showed that split application of sunhemp residue did not influence SPAD index. However, CHLT and N-total content were higher under FI at 38 DAP. Total dry matter production, including foliage and tubers at 85 DAP, was lower under SD than FI. Foliage and tubers N content as well as tubers N proportion was not influenced by splitting application. Moreover, NR, NRE and ANP were not influenced by SD as well. Marketable and total yield and dry matter production were increased by 7% and 9.3 %, respectively as a result of split residue application. In addition, N use efficiency (NUE) was greater under SD than FI. However, dry matter content (%) was not influenced by either FI or SD.

Potato N nutritional indices at 38 DAP showed linear responses to sunhemp N doses except CHLT which did not respond to N doses. Before senescence, dry matter production showed quadratic responses to N doses under FI and SD. However, foliage and tubers N content and N recovery were linearly increased by increasing N dose. Yield components, except classes 3 and 4, and dry matter production was linearly increased by increasing sunhemp N dose. However, dry matter content (%) was not affected by N doses. In addition, NUE lowered by increasing N rate.

Sunhemp treatments, as individuals, with MN control presented inferior SPAD values to MN control regardless of N dose. However, CHLT and N-total content did not differ from the MN control at 38 DAP. At 85 DAP, sunhemp treatments resulted in similar or higher dry matter production than MN. Foliage and tubers presented inferior N content to MN control with no significant difference. Meanwhile, the tuber N proportion ranged between 0.71 and 0.79 and did not differ from MN control. Potato N recovery under sunhemp treatments did not differ from MN control except FI75 and SD 75 which were significantly lower than MN. Fresh tuber yield and dry matter production, except classes 3 and 4 and DM (%), were alike or higher than MN control. In addition, sunhemp fertilization increased N productivity (NUE) than MN.

Total and marketable yield presented significant linear correlation with the tested leaf nutritional indices for sunhemp treatments particularly under FI prior planting. Furthermore, only SPAD index was correlated with total yield under SD. The study concluded that sunhemp could be accounted for adequate N supply in potato production. Moreover, the split residue application could be possible technical option to improve fresh tuber yield, dry matter production and N use efficiency. Moreover, N diag-

noses indices could be used to predict yield when sunhemp used a sole N source and fully incorporated prior to planting.

**Key words;** *Solanum tuberosum* L, Sunhemp incorporation, N nutrition and efficiency, productivity.

### 3.2. RESUMO

Há relatos da contribuição das leguminosas como adubo verde ao fornecimento de N e ao aumento da produtividade da batateira em diferentes sistemas da produção. No entanto, a eficiência da incorporação e dosagem da leguminosa como uma única fonte de N sobre o crescimento e rendimento de batata não foi estudado. O presente estudo teve como objetivo investigar a influência de época da incorporação e de doses de massa de crotalária (*Crotalaria juncea* L) sobre o crescimento e produtividade de batata. O experimento foi conduzido em Viçosa MG, Brasil, em 2011. Oito tratamentos (2 × 4) com aplicação da crotalária consistiram da incorporação da dose completa (FI) aos 15 dias antes do plantio ou incorporação parcelada (SD) sendo 50% antes do plantio e 50% aos 15 dias após o plantio, combinados com quatro doses equivalentes a 75, 150, 225 e 300 kg ha<sup>-1</sup> de N. Além disso, duas testemunhas, MN 250 kg ha<sup>-1</sup> da recomendação e zero N, foram incluídos. Os tratamentos foram dispostos em parcelas subdivididas em DBC com quatro repetições, sendo as doses de crotalária alocadas nas subparcelas. O estado nutricional da batata foi determinado na quarta folha expandida a partir do ápice empregando leituras SPAD, determinação de teor de clorofila total (CLT) e de N-total aos 38 DAP. O acúmulo de matéria seca, o acúmulo de N e sua partição entre folhas e tubérculos, a recuperação de N (NR) e a eficiência de recuperação (NRE), bem como a recuperação aparente de N (NAP) foram medidas e determinadas antes da senescência da parte aérea, aos 85 DAP. Na colheita, 103 DAP, a produtividade e o teor de matéria seca foram determinados, bem como foi calculada a eficiência de utilização de nitrogênio (EUN). Além disso, a mineralização de N e a decomposição da crotalária em cada tempo de aplicação foram acompanhadas usando *litter bags*. Os dados foram submetidos à análise de variância e de regressão (p < 0,05). A comparação entre os tratamentos da crotalária e a testemunha (MN) foi realizada pelo teste de Dunnett (p < 0,05). Foram ainda determinados coeficientes de correlação de Pearson entre os índices nutricionais e a produção comercial e total (p < 0,05).

A mineralização de N da crotalária em ambas as épocas da incorporação foi rápida, particularmente nas primeiras semanas após a incorporação. As taxas estimadas

da mineralização de N foram 0,0168 e 0,0140 g g<sup>-1</sup> dia<sup>-1</sup> e as taxas de decomposição de dos tecidos foram 0,0094 e 0,0075 g g<sup>-1</sup> dia<sup>-1</sup> para FI e SD, respectivamente. Além disso, a proporção de N mineralizado da crotalária até a colheita de batata representou 76% e 68,18% que corresponderam a 57, 114, 171 e 228 kg ha<sup>-1</sup> e 54, 108, 162 e 216 kg ha<sup>-1</sup> nas doses fornecidas para FI e SD, respectivamente.

Os resultados mostraram que o parcelamento da aplicação da crotalária não influenciou o índice SPAD. No entanto, os teores de CLT e N-total foram maiores sob FI aos 38 DAP. Aos 85 DAP a produção da matéria seca de folhas e tubérculos foi menor com SD do que com FI. O N acumulado na folhagem e nos tubérculos, bem como sua proporção nos tubérculos não foi influenciada pela aplicação parcelada. Além disso, a NR, NRE e ANP não foram influenciados pela SD também. Na colheita, aos 103 DAP, a produtividade comercial e total e produção de matéria seca aumentaram em 7% e 9,3%, respectivamente, como resultado da aplicação parcelada comparativamente à aplicação em dose única. Além disso, a EUN foi maior em SD do que em FI.

Os índices nutricionais aos 38 DAP aumentaram linearmente em função de doses de crotalária, exceto o teor de CLT que não foi afetado. Antes da senescência (85 DAP), a produção de matéria seca apresentou resposta quadrática às doses de N. No entanto, o conteúdo de N na folhagem e nos tubérculos e a recuperação de N aumentaram linearmente com o aumento da dose N. Na colheita (103 DAP) a produtividade comercial, exceto dos tubérculos classes 3 e 4, e produtividade de matéria seca aumentaram linearmente em função das doses de N da crotalária. No entanto, o teor de matéria seca não foi afetado. No entanto, a EUN foi reduzida pelo aumento da dose de N.

Comparativamente à testemunha MN, os tratamentos com aplicação de crotalária resultaram em valores inferiores de SPAD, independentemente da dose de N. No entanto, os teores de CLT e N-total foram similares aos obtidos com MN. Aos 85 DAP, os tratamentos com crotalária resultaram em produção de matéria seca similar ou superior à obtida com MN. O conteúdo de N na folhagem e nos tubérculos e a proporção de n nos tubérculos foram similares aos obtidos com MN. A recuperação de N nos tratamentos com crotalária não deferiu daquela obtida com MN, exceto FI75 e SD 75, que foram menores. A aplicação de crotalária resultou em produção comercial de tubérculos e de matéria seca, exceto naquelas classes 3 e 4 similares ou superiores às obtidas com MN. Além disso, a adubação com a crotalária resultou em maior EUN do que a MN.

As produtividades total e comercial apresentaram correlações lineares significativas com os índices nutricionais para os tratamentos com aplicação de crotalária, parti-

cularmente sob FI. Além disso, apenas o índice SPAD foi correlacionado com a produtividade total em SD. O estudo concluiu que a crotalária poderia ser utilizada para fornecer N adequadamente para batateira. Além disso, a aplicação parcelada da crotalária possivelmente poderia ser uma opção técnica para aumentar a produção comercial de tubérculos, a produção de matéria seca e eficiência de uso de N. Além disso, os índices de diagnóstico de N podem ser utilizados para prever o rendimento quando crotalária utilizada como uma fonte única de N e totalmente incorporada antes do plantio.

**Palavras-chave;** *Solanum tuberosum* L, *Crotalaria juncea* L., parcelamento da incorporação, nutrição e eficiência de N, produtividade.

### 3.3. INTRODUCTION

Potato crop (*Solanum tuberosum* L.) is highly responsive to N fertilizer therefore adequate nitrogen supply is necessary for growth and development. It is well known that excessive application of N fertilizer to potato crop generates excessive vine growth, lower tuber quality, delay maturity, decline dry matter content and nitrogen use efficiency. On the contrary, N deficiency substantially reduces growth development and limits yield (Alva, 2004). In addition, potato crop is known to have relatively low nitrogen use efficiency, up to 50 %, due to its naturally shallow and poorly developed root system leading to risks of N losses to the environment (Tyler *et al.*, 1983).

Studies on N uptake characteristics of potato have shown that applied N fertilizers usually exceed the optimal rate for maximum yield thus economic losses and environmental risks are common where potato production dominates (Munoz *et al.*, 2005). Attempts to optimize nitrogen supply in potato fields for sustained production as well as environment have developed different tools and strategies to better match N supply with potato uptake, improve N use efficiency, optimize yield and overcome environmental impacts (Goffart *et al.*, 2008).

From the fact that the establishment of provisional N application for the potato crop at planting time can never be accurate as soil mineral N supply of both fertilizers and previously incorporated organic residues is influenced by several predictable and unpredictable factors such as weather conditions, chemical and physical soil properties, the quality of the incorporated residues and agronomic practices. Therefore, sustainable fertilization programs have developed different approaches such as in-season crop nitrogen status monitoring. Such approaches are valuable to fine-tune crop N requirement and consequently help to decide the need for supplementary N application as topdress-

ing (Vos and MacKerron, 2000). In-season crop nitrogen status monitoring mainly relies on the plant to indicate its N status as plants are good integrators of reflecting various factors such as soil N availability, weather conditions and crop management (Schröder *et al.*, 2000).

From this context, different in-season methods to monitor potato N status have been developed and can be operated at different scales such as Kjeldahl digestion, Dumas combustion, NIR spectroscopy analysis, nitrate specific electrodes and nitrate test strips, chlorophyll meters, Croma meters, chlorophyll fluorescence and crops can (Goffart *et al.*, 2008). Most of these methods have been compared for their precision, time-consumption and utility in a commercial scale. Moreover, critical thresholds of N sufficiency for each employed method, particularly under conventional potato production, have been established to optimize nitrogen supply. Furthermore, some research investigations have reported the feasibility of using these indices in the tuber yield forecast.

In the USA, Jindong *et al.* (2007) compared the efficiency of SPAD, petiole nitrate and Quickbird satellite imaginary data in determining N status of potato plants. They reported petiole nitrate concentration as highly efficient, among the tested methods, to reflect potato N status. The feasibility of these indices and their critical levels were not reported. In Brazil, Coelho *et al.* (2010) conducted an experiment to determine the critical values of SPAD index, total chlorophyll and N-total content which corresponds to the economic N dose and maximum yield within two potato varieties. Besides, their efficacy to predict yield was tested. They reported 40.5 and 43.7 of SPAD index, 66.7 and 75.2 g N kg<sup>-1</sup> DM and 6.13 and 6.96 mg g<sup>-1</sup> FM of total chlorophyll for Agata and Astrix, respectively. Moreover, they reported positive correlations between these indices and yield levels. In Argentina, Giletto *et al.* (2010) reported 40 and 36 SPAD index readings as minimum thresholds during vegetative growth and tuber bulking for economical yield in three tested potato varieties, respectively.

In sustainable production systems such as organic production and in a particular case of the potato crop, employing nutritional indices to indicate plant N status is not well documented with the exception to the posterior investigation. Shukla *et al.* (2007) conducted experiments to improve potato yield under organic management. Potato N status was monitored during the growing season by chlorophyll meter and leaf N content from 28 to 70 DAS at 10–12 days interval. The obtained results showed highly significant linear and quadratic trends for the regression of leaf N content ( $r^2 = 0.59$  to

0.83) and tuber yields ( $r^2 = 0.43$  to  $0.65$ ) and chlorophyll meter readings. They concluded that employing these indices in organic production are promising tools to monitor potato N status in order to optimize tuber yield.

Another approach, which has been introduced and wide-spread to optimize N supply for crops, reduce N losses from the agro-system and maximize productivity, is the integration of synthetic N with organic fertilizers, e.g. animal manures, compost and green manures. This approach was reported to be sustainable, particularly under low fertile soil, and can substantially enhance N supply in relation to crop demand (Sikora and Enkiri, 2000). Among organic amendments, legume green manures have received considerable attention as an important component of sustainable cropping systems. Their use as a source of N for subsequent crops increased dramatically in the recent years due to high fertilizer costs and potential environmental impacts of mineral N fertilizer (Sanderson *et al.*, 1999). Legume green manures can supply substantial amounts of biologically fixed N, up to  $250 \text{ kg ha}^{-1}$ , and most of which is released during the first year after incorporation (Vanotti and Bundy, 1995). Moreover, the use of legumes as winter covers was reported to reduce  $\text{NO}_3$  leaching, protect soil from wind and water erosion and improve soil quality by returning C input, recycling N input, and improving water quality (Reicosky and Forcella, 1998). In addition, improve soil microbial community composition after frequent and long-term application (Elfstrand *et al.*, 2007).

Integration of green manures in potato rotation as a main crop has been reported to increase N availability, improve soil physical properties and suppress soil-borne diseases (Griffin and Hesterman, 1991). These factors have the potential to directly or indirectly improve tuber yield and quality and thus profitability and sustainability of the production system. Moreover, the impacts of several species such as legumes, cereals and grasses as mono crops or in combinations have been tested and their influences on subsequent potato crop have been reported. Griffin and Hesterman, (1991) found that potato after all legume green manures, alfalfa (*Medicago sativa* L) and red clover (*Trifolium pratense* L.), showed higher N uptake and produced higher plant dry matter than after non-legume species, birdsfoot trefoil (*Lotus corniculatus* L.) and corn (*Zea mays* L). Moreover, optimal doses for maximum productivity were declined from  $250 \text{ kg N ha}^{-1}$  to  $120\text{-}170 \text{ kg N ha}^{-1}$ . Li *et al.* (1999) obtained optimal potato production using only  $70 \text{ kg N ha}^{-1}$  following grasslands. However, Riley, (2000) found that the optimum level was  $80 \text{ kg N ha}^{-1}$  for a yield of  $15 \text{ t ha}^{-1}$  and up to  $120 \text{ kg N ha}^{-1}$  for a yield of  $40 \text{ t ha}^{-1}$ . Carter *et al.* (2003) found that the average tuber yield of potato was higher after

Italian rye-grass than after red clover in a 2 year rotation during 11 years. Honeycutt, (2006) reported 9 to 12% potato yield increases after soybean-canola than after soybean-barely. Moreover, potato productivity within rotation was higher than continuous potato in a 3 year rotation. Silva *et al.* (2007) quantified the effects of annual organic fertilization with animal manure and/or sunhemp (*Crotalaria juncea L*) on potato productivity and soil nutrient stocks. The undertaken treatments were combinations of sunhemp incorporation and goat manure. The results showed an increase in total N, P and available P and K in plots received a combination of green and goat manure compared to non amended plots. Moreover, potato production average significantly increased and was consistently higher in the combination of sunhemp and manure. Sharifi *et al.* (2009) studied the effect of different green manures, e.g. pea, white clover, oats and Italian ryegrass, in a 2-year rotation on soil nitrogen supply to potato. They reported that the potential N mineralization after pea-white clover and oats- Italian ryegrass (102-109 kg N ha<sup>-1</sup>) was higher than oats by 35 and 22%, respectively. Moreover, Potato N uptake was higher after pea-white clover (126 kg ha<sup>-1</sup>) and higher than the other rotations (67 kg ha<sup>-1</sup>). Mohr *et al.* (2011) investigated the influence of different crops, canola, wheat, oat, and alfalfa, on potato yield for twelve years. The study concluded that including alfalfa in the rotation helped to maintain high productivity and resulted in a more sustainable system.

Most of the previous studies highlighted the importance of legumes to sustainable potato production in the long run by providing a substantial proportion of N credit for subsequent potato as well as improve soil properties. On the other hand, the efficiency of green manures to improve potato yield and decrease N external inputs in the short term was reported as well. Nyiraneza and Snapp, (2007) examined the response of potato to fallow and/or winter rye cover crop with 0 and 5.6 Mg ha<sup>-1</sup> poultry manure. The inorganic N source was used with (manure, cover crop residues, or both) to adjust fertilizer rate downward to provide an estimated 224 kg N ha<sup>-1</sup> for all treatments. The obtained results showed that the integrated treatment (179 kg N ha<sup>-1</sup> fertilizer+ manure) after winter rye consistently increased tuber yield and N uptake efficiency by 20% compared with the unamended fallow plots (224 kg N ha<sup>-1</sup> fertilizer). In the absence of chemical fertilizer, rye cover crop and manure enhanced tuber yield by 40 to 210% compared with unamended plants. Sincik *et al.* (2008) compared three green manure species combined with three rates of mineral N fertilizers on potato yield. They found that potato tuber yield and its components significantly increased as N rates increased.

Moreover, the differential responses to N significantly varied according to green manure crops grown as pre-crop or cover crops. Potatoes following legume green manures and cover crops produced approximately 36% to 38% higher tuber yields compared with potatoes following winter wheat when 0N was applied.

The potential of different green manure species to provide N for the potato crop under organic management was studied by Campiglia *et al.* (2009). They investigated the efficiency of rapeseed (*Brassica napus* L.), Italian ryegrass (*Lolium multiflorum* L.), hairy vetch (*Vicia villosa* Roth.), snail medick (*Medicago scutellata* Mill) and subclover (*Trifolium subterraneum* L.) compared with mineral NPK. The results showed that potato fresh marketable yield following subclover and hairy vetch was similar to that obtained by mineral nitrogen fertilization (48.5 t ha<sup>-1</sup>). The study demonstrated the potential of legume green manures to provide potato with adequate nitrogen supply and yield equal to that of mineral nitrogen.

From the above mentioned literature, the contribution of legumes to potato nitrogen budget either as green manures or as cover crops when precede potatoes, in the short and long run, was evident. However, relying on legumes as a unique nitrogen source and the efficiency of different management practices on potato has not been studied. For that reason, the current investigation aimed to study the influence of the GM sunhemp (*Crotalaria juncea* L.) residue as a unique nitrogen source on the potato nitrogen nutrition status, nitrogen recovery and productivity. Furthermore, estimate the efficiency of different N doses from sunhemp residue as well as incorporation timing whereas full incorporation before planting and split application were compared. In addition, test the visibility of leaf nitrogen nutrition indices on potato nitrogen diagnoses as well as yield forecast under sunhemp nitrogen supply.

### **3.4. MATERIALS AND METHODS**

#### **3.4.1. Experimental site, soil properties and meteorological data**

A field experiment was conducted, from May 13<sup>th</sup> to September 9<sup>th</sup> in 2011, in the Horta Nova experimental farm of Universidade Federal de Viçosa, Minas Gerais state. The experimental farm is located at 693 m altitude, latitude 20°45' S and longitude 42°51' W. The region is characterized by dry moderate to cold winter and rainy hot summer. Meteorological data (Table 1) were obtained from a nearby weather station.

Before installing the experiment, 25 individual soil samples were collected from the top soil surface to 30 cm depth then homogenized and mixed in a composite sam-

ple. A sub sample was collected and submitted to mechanical and chemical analysis. Soil characteristics are shown in Table 2.

**Table 1.** Mean daily air and night temperatures and precipitation from May to September 2011

Variable	May	June	July	August	September
Mean air temperature ( $^{\circ}\text{C}$ )	17.6	15.6	15.5	18.5	20.3
Mean night temperature ( $^{\circ}\text{C}$ )	13.5	11.5	9.9	12.8	16.9
Precipitation (mm)	0	0	0	0	0

**Table 2.** Initial chemical characteristics of the experimental soil before sunhemp incorporation and potato plantation.

Property	Unit	Value	Method
OM	$\text{dag kg}^{-1}$	2.5	Walkely-Black =org C $\times$ 1,724
pH (in water 1:2.5)		5.7	
P	$\text{mg dm}^{-3}$	39	Mehlich-1 extractor
K	$\text{mg dm}^{-3}$	60	Mehlich-1 extractor
Ca $^{2+}$	$\text{cmol}_c \text{ dm}^{-3}$	2.4	KCL-1mol L $^{-1}$ extractor
Mg $^{2+}$	$\text{cmol}_c \text{ dm}^{-3}$	0.5	KCL-1mol L $^{-1}$ extractor
Al $^{3+}$	$\text{cmol}_c \text{ dm}^{-3}$	0.0	KCL-1mol L $^{-1}$ extractor
Effective CTC	$\text{cmol}_c \text{ dm}^{-3}$	3.05	
CTC in pH 7.0	$\text{cmol}_c \text{ dm}^{-3}$	6.02	
V	%	51	
Rem-P	$\text{mg L}^{-1}$	35.7	
Texture		Sandy clay	EMBRAPA, 2006

### 3.4.2. Green manure production and chemical characterization

The GM sunhemp (*Crotalaria juncea* L.) was sown in January 12<sup>th</sup>, 2011 in an adjacent area to the experimental site. Seeds were sown manually with 0.5 m row spacing and density of 40 per meter linear. Sunhemp was not fertilized across the growing season. Before full blooming, 85 DAP, the green manure was harvested manually, chopped and air dried in indirect sunlight and ambient temperature then stored until incorporation. Before planting potato, the dry green manure mass was homogenized and sampled for dry matter, total nitrogen and total carbon content determination. Samples were oven dried in a forced air oven at 60  $^{\circ}\text{C}$  until constant weight. Thereafter, samples were ground to pass through a 2 mm sieve. Total nitrogen content was determined by the micro-Kjeldahl method (Miyazawa *et al.*, 2009). Total carbon content (C %) was determined by Walkley-Black method. GM dry mass presented 85 % dry matter, 2% N-total, 20.9 % total carbon and 10.45 C/N ratio.

### **3.4.3. Treatments and experimental layout**

The experiment consisted of ten treatments arranged in a factorial scheme (2×4 +2 controls). Eight treatments were based on the GM sunhemp as a unique nitrogen source. Two incorporation timings, full dose incorporation (FI) at fifteen days before planting and split application into two equal splits (SD) as 50% at fifteen days before planting and 50% at fifteen days after planting, combined with four nitrogen rates (75, 150, 225 and 300 kg ha<sup>-1</sup>). Furthermore, two extra control treatments, zero N treatment (N0) and recommended MN (250 kg ha<sup>-1</sup>) were included. Green manure treatments were arranged in split plot design in a complete randomized block design with 4 replicates. Furthermore, the two control treatments were allocated within the main plots. Additionally, in the control (N0), plants did not receive any nitrogen fertilizer. Meanwhile, fertilizer N was supplied in the MN control as recommended by Fontes, (2005).

### **3.4.4. Soil preparation, potato planting and fertilization**

The soil was tilled and disc harrowed then divided into blocks and plots. One meter interspacing between blocks and 0.5 m distance between experimental units were left as borders to avoid contamination. Each experimental plot had an area of 7.5 m<sup>2</sup> (3×2.5 m) with 4 rows.

Fifteen days before planting potato, the GM dry mass was weighed, based on N doses, and then manually distributed on the soil surface. Thereafter, it was incorporated by rotary tiller. Consequently, the experimental area was lightly irrigated to afford humidity for decomposition commence. One day before potato planting, the soil was rotary ploughed again to eliminate weeds and furrowed with a spacing of 0.75 m apart.

Soil was amended with the following mineral nutrients; phosphorus (simple super phosphate, 18% P<sub>2</sub>O<sub>5</sub>), potassium (potassium chloride, 60% de K<sub>2</sub>O) and magnesium (magnesium sulfate, 9 % MgO) with the amount of 420, 220 and 200 kg ha<sup>-1</sup>, respectively. Micro-nutrients, boron (borax, 11% B), copper (copper sulfate, 24% Cu), zinc (zinc sulfate, 22% Zn), 10 kg ha<sup>-1</sup> of each and molybdenum (Sodium molybdate, 39% Mo), 250 g ha<sup>-1</sup> were supplied as well. The fertilizers were blended and applied in the furrows before planting.

The N0 control plots received all nutrients except nitrogen. The mineral N control treatment received 250 kg ha<sup>-1</sup> of N as ammonium sulfate. Mineral nitrogen was supplied as 70% spread in the furrows preceding planting and the rest 30% was supplied as a side dressing at 22 days after emergence (Fontes, 2005).

Homogeneous potato seeds of 'Ágata', which is a widespread commercial variety in the region, with a diameter of 35-50 mm were pre-germinated at room temperature and indirect sunlight until sprouts reached around 3 cm length. Tubers were hand planted with 0.25 m apart and covered with 10 cm of soil. Each experimental plot contained 40 plants. Additionally, all data were collected from the two central rows whereas the outer rows were neglected from evaluations.

Fifteen days after planting, the second half of sunhemp residue of SD treatments was distributed on the hillsides. Ten days thereafter when plants reached up to 15 cm height, soil was hilled up to adjust plants in the mid ridge, mix the green manure with soil and control weeds. Additionally, agronomic practices such as irrigation and control of pests and diseases were accomplished following the regional recommendations.

#### **3.4.5. Green manure decomposition and nitrogen mineralization estimation**

Sunhemp mass breakdown and nitrogen mineralization was measured from GM incorporation until potato tuber harvest. Two litter bag experiments were set up simultaneously with residue incorporation times. Fifteen grams of the air dried biomass (13 g DW) were placed in Nylon bags (25×25 cm and 2 mm mesh size) and then buried to the depth of 10-15 cm and 30 cm apart adjacent to the potato experiment. For the first experiment, litter bags were sampled at 0, 7, 14, 21, 35, 49, 70, 91 and 112 days after incorporation. In the second experiment, samples were collected at 0, 7, 28, 49, 70 and 91 days after incorporation.

At each sampling date, four random bags were retrieved and the remaining GM mass was cleaned to remove soil particles then transferred into paper bags and dried in air forced oven at 60 °C until constant weight. Samples were weighed then ground to pass through a 2 mm sieve and subjected to total nitrogen content determination by micro-Kjeldahl. The percent of the remaining dry matter (DM) and nitrogen for each sampling event was calculated as shown:

$$XR (\%) = (X_t/X_0) \times 100;$$
 Where XR is the percent DM weight or nitrogen remaining,  $X_t$  the DM weight or nitrogen content at each sampling time and  $X_0$  is the DM weight or nitrogen amount at decomposition start. Dry matter and nitrogen mineralization kinetics were fitted into the first-order single exponential model (Wieder and Lang, 1982):  $M_t = M_0 e^{-kt}$ , where  $M_t$  is the remaining dry matter or nitrogen amount after a time period "t", in days,  $M_0$  is the total dry matter or nitrogen at the beginning of the experiment and "k" is the decomposition rate constant. The half life of the sunhemp residue and nitrogen content was calculated by the equation  $t_{1/2} = \ln(0.5)/k$  as described

by Paul and Clark, (1996), where  $t_{1/2}$  is the time required for crop residue biomass or the nutrient content reduction to half of the initial value.

#### **3.4.6. Potato nitrogen status measurements**

The potential of sunhemp residue to provide potato plants with nitrogen was measured employing some widely used nutritional indices in conventional potatoes. At 38 days after planting (DAP) which corresponded to 21 days after emergence, SPAD index, total chlorophyll content (CHLT) and total nitrogen were measured on the fourth expanded leaf from the plant apex down.

Non-destructive or indirect chlorophyll measurement was measured using the portable SPAD-502, Minolta Japan, within five plants in each plot. Readings were collected in the morning between 8 to 11 o'clock. Five readings were collected from the terminal leaflet between leaf margin and midrib. The mean value of five readings represented one plant.

Destructive sampling was implemented for total chlorophyll quantification following the method described by Lichthenthaler, (1987). Just after SPAD reading collection, two circular 1 cm diameter discs from each leaflet were punched, frozen and transferred to the lab. Discs' fresh weights were recorded, then macerated in 5 ml hydrated acetone (80%) and calcium carbonate in a mortar. After sedimentation, the extract solutions were filtered. The filtrates were received in flasks lined by aluminum paper and then the volume was raised to 25 ml with acetone (80%). The absorbance was determined at 663 and 645 nm L using a spectrophotometer (UV-VIS FEMTO CIRRUS 80, São Paulo, Brazil). Total chlorophyll concentration ( $\text{mg L}^{-1}$ ) was obtained using the following equation:  $\text{CHLT} = 7.15 A_{663} + 18.71 A_{645}$ . Then, the obtained chlorophyll concentrations were converted into  $\text{g kg}^{-1}$  fresh weight.

For N-total content, the same sampled leaves were detached, dried in air forced oven at 60 °C until constant weight and ground to pass through a 2 mm sieve. Then, total nitrogen ( $\text{g kg}^{-1}$ ) content was determined by micro-Kjeldahl method (Miyazawa *et al.*, 2009).

#### **3.4.7. Dry matter production, nitrogen accumulation and nitrogen recovery quantification**

Total dry matter production and nitrogen accumulation and partitioning were determined before senescence, at 85 DAP. Sampling was implemented following the method described by Sullivan *et al.* (2008). Three plants from each plot were harvested, excluding roots. Plants were segmented into foliage and tubers. The foliage was

cleaned and dried in air forced oven at 60 °C until constant weight. Dry weights were recorded, then samples were ground to pass through a 2 mm sieve and the total nitrogen content (%) was determined. Tubers were weighed and a sample of five tubers was collected, washed by tap water followed by distilled water and left to dry. Tubers were cut into cubes of approximately 1 cm<sup>3</sup> and mixed. A sub sample of 200 g was dried at 60 °C until constant weight. Samples were ground to pass through a 2 mm sieve and N-total content (%) was determined. N content (%) was multiplied by dry matter weight to obtain the total nitrogen amount. Sum of foliage and tubers dry matter weights were expressed in kg per hectare basis (50,000.00 plants ha<sup>-1</sup>) to represent total dry matter per hectare.

Nitrogen recovery (kg ha<sup>-1</sup>) was obtained by summing foliage and tubers N-content to represent N-total per plant and then expressed in a per hectare basis.

Nitrogen Recovery Efficiency (NRE %) was calculated by the equation:

$$\text{NRE (\%)} = \frac{\text{N recovery for treatment (kg ha}^{-1}\text{)}}{\text{N applied in the same treatment (kg ha}^{-1}\text{)}} \times 100$$

Nitrogen Apparent Recovery (NAR %) was calculated following the equation described by Lynch *et al.*, (2008) as follow:

$$\text{NAR (\%)} = \frac{\text{N uptake for treatment} - \text{N upatke for N0 control}}{\text{N applied in the treatment}} \times 100$$

### 3.4.8. Yield and its component's determination

At harvest (103 DAP), 17 plants of the two central rows were harvested. Tubers were left two hours for curing then graded according to the standards of Ministério da Agricultura, Agropecuária e Abastecimento (Brazilian Ministry of Agriculture, 1995). Yield grades were as follow; class 1 (diameter > 8,5 cm), class 2 (8,5 ≤ tubers diameter ≤ 4,5 cm), class 3 (4,5 ≤ tubers diameter ≤ 3,3 cm) and class 4 included tubers with a diameter less than 3,3 cm and tubers with commercial disorders such as greenish, rotten and infected by insects or diseased. The sum of the classes 1, 2 and 3 represented commercial yield and, the sum of all classes represented total yield.

Dry matter content (%) was determined by collecting samples of 5 tubers from the commercial tubers. Tubers were washed by running water followed by distilled water and cut into cubes of 1 cm<sup>3</sup> and mixed. A composite sub sample of 200 g was dried in 60 °C until constant weight. Dry matter (%) content was multiplied by total yield to calculate dry matter production. Nitrogen use efficiency (NUE, kg tuber kg<sup>-1</sup> N) for total and commercial yield was calculated by dividing total and marketable yield by the applied nitrogen.

### 3.4.9. Statistical analysis

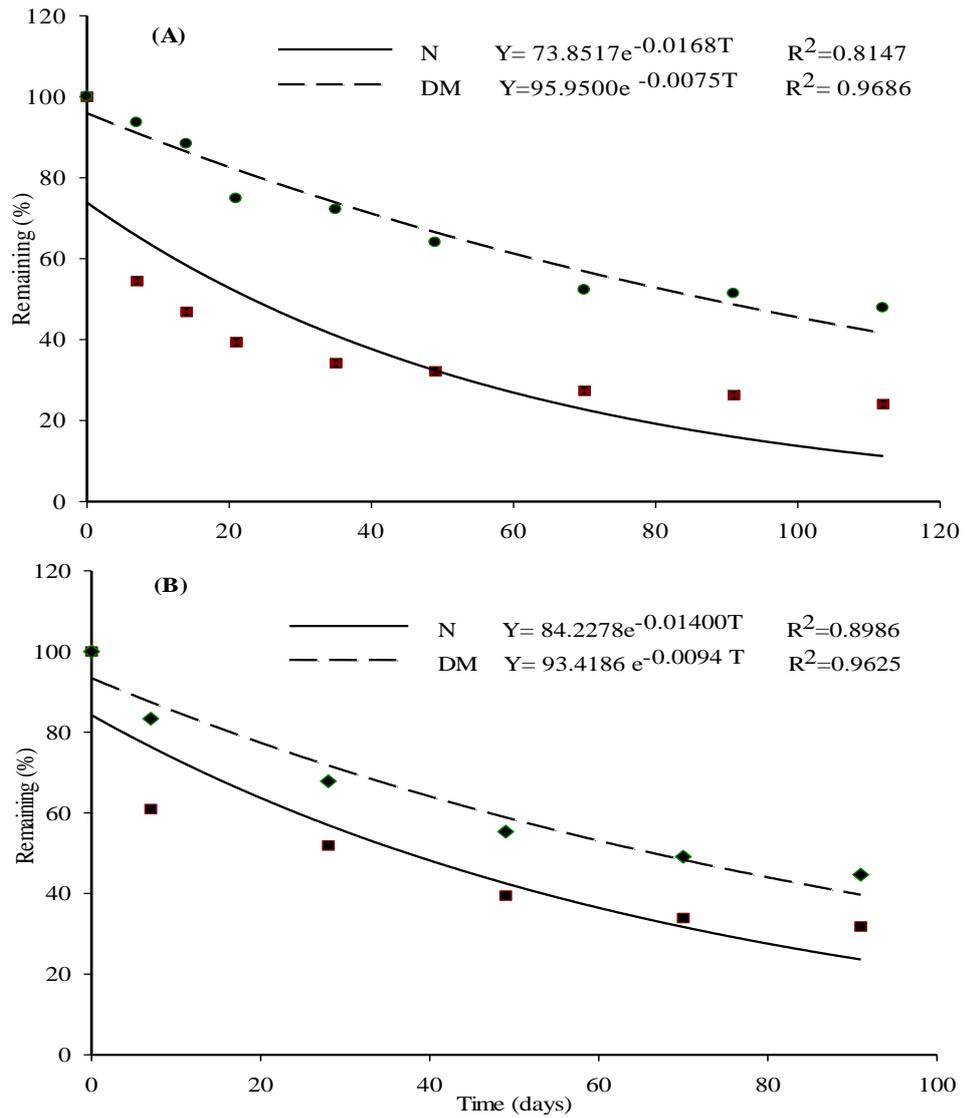
The obtained data were subjected to analysis of variance ( $P < 0.05$ ) using SAEG software (V, 9.1, 2007). ANOVA was implemented in sequential steps. Treatments were analyzed as a complete randomized block design. Additionally, comparison between the mineral nitrogen control (MN) and GM treatments was implemented by Dunnett test ( $P < 0.05$ ). Consequently, control treatments were separated to calculate their sum squares. Thereafter, green manure treatments were then subject to ANOVA in split plot design. Effect of GM nitrogen dose was subjected to regression analyses. Adequate models were selected according to significance, determination coefficient and biological behavior of attributes. In addition, Pearson correlation between total and commercial yield and nutritional indices was performed. Linear models were fitted between independent variables when correlation coefficient was significant. Finally, the three separated ANOVA were merged into combined ANOVA to correct experimental error (a) freedom degree and its sum square.

## 3.5. RESULTS

### 3.5.1. Green manure decomposition and nitrogen mineralization

Sunhemp dry matter decomposition and nitrogen mineralization in both incorporation timings presented similar patterns (Figure 1, A and B). Generally, nitrogen mineralization was faster than dry matter breakdown and had an accelerated rate during the first few weeks after incorporation. Incorporating sunhemp residue at 15 days before planting presented higher decomposition rate ( $k = 0.0168 \text{ g g}^{-1} \text{ day}^{-1}$ ) and shorter half life ( $t_{1/2} = 41.26$ ) than superficial application at 15 days after planting ( $k = 0.0140 \text{ g g}^{-1} \text{ day}^{-1}$  and  $t_{1/2} = 49.5$ ). However, the dry matter breakdown showed an inverse trend as residue incorporation after planting showed a higher decomposition rate ( $k = 0.0094 \text{ g g}^{-1} \text{ day}^{-1}$ ) and shorter half life ( $t_{1/2} = 73.74$ ) than incorporation before planting ( $k = 0.0075 \text{ g g}^{-1} \text{ day}^{-1}$  and  $t_{1/2} = 92.42$ ).

The proportions of released N from the sunhemp initial content within full incorporation and split application until potato harvest was 76% and 68.18 %, respectively. Considering these proportions and the supplied nitrogen doses, the released nitrogen amounts were 57, 114, 171 and 228  $\text{kg ha}^{-1}$  and 54, 108, 162 and 216  $\text{kg ha}^{-1}$  from the supplied doses 75, 150, 225 and 300  $\text{kg ha}^{-1}$  for FI and SD, respectively.



**Figure 1.** Patterns of sunhemp dry matter (DM) decomposition and N mineralization. (A): Sunhemp incorporated at 15 day before planting date and (B) sunhemp supplied at 15 days after planting date.

### 3.5.2. Potato nitrogen nutrition status

Comparison between control treatments and green manure incorporation timing which occupied the main plots (Table 3) showed significant difference between zero N control (N0) and MN control ( $p < 0.05$ ) for SPAD index whereas, N0 presented greater values than MN. Furthermore, the total chlorophyll content ( $\text{g kg}^{-1}$ ) on the fresh weight bases was higher within N0 but insignificant. On the other hand, N-total content in dry matter ( $\text{g kg}^{-1}$ ) showed the same trend of SPAD index as N0 control showed higher nitrogen content than MN control ( $p < 0.05$ ).

Under green manure incorporation timing (Table 3), SPAD index was not affected by the split residue application. However, FI resulted in a significant increase in

leaf total chlorophyll content (CHLT) in fresh tissues than SD ( $p < 0.05$ ). Moreover, leaf dry matter nitrogen content followed the same trend of CHLT as FI resulted in higher tissue nitrogen content than SD with significant difference by F test ( $p < 0.05$ ).

**Table 3.** Comparisons of potato nutritional indices between control treatments (N0 Vs MN) and sunhemp residue incorporation timing (FI Vs SD) at 38 days after planting

Treatment	SPAD Index	Total chlorophyll (g kg <sup>-1</sup> FM)	N-Total (g kg <sup>-1</sup> DM)
N0	43.28 a	7.55 a	59.65 a
MN	40.68 b	7.19 a	53.20 b
FI	38.12 a	7.38 a	54.92 a
SD	38.13 a	6.96 b	50.66 b
CV (%)	2.52	9.54	8.46

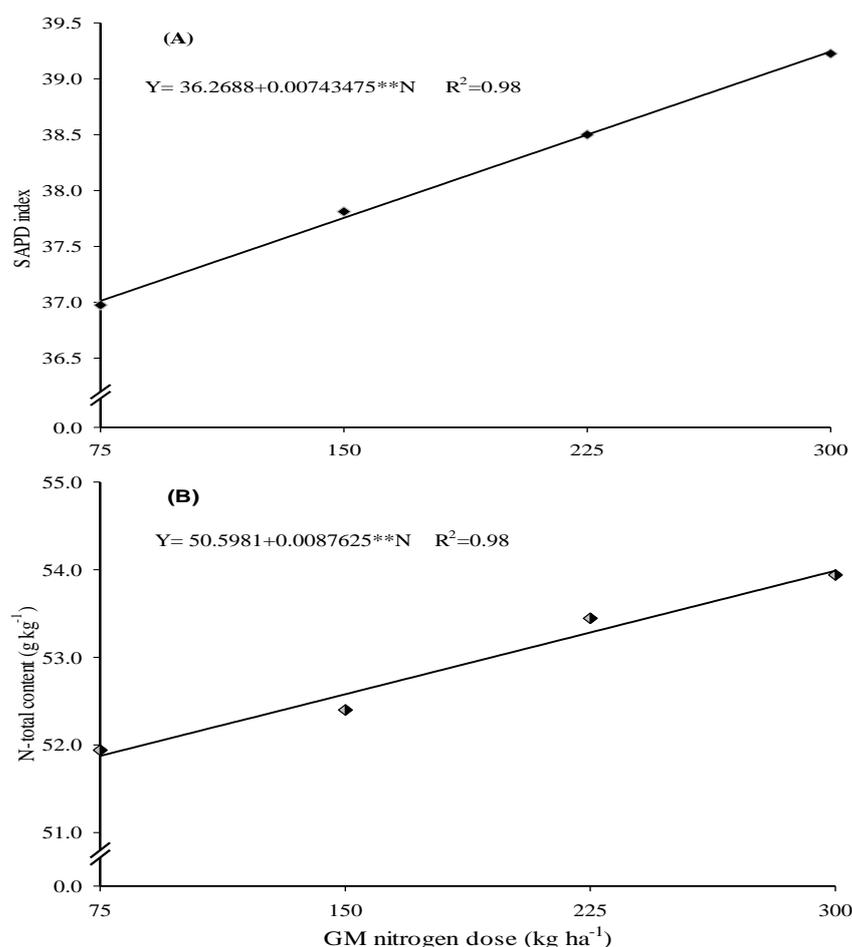
Means followed by the same letter in the column are not significantly different by F test ( $P \geq 0.05$ ).

\* N0; N zero treatment, MN; recommended mineral N dose, FI; full dose incorporation and SD; split incorporation.

Sunhemp nitrogen doses significantly influenced SPAD index whereas, SPAD values increased linearly by increasing N dose (Figure 1 A). SPAD values ranged between 36.8 and 38.6 for the low and high nitrogen dose, respectively. The interaction effect between the sunhemp incorporation timing and nitrogen doses was not significant.

Total chlorophyll content (CHLT) in potato fresh tissues was not influenced by GM nitrogen doses. The interaction effect between sunhemp nitrogen doses and incorporation timing was not significant as well. Meanwhile, leaves N-total content was increased linearly by increasing sunhemp nitrogen dose (Figure 2B) with high determination coefficient ( $R^2=0.98$ ). Total nitrogen content increased from 51.87 to 53.9 g kg<sup>-1</sup> in leaves dry matter by increasing N dose from 75 to 300 kg N ha<sup>-1</sup>. In addition, the interaction between incorporation timing and N doses was not significant and did not influence N-total content.

Comparison between green manure treatments, as individuals, and MN control showed lower SPAD values than MN control regardless of nitrogen dose (Table 4). Generally, a gradient increase as a result of increasing sunhemp N dose under both incorporation timing was observed. In spite of this increase, all sunhemp treatments were statistically lower than MN control except for the elevated nitrogen dose (300 kg ha<sup>-1</sup>) under FI and SD which was statistically similar to MN control by Dunnett test ( $p \geq 0.05$ ) despite the mathematical inferiority



**Figure 2.** Effect of sunhemp N dose on SPAD index (A) and N-total content (B) in potato fourth leaf at 38 DAP.

**Table 4.** Comparison of potato nutritional indexes between sunhemp treatments and mineral nitrogen treatment (MN) at 38 days after planting (DAP)

Treatment	SPAD Index	Total chlorophyll (g kg <sup>-1</sup> FM)	N-Total (g kg <sup>-1</sup> DM)
MN	40.68	7.20 ns	53.20
FI75	36.95 *	7.30	53.28
FI150	37.65 *	7.40	54.20
FI225	38.55 *	7.45	55.87
FI300	39.33	7.41	56.34
SD75	37.00 *	6.75	49.46 *
SD150	37.98 *	6.77	50.61
SD225	38.45 *	7.02	51.03
SD300	39.13	7.32	51.54
LSD	1.84	0.754	3.15
CV (%)	2.62	5.8	3.3

Means followed by \* in the column are statistically different from MN by Dunnett test ( $p < 0.05$ ), ns; not significant. FI; full incorporation, SD; split application and numerical values refer to N dose (kg ha<sup>-1</sup>).

Leaf total chlorophyll content (CHLT) was not influenced by GM fertilization whereas comparison with MN control did not show significant difference by Dunnett

test ( $p < 0.05$ ). Numerically, sunhemp residue incorporation before planting increased total chlorophyll contents more than split incorporation and MN control.

N-total for GM treatments did not show differential effects compared to MN control as no significant difference was found by Dunnett test ( $p \geq 0.05$ ) except for the low GM nitrogen dose (SD75) which was inferior to MN. In addition, split GM nitrogen application reduced leaf N-total content despite the insignificant difference from MN control.

### 3.5.3. Dry matter production and nitrogen accumulation at 85 DAP

Shoot and tuber dry matter production ( $\text{kg ha}^{-1}$ ) showed different patterns under N0 and MN treatments. The absence of N fertilizer in N0 reduced dry matter production (foliage and tubers), N tuber accumulation and its proportion of total accumulated nitrogen in the entire plant. These differences were significant by f test ( $p < 0.05$ ). However, N accumulated in the foliage was not affected by absence of N fertilizer (Table 5).

Total dry matter production was influenced by GM incorporation timing as FI increased dry matter than SD ( $p < 0.05$ ). However, N accumulated in potato foliage, tubers and N proportion were not affected by different incorporation timing (Table 5).

**Table 5.** Comparisons of potato dry matter production (DM) and nitrogen accumulation in foliage (FN), tubers (TN) and tubers` nitrogen proportion (TNP) between control treatments (N0 Vs MN) and GM incorporation timing (FI Vs SD) at 85 DAP

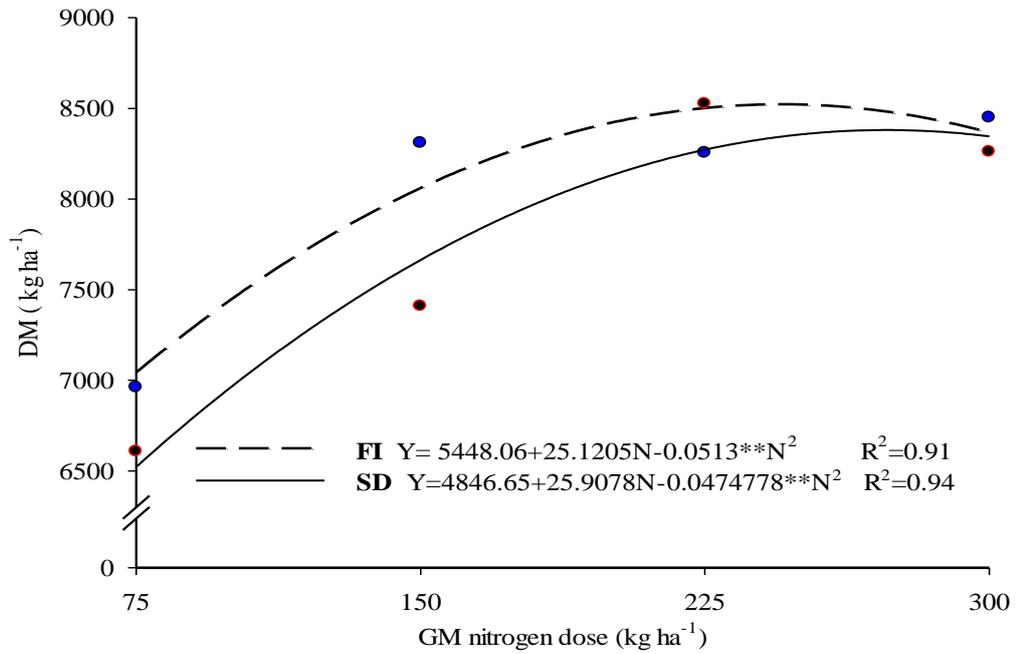
Treatment	DM ( $\text{kg ha}^{-1}$ )	FN ( $\text{kg ha}^{-1}$ )	TN ( $\text{kg ha}^{-1}$ )	TNP
N0	5930.75 b	25.73 ns	28.16 b	0.523 b
MN	7530.38 a	27.55	72.38 a	0.728 a
FI	7993.94 a	23.75 ns	65.88 ns	0.735 ns
SD	7701.31 b	22.35	65.81	0.747
CV (%)	4.6	26.04	13.15	5.68

Means followed by the same letter in the column are not significantly different by F test ( $p \geq 0.05$ ).

\* N0; N zero treatment, MN; recommended mineral N dose, FI; full dose incorporation and SD; split incorporation

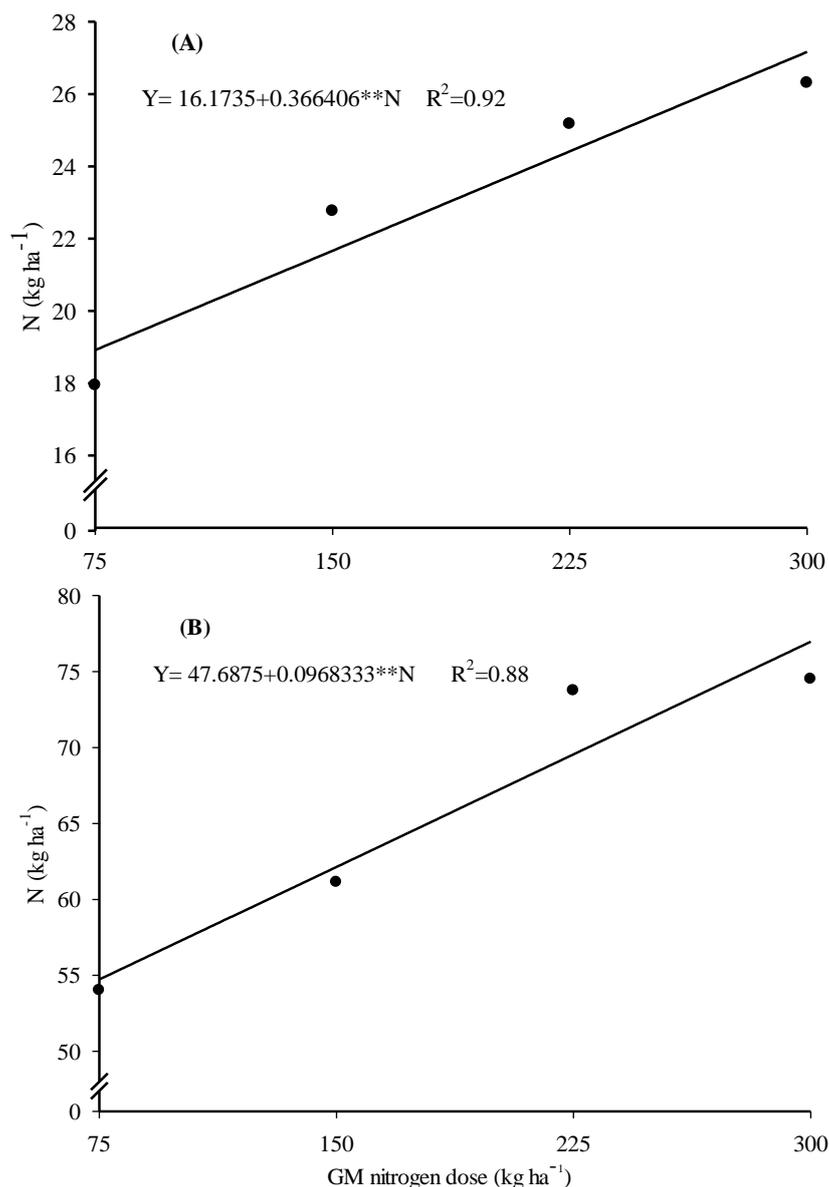
Dry matter accumulation at 85 DAP was influenced by the interaction of sunhemp N doses and incorporation timing ( $p < 0.05$ ). Under FI and SD, dry matter production was best fitted to a quadratic model as shown in figure (3).

Maximum dry matter production under FI was  $8523.3 \text{ kg ha}^{-1}$ , including foliage and tubers, and corresponded to  $244.84 \text{ kg N ha}^{-1}$ . However, maximum dry matter under SD was 8381 and corresponded to  $272.84 \text{ kg N ha}^{-1}$  from sunhemp residue incorporation.



**Figure 3.** Shoot and tuber dry matter production ( $\text{kg ha}^{-1}$ ) as affected by sunhemp nitrogen doses under GM incorporation timing. FI: GM whole dose incorporation at 15 days before planting and SD: GM supply at 15 days before planting and 15 days after planting, at 85 DAP.

Nitrogen accumulation ( $\text{kg ha}^{-1}$ ) in potato foliage (Figure 4A) and tubers (Figure 4B) at 85 DAP showed linear responses to sunhemp N doses. Foliage and tubers N content ranged from 19 to 27  $\text{kg ha}^{-1}$  and 55 to 78  $\text{kg ha}^{-1}$ , respectively. The interaction effect between incorporation timing and N dose did not affect the N partitioning between potato foliage and tubers at this growth stage. In addition, the N proportion within potato tubers to the total assimilated N ranged between 0.74 to 0.80 and was not influenced by either N doses or their interaction with incorporation timing.



**Figure 4.** Effects of sunhemp nitrogen doses on N-total accumulation in plant foliage (A) and tubers (B) at 85 DAP.

Dry matter production was influenced by sunhemp treatments, as individuals, whereas greater and significant increases than MN control were recorded by Dunnett ( $P < 0.05$ ), except for the treatments FI75 and SD150 which did differ from MN. However, SD75 was inferior to MN (Table 6). Generally, potato subjected to green manuring produced more dry matter than mineral N fertilization. On the other hand, N accumulation showed a different pattern whereas, low foliage N content under green manuring with no significant differences except for the dose SD 75 which was statistically inferior to MN. In addition, N accumulation within potato tubers followed the same pattern of foliage N. The majority of sunhemp treatments did not differ from MN ex-

cept the low N doses FI75 and SD75 which were statistically inferior to MN control. However, the tuber N proportion was not affected by sunhemp treatments as no significant differences from MN were detected by Dunnett test ( $p \geq 0.05$ ).

**Table 6.** Comparison of potato dry matter production (DM) and nitrogen accumulation in foliage (FN), tubers (TN), and tuber N proportion (TNP) between MN control and sunhemp treatments at 85 DAP

Treatment	DM (kg ha <sup>-1</sup> )	FN (kg ha <sup>-1</sup> )	TN (kg ha <sup>-1</sup> )	TNP
MN	7530.38	27.55	72.38	0.728 ns
FI75	6961.00	21.09	51.88 *	0.713
FI150	8309.50 *	21.17	63.38	0.750
FI225	8255.50 *	24.57	72.00	0.744
FI300	8449.50 *	28.18	76.25	0.732
SD75	6607.38 *	14.80 *	56.13 *	0.790
SD150	7410.13	24.34	58.88	0.709
SD225	8526.63 *	25.78	77.00	0.745
SD300	8261.13 *	24.42	71.25	0.745
LSD	594.54	8.76	16.2	0.078
CV (%)	4.32	20.43	14.3	6.04

Means followed \* in the column are statistically different from MN by Dunnett test at ( $p < 0.05$ ) and ns; not significant. MN; recommended mineral N, FI; full dose incorporation, SD; split incorporation and numbers adjacent refer to N dose (kg ha<sup>-1</sup>).

#### 3.5.4. Potato Nitrogen recovery, nitrogen recovery efficiency and nitrogen apparent recovery

Potato N recovery (Table 7) in N0 plots presented 59.5 kg N ha<sup>-1</sup>, from soil organic matter, and was inferior to mineral nitrogen supply (99.88 kg ha<sup>-1</sup>). Taking into account the supplied nitrogen per hectare, potato plants under MN presented 39.5% N recovery. However, the apparent recovered N, derived from the applied N amount after excluding N derived from soil organic matter, represented 16.15% of the mineral N.

Incorporation timing of sunhemp did not affect potato nitrogen recovery whereas, no significant difference between FI and SD ( $p \geq 0.05$ ). Potato crop has recovered approximately 86 kg N ha<sup>-1</sup> from SOM and incorporated sunhemp residue (Table 7). Nitrogen recovery efficiency (%) under both FI and SD was 57.1 and 56.91, respectively. Additionally, after removing the supplied N by SOM, N derived from sunhemp residue represented 15.76 and 15.7 % for FI and SD, respectively.

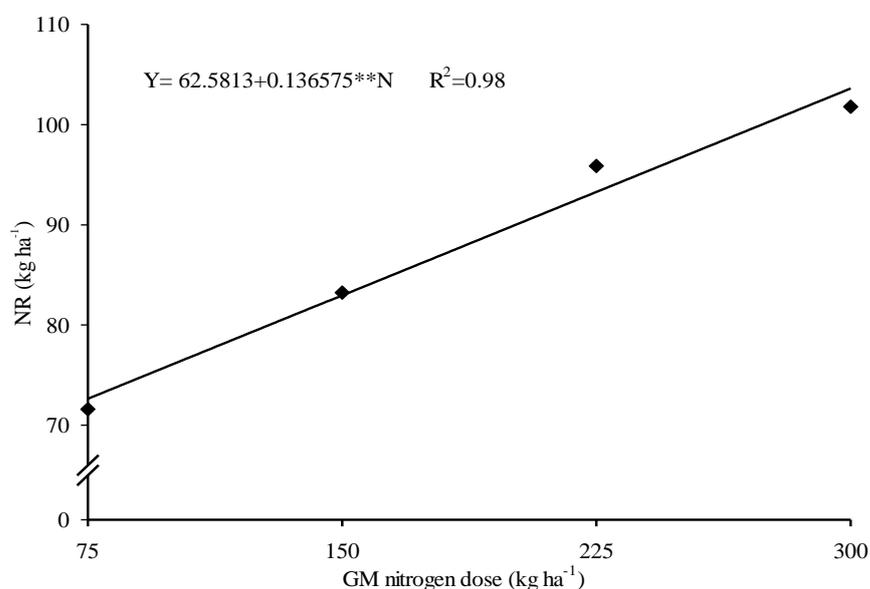
Nitrogen recovery (kg ha<sup>-1</sup>) was influenced by sunhemp nitrogen doses whereas, potato nitrogen acquisition increased linearly by increasing nitrogen doses (Figure 5) with a high determination coefficient ( $R^2=0.98$ ). Nitrogen recovery ranged between 70

and 104 kg N ha<sup>-1</sup> for sunhemp nitrogen doses. Meanwhile, the interaction between sunhemp incorporation timing and N doses did not influence potato N recovery.

**Table 7.** Comparisons of potato nitrogen recovery (NR), nitrogen recovery efficiency (NRE) and nitrogen apparent recovery (NAR) between control treatments (N0 vs. MN) and GM nitrogen incorporation timing (FI vs. SD) at 85 DAP

Treatment	NR (Kg ha <sup>-1</sup> )	NRE (%)	NAR (%)
N0	59.50 <sup>b</sup>	-	-
NM	99.88 <sup>a</sup>	39.95	16.15
FI	86.24 <sup>a</sup>	57.10	15.76
SD	86.23 <sup>a</sup>	56.91	15.37
CV (%)	17.91	-	-

Means followed by the same in the column are not significantly different by F test ( $P \geq 0.05$ ). N0; N zero treatment, MN; recommended mineral N dose, FI; full dose incorporation and SD; split incorporation.



**Figure 5.** Potato nitrogen recovery (kg ha<sup>-1</sup>) as affected by sunhemp nitrogen doses

Comparison between MN control and green manure treatments, as individuals, did not show significant differences of recovered N except for the dose (75 kg ha<sup>-1</sup>) under FI and SD. These treatments presented lower nitrogen recovery than MN (Table 8). However, the other GM doses presented similar N recovery to MN control, despite the numerical inferiority. In addition, the dose SD225 resulted in higher N recovery than MN but the difference was insignificant ( $p \geq 0.05$ ).

Potato plants within this experiment presented moderate nitrogen recovery efficiency from mineral nitrogen, around 40%. On the other hand, nitrogen recovery effi-

ciency as influenced by green manuring under both incorporation timing showed a gradient decrease by increasing N dose. NRE (%) ranged between 97.35 and 32 % of the total supplied N. However, nitrogen apparent recovery (NAR) which represents the amount of nitrogen derived from the supplied amount represented 16.15 % of mineral nitrogen and 12 to 19% for green manure nitrogen doses (Table 8).

**Table 8.** Comparison of potato nitrogen recovery (NR), nitrogen recovery efficiency (NRE) and apparent nitrogen recovery (ANP) between MN control and GM treatments at 85 DAP

Treatment	NR (kg ha <sup>-1</sup> )	NRE (%)	NAR (%)
MN	99.88	39.95	16.15
FI75	73.01 *	97.35	18.02
FI150	83.15	55.43	15.77
FI225	88.95	39.53	13.1
FI300	88.30	36.02	16.18
SD75	70.70 *	94.27	14.4
SD150	83.33	55.55	15.88
SD225	102.3	45.70	19.25
SD300	88.10	31.90	12.10
LSD	20.95	-	-
CV (%)	13.42	-	-

Means followed by \* in the column are statistically different from MN by Dunnett test ( $p < 0.05$ ). MN; recommended mineral N, FI; full dose incorporation, SD; split incorporation and numbers adjacent refer to N dose (kg ha<sup>-1</sup>)

### 3.5.5. Yield and its components

Potato fresh tuber yield and dry matter production showed a drastic reduction in the absence of N fertilizer (Table 9) except class 4 and dry matter content (%). Tubers` class 4 which includes small tubers, diameter less than 3.3 cm, and blemish ones, was not influenced by N absence and did not differ from mineral N ( $p \geq 0.05$ ). Mineral nitrogen supply increased tubers` classes 2 and 3. Consequently, commercial and total yield was increased. Fresh tubers` class 1 was not obtained within this experiment. Tubers dry matter content (%) was not influenced by mineral N supply. However, dry matter production (t ha<sup>-1</sup>) was higher under mineral N fertilization than in N0 duo to the elevated fresh tuber yield.

Splitting sunhemp residue application resulted in significant fresh tuber yield increases more than full incorporation before planting (Table 9) with the exception to classes 3 and 4. In spite of the higher numerical means, the difference was not signifi-

cant ( $p \geq 0.05$ ). Tubers class 2 ( $4.6 \leq \text{diameter} \leq 8.5$  cm) was increased by approximately 7.5 % as a result of splitting sunhemp N doses. This increase in tubers` class 2 was consequently reflected in marketable and total fresh tuber yield by approximately 7 % under SD than FI. Meanwhile, dry matter content (%) was not influenced by sunhemp incorporation timing ( $p \geq 0.05$ ) despite the higher content under SD. However, dry matter production ( $\text{t ha}^{-1}$ ) showed significant difference ( $p < 0.05$ ) between FI and SD whereas, SD augmented dry matter production by 9.3% more than FI.

**Table 9.** Comparison of potato yield classes, marketable yield (MY), total yield (TY), dry matter content (DM) and dry matter production (DM) between control treatments (N0 vs. MN) and GM incorporation timing (FI vs. SD) at 103 DAP

Treatment	Class 4 ( $\text{kg ha}^{-1}$ )	Class 3 ( $\text{kg ha}^{-1}$ )	Class 2 ( $\text{t ha}^{-1}$ )	MY ( $\text{t ha}^{-1}$ )	TY ( $\text{t ha}^{-1}$ )	DM (%)	DM ( $\text{t ha}^{-1}$ )
N0	1596.25 a	4700.00 b	25,577.50 b	30,277.50 b	31,873.80 b	13.46 a	4,020.34 b
MN	1522.50 a	6492.50 a	34,837.50 a	41,330.00 a	42,852.50 a	12.61 a	5,767.00 a
FI	1512.81 a	6456.13 a	34,463.80 b	40,919.90 b	42,432.70 b	14.00 a	6,046.51 b
SD	1670.00 a	6641.88 a	37,065.00 a	43,706.90 a	45,376.90 a	14.55 a	6,607.70 a
CV (%)	44.14	25.77	8.45	7.87	8.15	4.96	10.49

Means followed by the same letter are not significantly different by F test at ( $p \geq 0.05$ ).

N0; N zero treatment, MN; recommended mineral N dose, FI; full dose incorporation and SD; split incorporation.

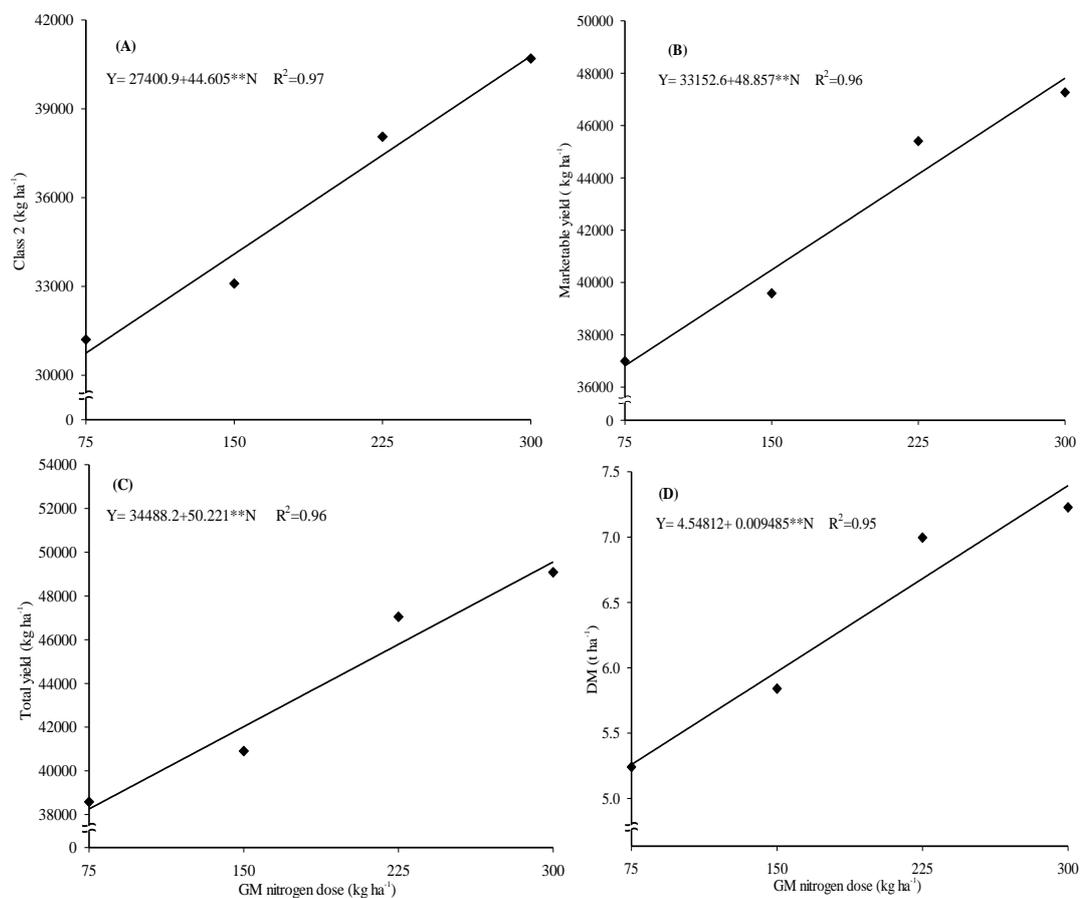
Sunhemp N doses did not influence tubers classes 3 and 4 productions as no increments in these grades were resulted from increasing nitrogen dose. As well as the interaction of sunhemp incorporation timing with N dose did not affect these grades proportions ( $p \geq 0.05$ ). However, tubers` class 2 showed a positive response to N dose whereas; it was linearly increased with increasing nitrogen dose (Figure 6A). In addition, the interaction between sunhemp incorporation timing and its N doses was not significant.

Marketable yield was influenced by sunhemp nitrogen dose. A strong linear increase ( $R^2=0.96$ ) was obtained (figure 6B). Meanwhile, the interaction between sunhemp incorporation timing and nitrogen dose did not affect marketable yield and was insignificant ( $p \geq 0.05$ ). Total yield followed the same trend of tubers` class 2 and marketable yield as it increased linearly by increasing sunhemp nitrogen dose (figure 6 C). Total fresh tuber yield ranged between 36 and 50  $\text{t ha}^{-1}$ . Meanwhile, the interaction with incorporation timing was not significant as well.

Tuber dry matter content (%) was not influenced by sunhemp N doses as well as their interaction with incorporation timing. Inversely, dry matter production ( $\text{t ha}^{-1}$ )

presented a linear increase pattern to N doses (figure 6D). In addition, the interaction between N doses and incorporation timing did not influence dry matter production

Fresh tuber yield components in relation to MN control showed that tubers` classes 3 and 4 were not affected by green manuring and were similar to MN ( $p \geq 0.05$ ) as shown in table (10). Meanwhile, some GM treatments showed significant differences from MN control for class 2 production. The GM nitrogen dose FI75 produced lower tuber yield than the control. However, the elevated N doses FI300, SD225 and SD300 were superior to MN. Besides, the rest of sunhemp treatments were statistically equivalent to MN.



**Figure 6.** Response of tuber class 2 (A), marketable yield (B), total yield (C) and dry matter production (D) to sunhemp N doses.

Marketable and total yield as affected by green manuring showed significant differences from MN whereas the elevated doses FI300, SD225 and SD300 resulted in greater yields than MN fertilization. However, the rest of sunhemp treatments were not statistically different from MN except FI75 which produced lower marketable and total yield than the control.

Dry matter content (%) was not influenced by green manuring as no significant difference by Dunnett test ( $p \geq 0.05$ ) were detected despite the superior dry matter content under sunhemp treatments. Meanwhile, dry matter production ( $t\ ha^{-1}$ ) was higher under FI 225, FI300, SD225 and SD300 sunhemp treatments and lower under FI75 than MN control.

**Table 10.** Comparison of potato yield classes, marketable yield (MY), total yield (TY), dry matter content (DM %) and dry matter production (DM  $t\ ha^{-1}$ ) between MN control and sunhemp treatments at 103 DAP

Treatment	Class 4 ( $kg\ ha^{-1}$ )	Class 3 ( $kg\ ha^{-1}$ )	Class 2 ( $t\ ha^{-1}$ )	MY ( $t\ ha^{-1}$ )	TY ( $t\ ha^{-1}$ )	DM (%)	DM ( $t\ ha^{-1}$ )
MN	1522.2 ns	6492.5 ns	34,837.50	41,330.50	42,852.50	13.46 ns	5,767.00
FI75	1683.75	5408.25	29,705.00 *	35,113.25 *	36,797.00*	13.29	4,886.48 *
FI150	1090.00	5846.25	32,131.25	37,977.50	39,067.50	14.34	5,564.00
FI225	1340.00	7145.00	37,487.50	44,632.50	45,972.50	14.81	6,807.80 *
FI300	1512.81	6456.13	38,531.25 *	45,956.25 *	47,893.75*	14.92	6,927.90 *
SD75	1491.25	6172.5	32,700.00 *	38,872.50	40,363.75	13.88	5,598.20
SD150	1540.00	7137.5	34,060.00	41,197.50	42,737.50	14.35	6,119.10
SD225	1948.75	7543.75	38,633.75 *	46,177.50 *	48,126.25*	14.98	7,184.10 *
SD300	1670.00	6641.88	42,866.25 *	48,580.00 *	50,280.00*	14.47	7,529.40 *
LSD	987.90	2463.68	3,163.25	3,634.73	3,785.46	1.87	769.33
CV (%)	34.53	21.46	5.06	4.91	4.92	7.34	7.05

Means followed by \* in the column are significantly different from MN by Dunnett test ( $p < 0.05$ ) and ns: not significant. MN; recommended mineral N, FI; full dose incorporation, SD; split incorporation and numbers adjacent refer to N dose ( $kg\ ha^{-1}$ ).

### 3.5.6. Nitrogen use efficiency (NUE)

Nitrogen use efficiency for total and marketable yield under green manuring presented the same trend (Table 11) whereas increasing GM nitrogen rates declined NUE. Comparing NUE values for both total and marketable yield under FI and SD of the same GM nitrogen dose showed a persistent trend as SD has increased the mean yield for each nitrogen unit. These results highlight the efficiency of splitting sunhemp incorporation in increasing N productivity. Taking into account the efficacy of MN, potato showed better nitrogen use efficiency when fertilized with GM nitrogen.

**Table 11.** Nitrogen use efficiency (NUE kg tuber kg<sup>-1</sup> N) of potato total and marketable yield as affected by GM nitrogen doses under different incorporation timing. FI: full GM incorporation before planting and SD: split dose

GM N dose (kg ha <sup>-1</sup> )	Total yield		Marketable yield	
	FI	SD	FI	SD
75	490.63	538.18	468.18	518.3
150	260.45	284.92	253.18	274.65
225	213.77	213.9	198.37	205.23
300	159.65	167.6	153.19	154.68
MN control	174.16		164.36	

### 3.5.7. Correlation between nutritional indices and total and marketable yield

The tested correlations between nutritional indices and total tuber yield presented different patterns and was influenced by incorporation timing. All nutritional indices presented significant coefficients with total and marketable yield under FI. Among the tested nutritional indices meanwhile, SPAD presented significant correlation coefficient with a total yield under SD. Based on the significance of these coefficients, linear equations were adjusted therefore they can help in yield prediction when sunhemp residue is incorporated before planting as a unique nitrogen source.

**Table 12.** Pearson correlation and linear equations between N nutritional indices (38 DAP) and potato total and marketable yield under green manure full incorporation before planting (FI) and split application (SD)

Variable	Total yield			
	FI		SD	
	r <sup>2</sup>	Estimated equation	r <sup>2</sup>	Estimated equation
SPAD	0.695**	$\hat{Y} = -74974.005 + 3080.025^{**} \text{SPAD}$	0.508**	$\hat{Y} = -30380.375 + 1986.421^{*}$
CHLT	0.589*	$\hat{Y} = -10611.557 + 7614.462^{*} \text{CHLT}$	0.173 ns	-
N-total	0.691**	$\hat{Y} = -39720.809 + 1495.809^{**} \text{NT}$	0.326ns	-
Marketable yield				
SPAD	0.712**	$\hat{Y} = -75462.010 + 3053.14^{**} \text{SPAD}$	0.496ns	-
CHLT	0.572*	$\hat{Y} = -6745.231 + 6842.29^{*} \text{CHLT}$	0.176ns	-
N-total	0.711*	$\hat{Y} = -40104.449 + 1475.248^{**} \text{NT}$	0.351ns	-

Correlation coefficients were obtained using 16 data sets.

\*, \*\* and <sup>ns</sup> are significant at 5%, 1% and not significant by t test, respectively,

## 3.6. DISCUSSION

### 3.6.1. Green manure decomposition and nitrogen mineralization

The fast decomposition and nitrogen mineralization rates of sunhemp residue can be attributed to its tissue high quality, particularly C/N ratio, as it presented lower C/N ratio. N mineralization of organic residues was reported to be controlled by nu-

merous factors such as climate, biochemical composition, carbon and nitrogen content. Chaves *et al.* (2004) reported that C/N ratio was the best predictor for the potential N mineralization from incorporated organic residues. They found that N mineralization rate after clover and Lucerne green manures (12 and 10 C/N ratio) incorporation was higher than after vetch and oat mixture (31 C/N ratio). Moreover, high quality residues which contain easily degradable compounds tend to increase soil microbial biomass and activity leading to net N mineralization (Stark *et al.*, 2007). Sunhemp residue presented fast decomposition rate particularly in the first few weeks after incorporation whereas approximately 35 % of initial N content was released during the first five weeks and 72% until potato harvest. These results are persistent with that obtained by Perin *et al.* (2006); Odhiambo, (2010) and Araujo, (2012).

The difference between decomposition velocity within FI and SD could be attributed to temperature since mean daily and night temperatures in May were higher by 2 degrees than in June. Climatic conditions, such as temperature and relative humidity, and application pattern were reported to influence organic residue degradation and N mineralization. This result is in accordance with that reported by Wilson and Hargrove, (1986). They reported that organic residue incorporation favors microbial activity and decomposition by increasing the exposed area for microbial attack and providing closer soil contact and adequate soil moisture better than surface or mulch application.

The obtained nitrogen half life in this experiment could be seen as protracted when compared with that obtained by Perin *et al.* (2006) and Araujo, (2012) in the same region and same species. They obtained shorter N half life (15 and 31.9, respectively). This prolonged half life could be attributed to phenological age at harvest and time of incorporation. In our experiment, sunhemp was harvested at 85 DAP and applied in the winter season whereas dry and cold weather. However in the previous investigations, sunhemp was harvested at approximately 70 DAP and applied during summer which is characterized by high temperature and intensive precipitation rate which led to higher decomposition rate and shorter half life.

### **3.6.2. Potato nitrogen nutrition status**

Nitrogen is influential of many plant cell components, including amino acids, nucleic acids as well as chlorophyll. Chlorophyll consists of tetrapyrrol which is derived from prothobilinogen, the precursor of tetrapyrroles synthesis, and formed by the condensation of  $\delta$ -amino levulinate which is produced by glutamate reduction. Glutamate is a major portion of reduced nitrogen in plant mesophyll. Normally, increased

nitrogen availability for plant assimilation enhances glutamate biosynthesis,  $\delta$ -amino levulinate and ultimately chlorophyll (Heldt, 2005). Therefore, leaf chlorophyll content as indicator for plant nitrogen status was reported in a variety of crops. In a particular case of potatoes, Botha *et al.* (2006) found an increment in chlorophyll concentration by increasing N supply. Besides, the SPAD meter measures the concentration of green color which is related to chlorophyll concentration and its values are relevant to plant N status indirectly as shown by Fonts, (2001) and Rodrigues, (2004).

The superiority of nutritional indices, particularly N-total content, within potato leaves under zero N to mineral N fertilization can be explained by the tendency of the potato crop under restricted N supply. As potato crop is a C3 plant and under restricted N availability, plant adapts to these conditions by accumulating more nitrogen within their leaves accompanied by reduction of leaf expansion to maximize solar radiation utilization. Moreover considering the experimental condition as the same irrigation regime was implemented and the addition of organic residue within GM treatments, it can be observed that control treatments, both MN and N0 were subject to partial water deficiency. Under these conditions, leaf nitrogen and chlorophyll content in N0 plots tend to be similar or higher than fertilized plots (Haverkort and Mackerron, 2006). Moreover, Shahnazari *et al.* (2008) found higher N-total content within potato leaves under deficit irrigation than full irrigation regime.

Under sunhemp residue incorporation timing, total chlorophyll and N-total content detected difference between FI and SD which could be attributed to N supplied by sunhemp residue prior to planting. Furthermore, the less sensitivity of SPAD to distinguish between treatments in the early stages as well. Although the second split of sunhemp N under SD was supplied 10 days before SPAD measurement, it can be speculated that potato plants did not benefit from that released nitrogen which was reflected in lower leaf chlorophyll and nitrogen content. These results are consistent with that obtained by Wu *et al.* (2007). They reported that the SPAD meter was much less sensitive to detect treatment differences than petiole nitrate content particularly before hilling.

Leaf nutritional indices response to nitrogen fertilization, for the same variety and the same region, were reported to be curve linear (Gil *et al.*, 2002; Coelho *et al.*, 2010 and Silva *et al.*, 2011). The optimum N dose corresponded to critical indices thresholds was approximately 200 kg ha<sup>-1</sup> mineral N in the base dressing. However in our study, SPAD index and leaf N-total content showed linear responses to sunhemp N

doses thus it was not possible to estimate critical thresholds. In addition, these linear responses can be attributed to the low amount of released N from sunhemp residue. From N release kinetic and at 8 weeks after incorporation which corresponded to 38 DAP, the released N from sunhemp residue accounted for 45% of the initial N. This represented approximately 135 kg N for the maximum N dose ( $300 \text{ kg ha}^{-1}$ ) which is less than the mentioned optimum dose for critical indices from mineral nitrogen. Moreover, the SPAD linear response in the current study is in accordance with that obtained by Zhu *et al.* (2012) who reported a progressive increase of SPAD value by increasing N rates in any growth stage except at 20 days after sowing.

Results of comparison with the MN control, sunhemp treatments presented lower SPAD values regardless of N dose. This inferiority can be attributed to other factors rather than N supply such as soil water content as explained by Haverkort and Mackerron, (2006). Green manure incorporation contributes to increase soil organic matter leading to improved water retention and supply consequently decreased SPAD values. These results are also in accordance with that obtained by Rodrigues *et al.* (2006) and Shahnazari *et al.* (2008). They reported inferior nutritional indices, particularly SPAD, under organic N fertilizers to mineral ones in sweet corn and potatoes, respectively. The similar content of total chlorophyll and N-total of potato leaves under sunhemp fertilization to MN control within this phenological age is not clear and could be seen as a physiological adaptation to N release dynamics of sunhemp residues to optimize radiation use efficiency and improve productivity.

### **3.6.3. Dry matter and nitrogen accumulation at 85 DAP**

The absence of nitrogen under N0 control resulted in reduced foliage and tuber dry matter accumulation when compared to MN treatment before senescence. As well as N accumulated within tubers was lower than in mineral N fertilization. Millard and Marshall, (1986) demonstrated limited potato growth and yield as a consequence to insufficient N supply. Moreover, Plotkin *et al.* (2000); Macqueen *et al.* (2007) and Campiglia *et al.* (2009) reported low dry matter production and nitrogen accumulation by potato plants in N0 plots before the senescence due to limited N supply.

Under sunhemp residue incorporation timing, reduction in total dry matter production (foliage and tuber) was observed under split application. This reduction can be attributed to the low supplied N before planting. Vos, (1999) reported that potato dry matter production tends to be low when little nitrogen is applied at the beginning leading to low foliage development. Meanwhile, foliage and tuber N content as well as tu-

ber N proportion of total N uptake were not affected by split residue incorporation. This result can be attributed to the fast N release from sunhemp residue. Additionally, it can be speculated that nitrogen of SD was synchronized with potato uptake and contributed to enhance N recovery without contribution to dry matter production. These results are in accordance with that mentioned by Vos, (1999). He reported that split N application up to 60 DAP slightly improved the absorption and utilization of nitrogen by potatoes. However, under organic fertilization, no investigations have reported the efficiency of splitting organic fertilizers in potato dry matter production and N accumulation.

The interaction effect between sunhemp incorporation and N doses showed similar quadratic patterns of dry matter production. Generally, this quadratic response is attributed to the equilibrium between N absorption and C fixation to produce dry matter. Furthermore, it is a variety-based attribute (Gastal and Lemaire, 2002). Full incorporation of sunhemp residue before planting increased dry matter production at 85 DAP. Meanwhile, foliage and tuber N content were increased linearly by increasing N rate. Similar results were obtained by Plotkin *et al.* (2000). They reported that potato foliage and tuber N content were linearly increased by increasing N rate from 67 to 268 kg ha<sup>-1</sup> under different green manures. Moreover, shoot and tuber N uptake ranged between 34 to 102 and 64 to 75 kg ha<sup>-1</sup>, respectively. Our results are not comparable with these results due to different climatic conditions, soil fertility and employed variety.

The majority of sunhemp treatments produced similar dry matter to MN control, even under low N doses. This similarity can be attributed mainly to the non-nitrogen effects of organic residue incorporation preceding potato. These observations were mentioned by Honeycutt, (1997) and Bath, (2000). Furthermore, foliage and tuber N content and tuber N proportion under sunhemp treatments were similar to MN control with the exception to low N rates. This similarity can be attributed to the fast release of sunhemp nitrogen and its synchrony with potato uptake. Honeycott, (1997) observed an increased N uptake preceding green manures. He regarded this increase to the improved soil water retention which enhances potato N acquisition.

The tuber nitrogen content proportion of the total accumulated N was not affected by green manuring and ranged from 0.72 to 0.79. These ranges are in accordance with that reported by Li *et al.* (2003) and Zebarth *et al.* (2004). They reported that N present in tubers ranges from 0.7 to 0.85. Moreover, the low N proportion occurs under elevated fertilizer rates or in immature crops.

### 3.6.4. Potato nitrogen recovery, nitrogen recovery efficiency and apparent nitrogen recovery

The potential of soil organic matter to provide potato crop with N in the absence of external N inputs was reported to range between 24 to 114 kg ha<sup>-1</sup> (Zebarth *et al.* 2008) depending on SOM level and climatic conditions. In this experiment, around 60 kg N ha<sup>-1</sup> was obtained from SOM. Reports on potato N acquisition from SOM have registered wide variations from 48 to 88 kg N ha<sup>-1</sup>. Zebrath *et al.* (2009) reported 74 to 78 kg N ha<sup>-1</sup>. However, Nyiraneza and Snapp, (2007) reported 22 kg N ha<sup>-1</sup>. Meanwhile, potatoes under MN control has recovered approximately 100 kg ha<sup>-1</sup> which represents (~ 40%) of the applied N fertilizer. Generally, potato N recovery from MN fertilizer varies according to various factors, e.g. location, season, applied N rate and variety. Potato N recovery was reported to range from 29-77% (Zebarth *et al.*, 2009). In addition, Shrestha *et al.* (2010) reported that N recovery from MN ranged between 110 to 250 kg ha<sup>-1</sup> and N recovery efficiency up to 50%. Our results are in the cited ranges.

Sunhemp incorporation timing did not influence N recovery and N recovery efficiency as well as apparent N recovery. Generally, the split fertilizer N application is a common approach under mineral fertilization to improve crop fertilizer N assimilation by improving the synchrony between N supply and crop demand. Several studies have reported increased N recovery by splitting N in potato crop when compared with full application at planting (Vos, 1999 and Maidl *et al.*, 2002). On the other hand, other studies showed no effect of splitting N on potato recovery (Joern and Vitosh, 1995). In this study, splitting sunhemp N did not influence potato N recovery and recovery efficiency. Meanwhile, Potato N recovery showed a linear response to sunhemp N doses. This result is in accordance with that obtained by Plotkin *et al.* (2000). They reported linear responses of potato N recovery to N doses after incorporation of Oat, Lupin, Vetch and Pea, either solely or in combinations.

Comparing potato N recovery of sunhemp treatments with MN control, low N recovery under organic fertilization was observed with the exception to one treatment, SD 225, despite the lack of significant differences. N recovery and N recovery efficiency of crops from organic fertilizers were reported to be lower than mineral fertilizers. Rodrigues *et al.* (2006) reported 40% decline in N recovery by triticle and maize after amending soil with vegetumus in relation to ammonium nitrate during three years. Moreover, N apparent recovery ranged between 5 and 13.6% for organic fertilizers and 58.2 % for ammonium nitrate.

### 3.6.5 Yield and its components

Reduced fresh tuber yield in the zero N plots was obtained. This reduction is due to the lack of nitrogen fertilization. Limited potato yield in the absence of N fertilizer was reported by Millard and Marshall, (1986), Plotkin *et al.* (2000), Macqueen *et al.* (2007) and Campiglia *et al.* (2009). This decline was attributed to reduced vegetative growth and dry matter accumulation in tubers. Meanwhile, marketable and total yield in the presence of MN in this experiment was 41.3 and 42.85 t ha<sup>-1</sup>, respectively and were greater than (33.1 and 36 t ha<sup>-1</sup>) that obtained by Fontes *et al.* (2010) in the same region using the same variety. However, the obtained yield levels are comparable to that mentioned by Coelho *et al.* (2010) in the same region and variety.

Split application of sunhemp residue N increased fresh tuber yield (class 2, marketable and total yield) and dry matter production. The efficiency of splitting organic fertilizers in potato yield has not been studied yet because of the split application constraints. Inversely, studies dealt with splitting mineral nitrogen highlighted its advantages to improve potato yield. Westermann *et al.* (1988); Vos, (1999); Maidl *et al.* (2002) and Zebarth and Rosen, (2007) recommended split N application as an approach to increase potato tuber yield in irrigated potato farms by providing better synchrony between N supply and potato N demand. Moreover, reducing nitrate leaching losses.

Marketable and total yield as well as dry matter production was linearly increased with sunhemp nitrogen doses. These results are in accordance with that obtained by Plotkin *et al.* (2000), Sincik *et al.* (2008) and Campiglia *et al.* (2009). They reported increases in potato yield and its components after incorporation of legume green manures when compared with non-legume crops. These increases were attributed to the high added N rates by legumes. On the other hand, Fontes *et al.* (2010) and Coelho *et al.* (2010) reported quadratic yield response for the same variety to mineral nitrogen rates which is different from our case. These differential responses can be attributed to N supplied. They have recommended N dose, in the base dressing, for maximum yields more than 200 kg ha<sup>-1</sup>. However, the highest N level which was released from sunhemp residue until harvest was 224 kg ha<sup>-1</sup>. Furthermore, taking into account N dynamics and mineralization-immobilization balance of the GM residue, it can be speculated that not all mineralized N from GM residue was readily available for plant uptake and was less than the critical dose for maximum yields.

Sunhemp residue incorporation as N source resulted in similar and higher potato fresh tuber yield and dry matter production when compared with the MN control de-

spite the inferior N recovery. These results are similar to that obtained by Campiglia *et al.* (2009). He obtained a yield of 48.5 t ha<sup>-1</sup> after subclover and hairy vetch incorporation which were similar to MN fertilization. In addition, different experiments showed potato yield increases prior to legumes incorporation when additional mineral N fertilizer was supplied. Carter *et al.* (2003) reported yield increases by 7.5% when the green manure preceded potato than mineral nitrogen alone. Nyiraneza and Snapp, (2007) reported 18% yield increase when amending soil with green manure more than in unamended soil. Sincik *et al.* (2008) reported 12.7 % and 15 % more tuber yield prior to incorporation of common vetch and Faba bean than mineral N fertilizer alone. Yield increases after green manures incorporation were also regarded to other factors rather than nitrogen such as improved soil properties, water retention, cation exchange capacity and water infiltration, augmented microbial activity and reduced crop pathogens, which ensure better circumstances for better growth and high yields (Bath 2000; Rodrigues *et al.* 2006; Carter *et al.* 2003; Sincik *et al.* 2008 and Campiglia *et al.* 2009).

### **3.6.6. Nitrogen use efficiency**

Nitrogen use efficiency (kg tuber kg<sup>-1</sup> N) or N fertilizer productivity for total and marketable was decreased by increasing N dose. This trend is in agreement with the observations of Darwish *et al.* (2006); Kumar *et al.* (2007); Goffart *et al.* (2008); Fontes *et al.* (2010) and Irena *et al.* (2011). The decline of fertilizer productivity is attributed to the low ability of potato to assimilate mineral soil nitrogen. Comparing NUE values of FI and SD for the same GM nitrogen dose for both total and marketable yield showed a persistent trend. SD presented great N productivity of total and marketable yield. The obtained result highlights the efficiency of splitting sunhemp N application in increasing potato yield. Moreover, it can be speculated that splitting sunhemp residue in this experiment provided better N synchrony to potato N uptake and contributed to increase yield.

Comparing N productivity of sunhemp treatments with MN control showed a higher efficiency of sunhemp N than mineral N even when the same doses were applied. This high efficiency can be attributed to the non-nitrogen benefits of sunhemp incorporation as mentioned by Bath, (2000) and Campiglia *et al.* (2009). In addition, Fontes *et al.* (2010) reported nitrogen productivity values of 115 and 99 (in the same region and variety) for total and marketable yield, respectively when 300 kg N ha<sup>-1</sup> was supplied. In our study, the mean values for NUE of sunhemp nitrogen doses exceeded

the mentioned ranges suggesting that green manures represent a higher NUE in potato production than mineral nitrogen fertilizers.

### **3.6.7. Correlation between N nutritional indices and yield**

The obtained correlation coefficients are similar to those mentioned by Minotti *et al.* (1994) who mentioned coefficients between SPAD and yield from 0.61 to 0.48, Majic *et al.* (2008) who mentioned 0.61 to 0.48 between SPAD and yield and Coelho *et al.* (2010) who obtained correlation coefficients of 0.63, 0.64 and 0.42 for total yield and 0.59, 0.6 and 0.35 for marketable yield with SPAD index, N-total and total chlorophyll content, respectively. In the present study, the correlation between leaf nutritional indices and yields was affected by green manure incorporation timing. A possibility to use fourth leaf N indices to predict yield when sunhemp accounts for the potato N supply, particularly when incorporated prior to planting, was found.

## **3.7. CONCLUSIONS**

The green manure sunhemp (*Crotalaria juncea* L) could be accounted for adequate N supply when used as a unique nitrogen source in potato production whereas similar or higher yields than MN fertilization were obtained.

Potato N status as influenced by sunhemp showed lower SPAD values and was not affected by split N supply. However, CHLT and N-total content showed similar ranges to mineral N fertilizer.

Dry matter production under sunhemp fertilization was higher than mineral N. Meanwhile, nitrogen partitioning in plant foliage and tubers showed a little decline. As well as N recovery was quite low under sunhemp than mineral nitrogen and was not affected by split application. In addition, N recovery efficiency and apparent N recovery depended on the sunhemp N dose and were higher than MN.

Yield and its components were similar or higher under sunhemp fertilization than mineral N and were influenced by splitting application whereas, marketable and total yield were higher by 7% than full incorporation before planting. In addition, DM production was increased by 9.3% as a result of split application. Furthermore, split application of the GM nitrogen doses improved nitrogen use efficiency.

The GM sunhemp increased nitrogen use efficiency than mineral nitrogen. Moreover, the split sunhemp application showed higher N use efficiency than full incorporation before planting.

Employing N indices provided us with opportunity to monitor potato N status and predict yield, particularly when sunhemp is used as N source and fully incorporated before planting.

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## 4. CHAPTER 3

### Potato Yield, Dry Matter and Nitrogen Accumulation and Use Efficiency under Green Manuring

#### 4.1. ABSTRACT

Potato productivity improvement succeeding green manures incorporation has been documented. Meanwhile, patterns of dry matter and nitrogen accumulation as well as yield increment are still scarce. For that reason, a field experiment was conducted in Viçosa MG, Brazil in 2012 to investigate potato yield and dry matter and nitrogen accumulation patterns under green manure (GM) supply as a nitrogen source. The experiment consisted of 5 treatments whereas; 3 N doses (100, 200 and 400 kg ha<sup>-1</sup>) from the GM sunhemp (*Crotalaria juncea* L.), the recommended mineral N dose (250 kg ha<sup>-1</sup>) and zero N treatment. Treatments were arranged in a complete randomized block design in a split-plot scheme with four replicates. Treatments composed the main plots while plant sampling dates, from 35 to 80 days after planting (DAP), comprised the subplots. Furthermore, a litter bag experiment was set up simultaneously with the GM incorporation to monitor dry matter decomposition and N mineralization over time. Data were subjected to regression analysis to determine dry matter, N and yield accumulation patterns over time. Meanwhile, mean comparison between treatments in each sampling date was implemented by Tukey test ( $p < 0.05$ ).

Nitrogen mineralization presented higher rate (0.0215 g g<sup>-1</sup> day<sup>-1</sup>) than dry matter decomposition (0.0116 g g<sup>-1</sup> day<sup>-1</sup>). After 6 weeks of incorporation, 69% of the GM initial nitrogen was mineralized. Furthermore, 88.2% of initial N was mineralized until 80 DAP.

Dry matter and nitrogen accumulation in potato foliage presented quadratic patterns with higher accumulation and estimated rates for GM treatments than MN. Additionally; nitrogen accumulation peaked prior to dry matter whereas maximum accumulated N was observed between 52.5 and 56.5 DAP while dry matter peaked between 57.5 and 71.65 DAP. Fresh tuber yield, dry matter and nitrogen accumulation as well as dry matter and nitrogen accumulation in the whole plant presented linear patterns over time with higher means and greater estimated rates for GM treatments than MN treatment at sampling dates. Additionally, potato under GM treatments accumulated 92.4 to 98.5% of the harvested tubers until 80 DAP, which was higher than MN (87.7%).

Harvest index (HI) was not influenced by treatments. Unlikely, nitrogen harvest index (NHI) was greater under the low GM nitrogen dose (GM100) than the other GM treatments, which were similar to MN treatment. Nitrogen recovery increased by increasing GM nitrogen dose and was higher under the elevated dose despite the similarity to MN treatment. Nitrogen recovery efficiency (NRE) and apparent recovery (NAR) presented an inverse trend to GM nitrogen dose. The elevated GM nitrogen dose presented similar efficiencies to MN. However, the lower dose presented higher efficiencies than MN.

Marketable and total yield increased by increasing GM nitrogen dose. Meanwhile, dry matter content at harvest was not affected by N source and doses as well. Inversely, dry matter yield increased by increasing GM nitrogen dose and was superior to MN control. NUE declined with increasing GM nitrogen dose. Additionally, the lower and intermediate GM doses presented higher NUE than the higher GM nitrogen dose as well as MN fertilizer. The results revealed that green manuring stimulated potato growth early in the season leading to high fresh tuber yield and DM accumulation in early stages

**Key words;** *Solanum tuberosum* L, Sunhemp, yield and dry matter accumulation, N uptake and efficiencies.

#### 4.2. RESUMO

Há relatos do aumento da produtividade da batata seguindo a incorporação de adubos verdes. No entanto relatos dos padrões de acúmulo da matéria seca e de nitrogênio sob adubação verde ainda são escassos. O objetivo do experimento foi investigar os padrões de acúmulo da matéria seca e do nitrogênio e da produtividade na batata sob adubação verde, como fonte de nitrogênio. O experimento foi realizado em Viçosa MG, Brasil, em 2012. O experimento consistiu de cinco tratamentos sendo 3 doses de massa de crotalária (*Crotalaria juncea* L.) equivalentes a 100, 200 e 400 kg ha<sup>-1</sup> de N, aplicação da dose recomendada de N mineral (250 kg ha<sup>-1</sup>) e tratamento com zero N. Os tratamentos foram dispostos em DBC, em esquema de parcelas subdivididas com quatro repetições. Os tratamentos compuseram as parcelas enquanto as datas de amostragem, de 35 a 80 dias após o plantio (DAP), compreenderem as subparcelas. Além disso, um experimento de *litter bags* foi conduzido simultaneamente com a incorporação da massa da crotalária para monitorar sua decomposição e a mineralização de N ao longo do período experimental. Os dados foram submetidos à análise de regressão para determi-

nar os padrões do acúmulo da matéria seca, N e rendimento ao longo do tempo. Além disso, a comparação entre as médias dos tratamentos em cada data de amostragem foi feita pelo teste de Tukey ( $p < 0,05$ ).

A mineralização do nitrogênio apresentou maior taxa ( $0,0215 \text{ g g}^{-1} \text{ dia}^{-1}$ ) do que a decomposição da massa ( $0,0116 \text{ g g}^{-1} \text{ dia}^{-1}$ ). Após 6 semanas da incorporação, 69% do nitrogênio originalmente presente na massa da crotalária foram mineralizados e 88,2% foram mineralizados até 80 DAP.

Os acúmulos de matéria seca e de nitrogênio na folhagem apresentaram comportamentos quadráticos, com maiores máximos e taxas com aplicação da crotalária do que com MN. Adicionalmente, o máximo acúmulo de nitrogênio ocorreu entre 52,5 e 56,5 DAP que foi antes de que matéria seca (57,5 e 71,65 DAP). O acúmulo de matéria fresca e seca e o acúmulo de nitrogênio nos tubérculos e na planta inteira aumentaram linearmente ao longo do tempo, com taxas maiores com a aplicação de crotalária do que com MN. Além disso, a batata sob adubação verde acumulou 92,4-98,5% da massa colhida aos 80 DAP, enquanto que nessa data plantas que receberam MN tinham acumulado 87,7% da massa colhida.

O índice da colheita não foi influenciado pelos tratamentos. Porém, o índice da colheita de N foi maior sob a menor de N com crotalária do que os outros tratamentos, que resultaram em valores similares ao obtido com MN. A recuperação do N aumentou linearmente em função da dose da crotalária, sendo similar ao obtido com MN. A eficiência de recuperação e recuperação aparente de N apresentaram decréscimos lineares em função do aumento da dose de crotalária, sendo que a dose mais elevada apresentou valores similares ao obtido com MN.

As produtividades comercial e total aumentaram com o aumento da dose de crotalária. Enquanto isso, o teor de matéria seca na colheita não foi afetado pela fonte de N e nem pelas doses. Inversamente, a produtividade de matéria seca aumentou com o aumento da dose de crotalária e foi superior à obtida com MN. A EUN diminuiu com o aumento da dose de crotalária. Além disso, as duas menores doses de crotalária resultaram em maior EUN do que a mais elevada e MN. Os resultados indicam que a adubação verde estimulou o crescimento da batata levando ao maior acúmulo de matéria seca nas fases iniciais e a uma alta produtividade de tubérculos.

**Palavras-chave:** *Solanum tuberosum* L, *Crotalaria juncea* L., acúmulo de matéria seca, recuperação e eficiência de N.

### 4.3. INTRODUCTION

Patterns of potato growth over the season and distribution of dry matter and nitrogen among different plant parts are important parameters to understand and adjust proper management practices leading to improved nitrogen uptake, yield and tuber quality. Furthermore, plant growth and N availability in the soil influence the pattern and magnitude of nutrient uptake, accumulation and partitioning of nitrogen and dry matter into plant parts. In this regard, many researchers have developed different simulation models to predict potato growth, tuber production and rational fertilization programs under different management practices. Biemond and Vos, (1992) carried out an experiment on pots (Potato cv. Bintje) with N rates of 2.5, 8 and 16 g plant<sup>-1</sup>. They found that plants under high N rates have accumulated dry matter within foliage four-folds greater than under low N rates during 30 to 80 days after emergence. Additionally, the foliage dry weight began to decrease during 80 to 120 days after emergence. Meanwhile, dry matter content per plant was greater at high N rates than low rate and increased rapidly during 100 to 120 days after planting. Additionally, harvest index showed a marginal response to different N rates and decreased with the N rate increase. The harvest index averaged 86% across all N rates at maturity. Vos, (1999) evaluated the time span during which potato crop can respond efficiently to additional nitrogen and studied the effect of split N application on the efficiency of nitrogen utilization for four years. The study used two potato varieties (Prominent and Vebece) combined with different N rates and an additional N zero plot. He reported yield increases by increasing N rate. In addition, splitting N dose into two or three applications did not affect dry matter content and yield at maturity but improved N utilization. Furthermore, increasing applied N before planting led to increased foliage dry matter and N content early after emergence. In addition, total dry matter accumulation continued throughout the season until 100 DAE, but total N reached a maximum at 60 DAE with a little gain thereafter. The study concluded that N application can be adjusted to meet the estimated requirements extends to (at least) 60 days after emergence. Alva *et al.* (2002) evaluated dry matter and nitrogen accumulation and partitioning of two potato cultivars, (Russet Burbank and Hilite Russet) during the growing period for four years. The results showed that dry matter allocation into tubers presented similar sigmoid patterns for both cultivars, reaching a maximum at 100 DAP. Meanwhile, canopy dry matter and N accumulation followed quadratic patterns and peaked at 90-100 DAP. Tuber weight increased rapidly during 60 to 100 days after planting and accounted for 76 to

87% of total plant weight at maturity. Tuber nitrogen content followed the same trend of dry matter accumulation and accounted for 81 to 86 and 83 to 89%, for the Hilite Russet and Russet Burbank, respectively.

Zebarth *et al.* (2004b) quantified nitrogen and dry matter accumulation as well as nitrogen use efficiency of 20 commercial potato cultivars in response to different N rates (0, 100 and 200 kg ha<sup>-1</sup>) for two sequential years. They found that dry matter accumulation increased linearly with increasing N supply with significant differences between cultivars. Meanwhile, nitrogen accumulation presented a linear increase to increased N supply with significant differences between cultivars as well as between study years. Furthermore, harvest index (HI) and nitrogen harvest index (NHI) showed an inverse trend to N rates. These indexes were declined by increasing N rates with wide variations among varieties. Similarly, nitrogen use efficiency was declined by increasing N rate. Additionally, potato cultivars presented different potentials to assimilate soil N under the same fertilization regimes and environment. Under Brazilian conditions, Fernandes *et al.* (2010 and 2011) evaluated nitrogen, dry matter and fresh yield accumulation of five potato cultivars (Agata, Asterix, Atlantic, Markies and Mondial) over the growing season under one fertilization regime. The undertaken cultivars presented sigmoid patterns of N, dry matter and fresh tuber yield accumulation over time with relative variations of accumulation rates between cultivars, depending on maturity. Furthermore, cultivars presented different HI, ranging from 79.6 to 86.8, at 97 DAP. Additionally, NHI ranged from 68.7 to 84 of the total accumulated N. the study concluded that the peak of nutrients supply, particularly nitrogen, is between 42 to 70 DAP and each variety requires proper nitrogen management.

In the recent past, integrating green manures in farming systems for more sustainability issues has been renewed. Furthermore, potato yield increases after green manure incorporation has been reported. Nyiraneza and Snapp, (2007) found that potato yield and N uptake efficiency after winter rye was greater by 20% than after fallow plots in the presence of mineral N supply. Meanwhile, in the absence of mineral fertilizer, rye cover crop increased tuber yield by 40 to 210% than in bare soil. Sincik *et al.* (2008) found that potato crop following legume green manures and cover crops produced approximately 36% to 38% higher tuber yields than following winter wheat in the absence of external N supply. Campiglia *et al.* (2009) found that potato fresh marketable yield following subclover and hairy vetch was similar to that obtained by mineral nitrogen fertilization (48.5 t ha<sup>-1</sup>). The study demonstrated the potential of green

manures to provide potato with adequate nitrogen supply and yield equal to that of mineral nitrogen. Moller and Reents, (2009) reported potato higher fresh yield after legumes by 10 % than after non-legumes. Similarly, tuber N uptake was higher by 30%.

Investigations dealt with potato yield improvement following organic fertilizers have attributed these results to non-nitrogen effects. Meanwhile, reports concerning growth patterns are still limited. Macqueen, (2007) carried out an investigation in 12 organically managed potato farms to estimate dry matter production and N uptake, and fresh tuber yield accumulation in order to optimize fertilization programs. The results showed different estimated rates of dry matter accumulation ( $150 - 200 \text{ kg ha}^{-1} \text{ day}^{-1}$ ) and harvest index ( $0.53 - 0.83$ ) before senescence. The N uptake pattern presented different models (linear, quadratic, cubic and sigmoid). However, N accumulation in tubers presented a linear model in all farms with different estimated rates ( $1.6 - 2 \text{ kg ha}^{-1} \text{ day}^{-1}$ ). Similarly, the fresh tuber yield increase had similar estimated rates ( $0.60$  to  $1 \text{ Mg ha}^{-1} \text{ d}^{-1}$ ) in all farms. The differences between farms were regarded to management and fertilization history, which influenced nitrogen availability. Additionally, differences regarding the maturity time of the potato varieties resulted in relative differences in growth parameters and N uptake. Najm *et al.* (2010) compared the effect of different rates of cattle manure (5, 10, 15 and  $20 \text{ t ha}^{-1}$ ) and mineral nitrogen doses (50, 100,  $150 \text{ kg ha}^{-1}$ ) and their interaction on potato shoots dry matter (at 75 DAP) and tuber yield. The results showed that the shoot dry matter presented linear increases due to manure and nitrogen rates with no interaction effect between manure and mineral nitrogen on this attribute. However, fresh tuber yield was influenced by the interaction between manure rate and N dose, and the highest yield was obtained from the high manure rate and N dose.

It is evident that potato growth, N uptake and dry matter accumulation under organic N fertilization, particularly green manuring, are marginally studied. The current study aimed to evaluate dry matter, nitrogen and yield accumulation patterns of potato subjected to different N rates from the green manure sunhemp (*Crotalaria juncea* L.).

#### **4.4. MATERIALS AND METHODS**

##### **4.4.1. Site description, soil properties and meteorological data**

A field experiment was conducted from May 21<sup>st</sup> to September 27<sup>th</sup> 2012, in the Horta Nova experimental farm of Universidade Federal de Viçosa, MG. The experi-

mental farm is located at 693 m altitude, latitude 20°45` S and longitude 42°51' W. The region is characterized by dry moderate to cold winter and rainy hot summer. Meteorological data (Table 1) during the experimental time was obtained from a weather station nearby the experimental farm.

**Table 1.** Mean daily air and night temperatures and precipitation rate during potato plantation from May to September 2012

Variable	May	June	July	August	September
Mean daily temperature (°C)	23.6	24.0	24.8	25.2	27.8
Mean night temperature (°C)	14.3	14.0	10.9	12.0	13.6
Precipitation (mm)	3.04	0	0	0	1.56

The experimental area was planted with two sequential corn plantations in order to reduce soil mineral N content. After corn harvest and before installing the experiment, soil samples were collected from the top soil surface to 30 cm, homogenized and mixed in a composite sample. A sub sample was collected and submitted to analysis. Soil characteristics are shown in Table (2).

**Table 2.** Initial characteristics of the soil of experimental before sunhemp incorporation and potato plantation

Property	Unit	Value	Method
OM	dag kg <sup>-1</sup>	3.0	Walkely-Black =org C×1.724
pH (in water1:2.5)		5.3	
P	mg dm <sup>-3</sup>	37.7	Mehlich-1 extractor
K	mg dm <sup>-3</sup>	45.0	Mehlich-1 extractor
Ca <sup>2+</sup>	cmol <sub>c</sub> dm <sup>-3</sup>	3.0	KCL-1mol L <sup>-1</sup> extractor
Mg <sup>2+</sup>	cmol <sub>c</sub> dm <sup>-3</sup>	0.5	KCL-1mol L <sup>-1</sup> extractor
Al <sup>3+</sup>	cmol <sub>c</sub> dm <sup>-3</sup>	0.0	KCL-1mol L <sup>-1</sup> extractor
Effective CTC	cmol <sub>c</sub> dm <sup>-3</sup>	3.66	
CTC in pH 7.0	cmol <sub>c</sub> dm <sup>-3</sup>	8.61	
V	%	43.0	
Rem-P	mg L <sup>-1</sup>	37.3	
Texture		Sandy clay	EMBRAPA, 2006

#### 4.4.2. Green manure production and chemical characterization

The green manure sunhemp (GM) was sown on February 10<sup>th</sup>, 2012 in an area next to the experimental site. Sunhemp seeds were sown manually with 0.5 m row spacing and density of 40 per meter linear. Plants did not receive any fertilizers before sowing and during growing season. Before full blooming (At 65 Days after Planting - DAP), the green manure was harvested manually, chopped and air dried at room tem-

perature then stored until incorporation. Before incorporation, the dry GM mass was homogenized, and sampled for dry matter and total nitrogen determination. Samples were oven dried in a forced air oven at 60 °C until constant weight. Thereafter, samples were ground to pass through a 2 mm sieve. Total nitrogen content was determined by micro-Kjeldahl method (Miyazawa *et al.*, 2009). GM dry mass presented 87.5 % dry matter and 2.37% N-total content.

#### **4.4.3. Treatments and layout of the experiment**

The experiment consisted of 5 treatments whereas 3 treatments received N doses equivalent to 100, 200 and 400 kg ha<sup>-1</sup> from the GM sunhemp residue. The two other treatments were a zero N treatment (N0) and recommended mineral N (250 kg ha<sup>-1</sup>). Treatments were arranged in a complete randomized block design with 4 replicates. Sunhemp dry mass corresponding to N doses was incorporated one week before potato planting. Meanwhile, mineral nitrogen was supplied in the form of ammonium sulfate whereas 70% spread in the furrows before planting, and 30% was supplied as a side dressing at 22nd day after emergence (Fontes, 2005).

Before planting, the soil was tilled and disc harrowed. One meter interspacing between blocks and 0.5 m distance between each experimental plot were left to avoid the risk of green manure contamination during incorporation. Each experimental plot had 9.4 m<sup>2</sup> (3.75×2.5 m). Each experimental plot contained 50 plants. The outer two rows were considered as border and were excluded from data collection.

Soil was amended with the following mineral nutrients; super phosphate, potassium chloride and magnesium sulphate with the amount of 420, 220 and 200 kg ha<sup>-1</sup>, respectively. In addition, Micro-nutrients, boron, copper and zinc 10 kg ha<sup>-1</sup> of each and 250 g ha<sup>-1</sup> molybdenum. The fertilizers were blended and applied in the furrows.

Homogeneous tubers of 'Ágata' potato, with a mean weight of 25g were pre-germinated at room temperature and indirect sunlight. When the sprouts reached around 3 cm length, tubers were hand planted with 0.25 m apart and covered with 10 cm of soil. In addition, agronomic practices such as irrigation and control of pests and diseases were implemented following the regional recommendations for potato plantation.

#### **4.4.4. Green manure decomposition and nitrogen mineralization**

A litter bag experiment was set up simultaneously with the GM incorporation. Determination of sunhemp mass breakdown and nitrogen mineralization was measured from green manure incorporation until potato harvest. Fifteen grams of the air-dried biomass (14 g DW) were placed in Nylon bags (2 mm mesh size and 25×25 cm) and

buried to the depth of 10-15 cm in the soil adjacent to the experiment. Bags were randomly sampled at 0, 7, 14, 21, 35, 49, 70, 91 and 112 days after incorporation.

At each sampling date, three random bags were retrieved. The remaining GM mass was cleaned from soil particles then dried in air forced oven at 60 °C until constant weight. Samples were weighed, ground to pass through a 2 mm sieve and subjected to total nitrogen content determination by micro-Kjeldahl method. The percent of the remaining dry matter (DM) and nitrogen was calculated as shown:

$$XR (\%) = (X_t/X_o) \times 100;$$

The XR is the percent DM weight or nitrogen remaining;  $X_t$  is the DM weight or the nitrogen content at each sampling time and  $X_o$  is the DM weight or nitrogen amount at the decomposition start.

Dry matter and nitrogen mineralization kinetics were fitted into the first-order single exponential model (Wieder and Lang, 1982):  $M_t = M_0 e^{-kt}$ , where;  $M_t$  is the remaining dry matter or nitrogen amount after a time period “t”, in days,  $M_0$  is the total dry matter or nitrogen at the beginning of the experiment, “k” is the decomposition rate constant.

Half-life of the sunhemp residue and nitrogen content was calculated by the equation  $t_{1/2} = \ln(0.5)/k$  as described by Paul and Clark., (1996); where  $t_{1/2}$  is the time required for crop residue biomass or the nutrient content reduction to half of the initial value.

#### **4.4.5. Potato sampling, dry matter accumulation and nitrogen uptake determination**

Dry matter accumulation and nitrogen uptake were quantified over the growing season in the above and underground plant parts, excluding roots, beginning at an early stage of tuber set until senescence. Sampling was carried out at 35, 50, 65 and 80 DAP. All calculations were based on 0.75 m row spacing and 0.25 m between plants, equating a plant population of 50,000.00 plant ha<sup>-1</sup>. Sampling was conducted following the method described by Sullivan *et al.* (2008). At each sampling date, two plants from the inner rows surrounded by competitive plants were harvested, excluding roots, and separated into foliage and tubers. The foliage was cleaned, washed and dried in air forced oven at 60 °C until constant weight and then dry weight was recorded. Samples were ground to pass through a 2 mm sieve, and total nitrogen content (%) was determined. Tubers were weighed and a sample of five tubers was collected, washed by tap water followed by distilled water and left to dry. Tubers were cut into cubes of approximately

1 cm<sup>3</sup> and mixed. A sub-sample of 200 g was dried at 60 °C until constant weight then ground to pass through a 2 mm sieve, and N-total content (%) was determined by micro-Kjeldahl method. N uptake was calculated by the following equation:

$$\text{N uptake (kg ha}^{-1}\text{)} = \text{dry biomass (kg ha}^{-1}\text{)} \times \text{N\%}$$

Sum of mean foliage and tubers dry matter and nitrogen were expressed in kg per hectare basis to represent the total amount per hectare.

Within the last sampling date (at 80 DAP), Harvest index (HI) was obtained by dividing tuber dry matter (kg ha<sup>-1</sup>) to total dry matter (kg ha<sup>-1</sup>).

Nitrogen recovery (kg ha<sup>-1</sup>) was obtained by summing foliage and tubers N-content to represent N-total per plant and then expressed in a per hectare basis.

Nitrogen Harvest Index (NHI) was calculated as described by Gholipouri and Kandi, (2012) by the following equation:

$$\text{NHI} = \frac{\text{N uptake by tubers (kg ha}^{-1}\text{)}}{\text{N uptake by the plant (kg ha}^{-1}\text{)}}$$

Nitrogen Recovery Efficiency (NRE %) was calculated by the equation:

$$\text{NRE (\%)} = \frac{\text{N recovery for treatment (kg ha}^{-1}\text{)}}{\text{N applied in the same treatment (kg ha}^{-1}\text{)}} \times 100$$

Nitrogen Apparent Recovery (NAR %) was calculated following the equation described by Lynch *et al.* (2008) as follow:

$$\text{NAR (\%)} = \frac{\text{N uptake for treatment} - \text{N uptake for 0N treatment}}{\text{N applied in the treatment}} \times 100$$

#### 4.4.6. Yield component's determination

At harvest (103 DAP), 10 plants of the central rows were harvested. Tubers were left for curing then graded according to the standards of Ministério da Agricultura, Agropecuária e Abastecimento (Brazilian Ministry of Agriculture, 1995). Yield grades were as follows; class 1 (diameter > 8,5 cm), class 2 (8,5 ≤ tubers diameter ≤ 4,5 cm), class 3 (4,5 ≤ tubers diameter ≤ 3,3 cm) and class 4 included tubers with a diameter less than 3,3 cm and tubers with any commercial disorders such as greenish, rotten and infected by insects or diseased. The sum of the classes 1, 2 and 3 represented commercial yield and the sum of all classes represented total yield.

Dry matter content (%) was determined by collecting samples of 5 tubers from the commercial tubers. Tubers were washed by running water followed by distilled water and cut into cubes of 1 cm<sup>3</sup> and mixed. A composite sub-sample of 200 g was dried in 60 °C until constant weight. Dry matter (%) content was multiplied by total yield to calculate dry matter production. Nitrogen use efficiency (NUE, kg tuber kg<sup>-1</sup>N) for the

total and commercial yield was calculated by dividing the total and marketable yield by the applied nitrogen.

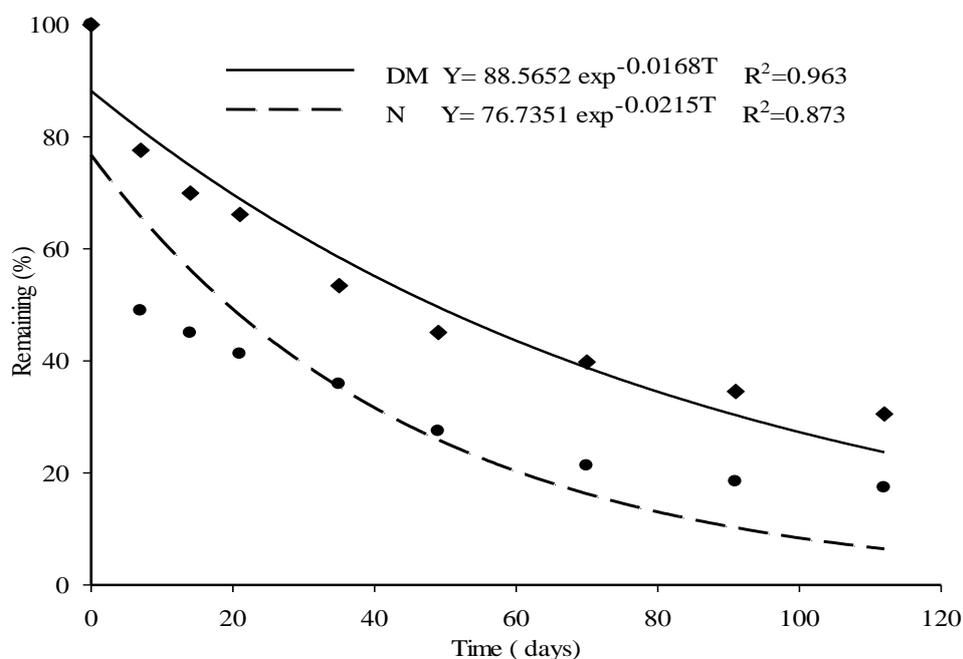
#### 4.4.7. Statistical analysis.

The data were subjected to analysis of variance ( $P < 0.05$ ) using SAEG software (V, 9.1, 2007). Data were analyzed adopting split-plot arrangement whereas treatments were comprised the main plots and plant sampling dates comprised subplots. Dry matter, nitrogen and yield accumulation were subjected to regression analysis whereas treatments were fixed, and time was considered as a dependent factor. The regression models were selected according to coefficient significance, determination coefficient and biological behavior of each variable. Furthermore, means comparison within each sampling event as well as harvest variables were performed by Tukey test ( $p < 0.05$ ) within each date individually to maximize the least significant difference.

### 4.5. RESULTS

#### 4.5.1. Green manure decomposition and nitrogen mineralization

Dry matter decomposition and nitrogen mineralization of sunhemp (Figure 1) presented high decomposition rates,  $k = 0.0168$  and  $0.0215 \text{ g g}^{-1} \text{ day}^{-1}$ . Nitrogen mineralization was higher than dry matter decomposition and presented shorter half life (32.24) than dry matter (59.75) as well.



**Figure 1.** Dry matter decomposition and nitrogen mineralization of sunhemp form biomass incorporation until potato harvest.

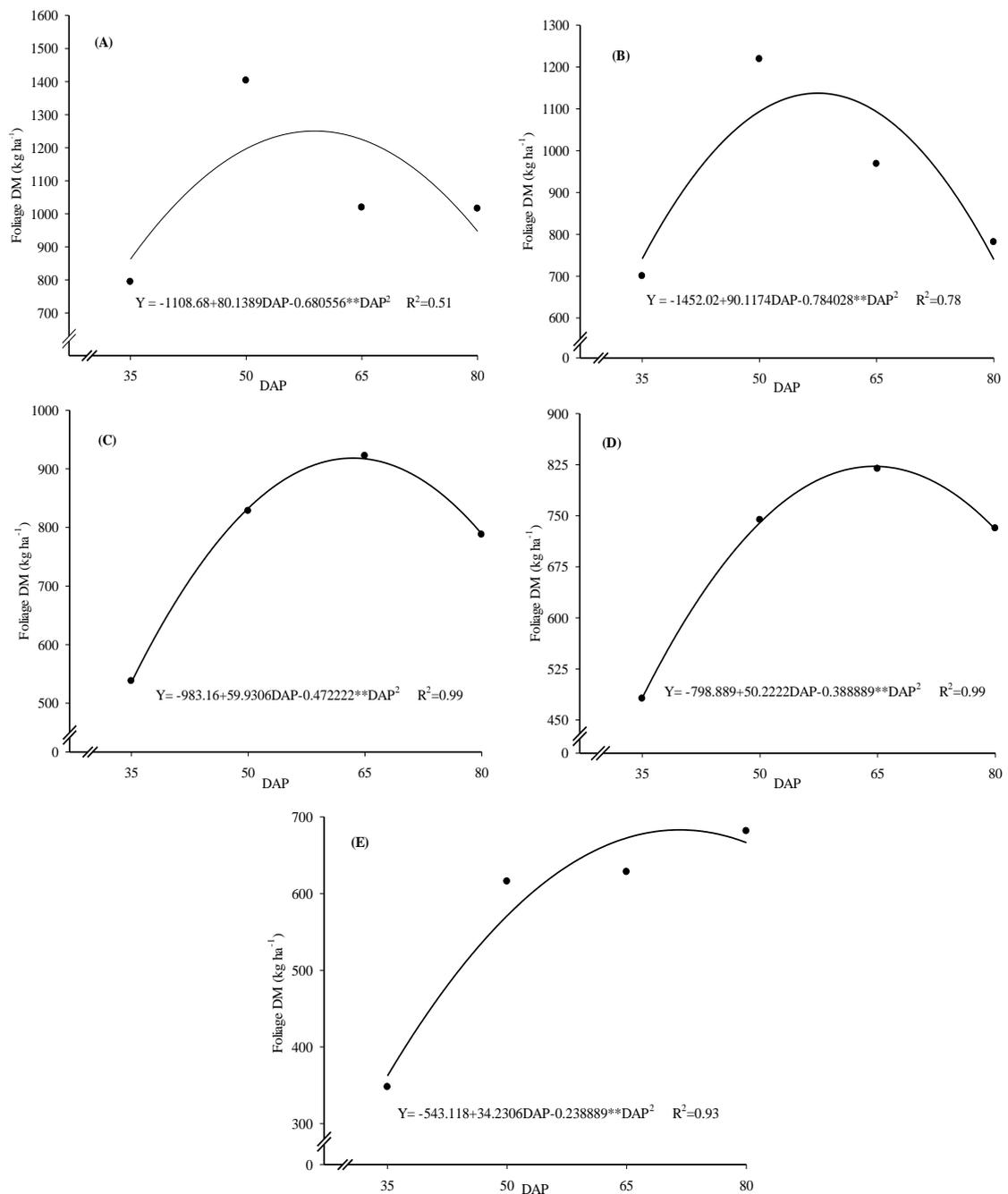
Nitrogen mineralization presented accelerated rate, particularly in the first days, whereas around 70% of the GM was mineralized in 6 weeks after incorporation. Estimates of the mineralized nitrogen from GM doses at each potato sampling date are shown in Table 3.

**Table 3.** Estimates of mineralized nitrogen amounts ( $\text{kg ha}^{-1}$ ) from GM doses at each potato sampling date after planting and until harvest

DAP	GM nitrogen doses ( $\text{kg ha}^{-1}$ )		
	100	200	400
35	68.90	137.79	275.58
50	77.47	154.94	309.88
65	84.03	168.06	336.11
80	88.18	176.36	352.72
112	93.03	186.18	372.38

#### 4.5.2. Dry matter and N accumulation

Foliage dry matter accumulation under all treatments fitted in quadratic functions (Figure 1). Green manure stimulated dry matter accumulation within the potato foliage more than mineral N fertilization. Dry matter accumulation peaked at 59, 57.5 and 63.5 DAP for GM nitrogen doses 400, 200 and 100  $\text{kg ha}^{-1}$ . Additionally, maximum accumulation was 1250.5, 1137.53 and 918.32  $\text{kg ha}^{-1}$ , respectively. Meanwhile, maximum DM accumulation under mineral nitrogen was 822.57  $\text{kg ha}^{-1}$  at 64.57 DAP. Potato grown on N0 plots presented low DM accumulation (683.12  $\text{kg ha}^{-1}$ ) late in the season (at 71.65 DAP).



**Figure 2.** Dry matter accumulation ( $\text{kg ha}^{-1}$ ) as a function of days after planting (DAP) under different fertilization treatments. (A): green manure ( $400 \text{ kg ha}^{-1}$ ), (B): green manure ( $200 \text{ kg ha}^{-1}$ ), (C): green manure ( $100 \text{ kg ha}^{-1}$ ), (D): mineral nitrogen ( $250 \text{ kg ha}^{-1}$ ) and (E): zero nitrogen.

Comparison between treatments means at each sampling date (Table 4) showed significant differences by Tukey test ( $p < 0.05$ ). At 35 DAP, potatoes under GM accumulated more DM than under MN fertilizer. Furthermore, increasing GM nitrogen dose resulted in significant increases in dry matter accumulation. Potato grown in zero N plots presented lower DM accumulation than all other treatments. The same trend was observed at 50 DAP. Meanwhile, at 65 and 80 DAP, despite potato growth on GM

plots remained higher than MN, no significant differences were obtained except on N0 treatment which resulted in the lower dry matter accumulation.

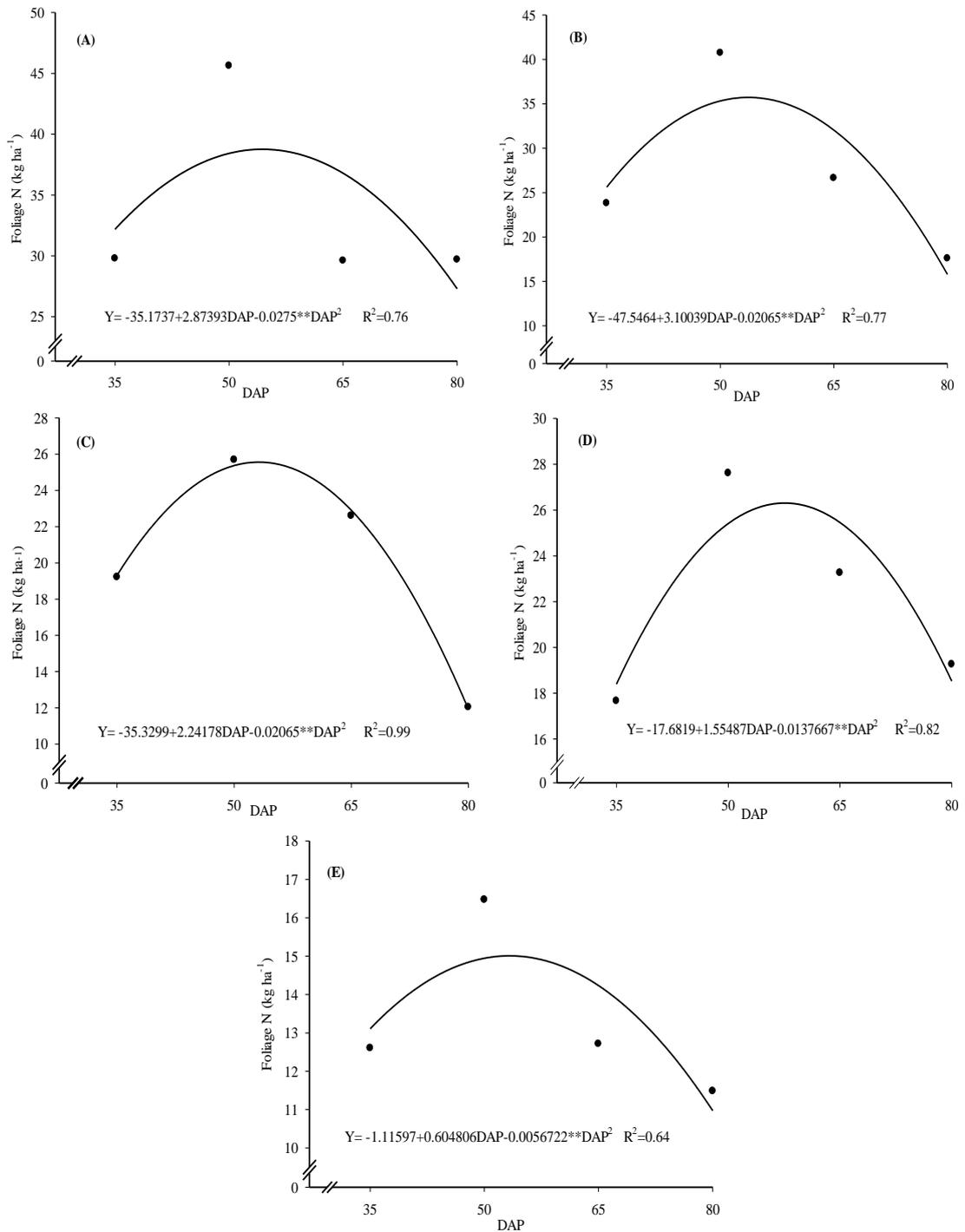
**Table 4.** Potato foliage dry matter (DM) accumulation under different N treatments at four sampling dates after planting (DAP)

Treatment	Foliage DM (kg ha <sup>-1</sup> )			
	35 DAP	50 DAP	65 DAP	80 DAP
GM400	793.75 <sup>a</sup>	1403.13 <sup>a</sup>	1018.75 <sup>a</sup>	1015.63 <sup>a</sup>
GM200	700.00 <sup>b</sup>	1218.75 <sup>b</sup>	968.130 <sup>a</sup>	781.250 <sup>ab</sup>
GM100	537.50 <sup>c</sup>	828.130 <sup>c</sup>	921.880 <sup>a</sup>	787.500 <sup>ab</sup>
MN	481.25 <sup>d</sup>	743.750 <sup>d</sup>	818.750 <sup>ab</sup>	731.250 <sup>ab</sup>
N0	347.50 <sup>e</sup>	615.630 <sup>e</sup>	628.130 <sup>b</sup>	681.250 <sup>b</sup>
LSD	53.73	66.19	214.19	292.89
CV (%)	4.09	3.05	10.9	16.23

Means followed by the same letter in the column are not statistically different by Tukey test ( $p \geq 0.05$ ).

GM: green manure, MN: mineral N fertilization 250 kg ha<sup>-1</sup> N, N0: zero N treatment. Numerical values refer to N dose (kg ha<sup>-1</sup>).

Nitrogen accumulation within the plant foliage under all treatments presented similar quadratic functions of dry matter accumulation (Figure 3). However, maximum N accumulation preceded the maximum dry matter accumulation by approximately one week except under N0 treatment. Maximum accumulated N for GM treatments and MN was observed between 52.5 and 56.5 DAP. Additionally, the high GM doses (400 and 200 kg N ha<sup>-1</sup>) resulted in more N accumulation (39.13 and 35.73 kg ha<sup>-1</sup>, respectively) than the 100 kg N ha<sup>-1</sup> dose and MN fertilizer (25.51 and 26.22 kg ha<sup>-1</sup>, respectively). Moreover, the maximum accumulated N in N0 plots was 15 kg ha<sup>-1</sup>.



**Figure 3.** Foliage dry matter accumulation ( $\text{kg ha}^{-1}$ ) as a function of days after planting (DAP) under different fertilization treatments. (A): green manure ( $400 \text{ kg ha}^{-1}$ ), (B): green manure ( $200 \text{ kg ha}^{-1}$ ), (C): green manure ( $100 \text{ kg ha}^{-1}$ ), (D): mineral nitrogen ( $250 \text{ kg ha}^{-1}$ ) and (E): zero nitrogen

Comparison of N accumulation within the foliage over time (Table 5) showed significant differences between treatments by Tukey test ( $p < 0.05$ ). The GM400 treatment resulted in higher amounts of accumulated N than all other treatments in all sampling dates. However, at 50 and 65 DAP those values were similar to GM200. Potato plants under N0 plots presented the smaller N accumulation.

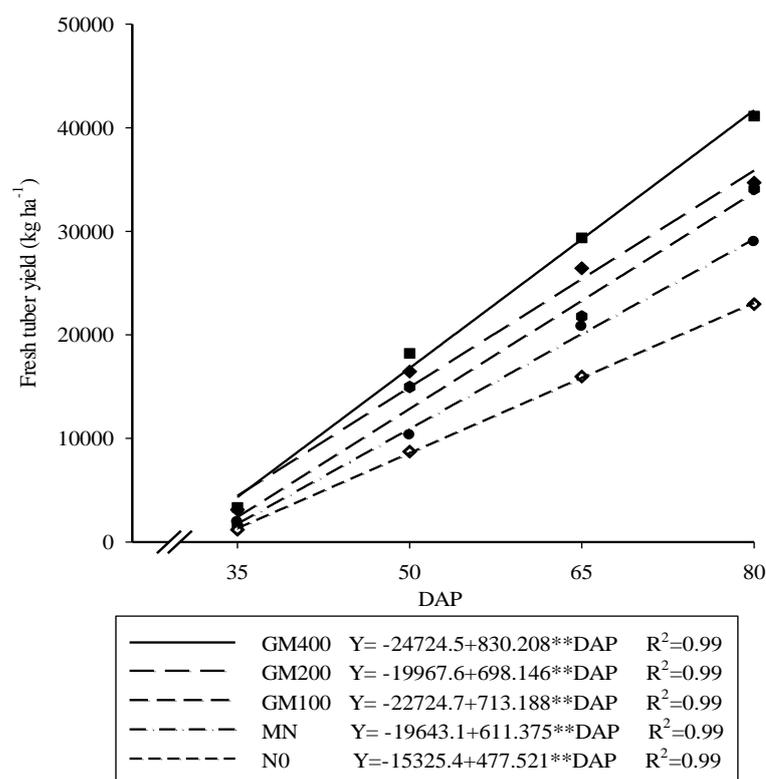
**Table 5.** Potato foliage N accumulation (kg ha<sup>-1</sup>) under different N treatments at four sampling dates after planting (DAP)

Treatment	Foliage N (kg ha <sup>-1</sup> )			
	35 DAP	50 DAP	65 DAP	80 DAP
GM400	29.78 <sup>a</sup>	45.62 <sup>a</sup>	29.60 <sup>a</sup>	29.69 <sup>a</sup>
GM200	23.82 <sup>b</sup>	40.73 <sup>a</sup>	26.65 <sup>ab</sup>	17.59 <sup>b</sup>
GM100	19.22 <sup>c</sup>	25.68 <sup>b</sup>	22.60 <sup>b</sup>	12.04 <sup>c</sup>
MN	17.66 <sup>c</sup>	27.61 <sup>b</sup>	23.26 <sup>b</sup>	19.26 <sup>b</sup>
N0	12.60 <sup>d</sup>	16.47 <sup>c</sup>	12.71 <sup>c</sup>	11.48 <sup>c</sup>
LSD	3.89	8.02	5.23	6.6
CV (%)	8.37	11.39	10.1	18.6

Means followed by the same letter in the column are not statistically different by Tukey test ( $p \geq 0.05$ ). GM; green manure, MN; recommended mineral N fertilization, N0; zero N treatment and numerical values refer to N dose (kg ha<sup>-1</sup>).

Fresh tuber yield accumulation under all treatments increased linearly over time until senescence at different rates (Figure 4). GM treatments resulted in higher rates of tuber biomass accumulation (from 698.15 to 830.21 kg ha<sup>-1</sup> day<sup>-1</sup>), than MN (611.4 kg ha<sup>-1</sup> day<sup>-1</sup>). Potato plants in the absence of N fertilizer presented the lowest yield increase rate (477.5 kg ha<sup>-1</sup> day<sup>-1</sup>).

Comparison of fresh tuber biomass accumulation at each sampling date (Table 6) showed significant differences between treatments ( $p < 0.05$ ). Generally, potato grown on GM400 plots presented greater tuber accumulation than all treatments over sampling. At 50 and 65 DAP, the treatment GM200 was similar to GM400 treatment and higher than MN treatment. At 65 DAP. Meanwhile, the difference between the elevated GM treatments (GM400 and GM200) was not significant which remained higher than GM100 and MN treatment. At the last sampling date, the difference between MN treatment and GM100 and GM200 was not significant and the anterior treatments remained lower the elevated GM dose. Additionally, in the absence of nitrogen (N0) presented the lowest fresh tuber accumulation over sampling dates.



**Figure 4.** Potato fresh tuber yield accumulation (kg ha<sup>-1</sup>) as a function of days after planting (DAP) under different fertilization treatments

**Table 6.** Potato accumulated fresh tuber yield (kg ha<sup>-1</sup>) under different N treatments at four sampling dates after planting (DAP)

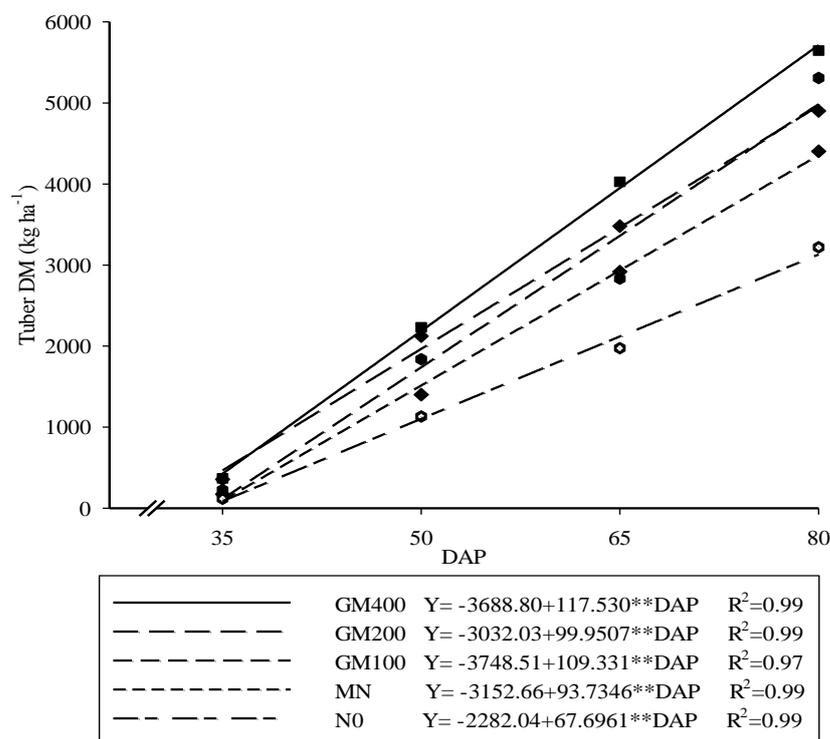
Treatment	Fresh tuber yield (kg ha <sup>-1</sup> )			
	35 DAP	50 DAP	65 DAP	80 DAP
GM400	3340.63 <sup>a</sup>	18209.38 <sup>a</sup>	29368.75 <sup>a</sup>	41131.25 <sup>a</sup>
GM200	3118.75 <sup>b</sup>	16453.13 <sup>ab</sup>	26431.25 <sup>a</sup>	34700.00 <sup>b</sup>
GM100	1500.00 <sup>c</sup>	14965.63 <sup>b</sup>	21781.25 <sup>b</sup>	34087.50 <sup>b</sup>
MN	1918.75 <sup>d</sup>	10325.00 <sup>c</sup>	20806.25 <sup>b</sup>	28993.75 <sup>bc</sup>
N0	1184.38 <sup>e</sup>	8725.000 <sup>c</sup>	15975.00 <sup>c</sup>	22643.75 <sup>c</sup>
LSD	148.95	1789.03	3284.43	6446.06
CV (%)	2.99	5.78	6.37	8.8

Means followed by the same letter in the column are not statistically different by Tukey test ( $p \geq 0.05$ ). GM; green manure, MN; recommended mineral N fertilization, N0; zero N treatment and numerical values refer to N dose (kg ha<sup>-1</sup>).

Taking into account fresh tuber yield at harvest and its proportion at 80 DAP, GM treatments accumulated between 92.35 to 98.5% of yield before senescence which was higher than MN treatment. Potato under MN accumulated 87.7% of tuber yield before senescence. Furthermore under zero N plots, tuber yield increased by 14.73% from 80 days until harvest.

Tuber dry matter accumulation presented a similar pattern of fresh yield increase over time (Figure 5). Furthermore, GM treatments presented higher rates of dry

matter accumulation (from 99.96 kg ha<sup>-1</sup> day<sup>-1</sup> to 117.53 kg ha<sup>-1</sup> day<sup>-1</sup>) than MN fertilization (93.74 kg ha<sup>-1</sup> d<sup>-1</sup>) and absence of N fertilization (67.7 kg ha<sup>-1</sup> day<sup>-1</sup>).



**Figure 5.** Tuber dry matter (DM) accumulation (kg ha<sup>-1</sup>) as a function of days after planting (DAP) under different fertilization treatments

Comparison of the accumulated dry matter at each sampling event (Table 7) showed significant differences between treatments by Tukey test ( $p < 0.05$ ). At 35 and 50 DAP, GM treatments presented higher accumulated dry matter than MN and N0 treatments. Meanwhile, the difference between GM400 and GM200 was not significant. At 65 and 80 DAP, the trend changed to some extent whereas the difference between MN fertilization and the low GM N doses (100 and 200) was not significant.

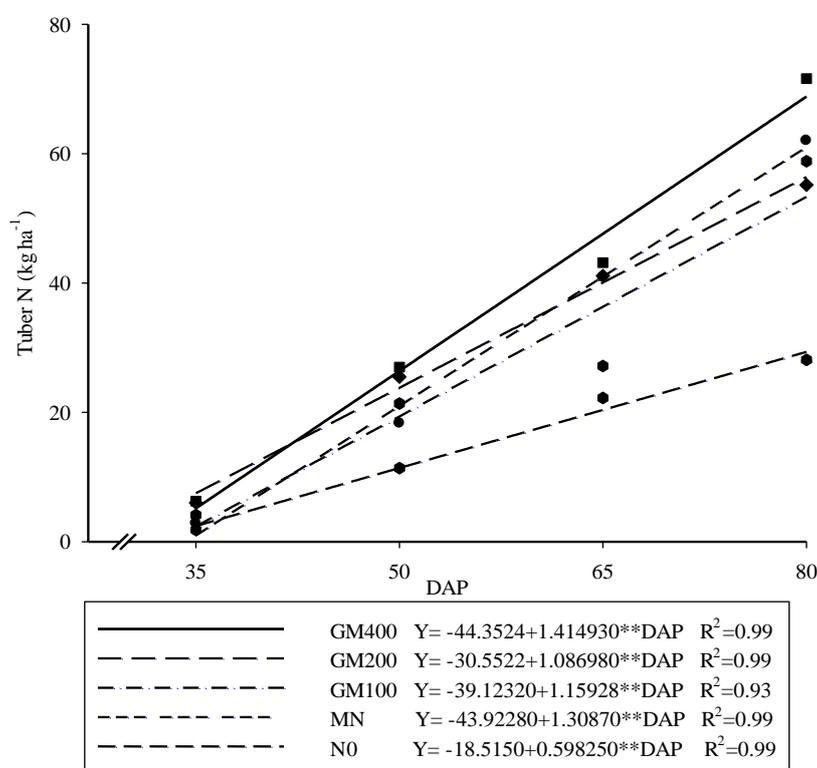
**Table 7.** Potato tuber DM accumulation (kg ha<sup>-1</sup>) under different N treatments at four sampling dates after planting (DAP)

Treatment	Tuber DM (kg ha <sup>-1</sup> )			
	35 DAP	50 DAP	65 DAP	80 DAP
GM400	368.97 <sup>a</sup>	2232.65 <sup>a</sup>	4028.11 <sup>a</sup>	5646.99 <sup>a</sup>
GM200	356.30 <sup>a</sup>	2122.47 <sup>a</sup>	3480.67 <sup>ab</sup>	4901.10 <sup>ab</sup>
GM100	224.02 <sup>b</sup>	1837.86 <sup>b</sup>	2833.15 <sup>b</sup>	5307.98 <sup>ab</sup>
MN	173.18 <sup>c</sup>	1400.56 <sup>c</sup>	2919.19 <sup>b</sup>	4404.54 <sup>b</sup>
NO	116.99 <sup>d</sup>	1130.55 <sup>c</sup>	1973.67 <sup>c</sup>	3220.75 <sup>c</sup>
LSD	36.21	282.58	708.29	1071.54
CV (%)	6.48	7.18	10.31	10.12

Means followed by the same letter in the column are not statistically different by Tukey test ( $p \geq 0.05$ ). GM; green manure, MN; recommended mineral N fertilization, N0; zero N treatment and numerical values refer to N dose (kg ha<sup>-1</sup>).

In addition, MN treatment remained lower than GM400 ( $P < 0.05$ ). Furthermore, N0 treatments presented inferior tuber dry matter accumulation to all treatments over sampling.

Tuber N accumulation presented the same linear pattern of fresh yield and dry matter (figure 6). GM100 and GM200 treatments presented lower estimated rates of N accumulation within tubers (from  $1.16 \text{ kg ha}^{-1} \text{ day}^{-1}$  and  $1.09 \text{ kg ha}^{-1} \text{ day}^{-1}$ ) than MN treatment ( $1.31 \text{ kg ha}^{-1} \text{ day}^{-1}$ ). Meanwhile, the GM400 treatments presented higher estimated rate ( $1.42 \text{ kg ha}^{-1} \text{ day}^{-1}$ ) than MN. In addition, N0 treatment presented the lowest estimated rate of N accumulation ( $0.59 \text{ kg ha}^{-1} \text{ day}^{-1}$ ).



**Figure 6.** Tuber N accumulation ( $\text{kg ha}^{-1}$ ) as a function of days after planting (DAP) under different fertilization treatments

Accumulated N within potato tubers at each sampling event (Table 8) showed significant differences between treatments ( $p < 0.05$ ). At 35 and 50 DAP, GM treatments presented higher accumulated dry matter than MN and N0 treatments. Meanwhile, the difference between GM400 and GM200 was not significant. However, At 65 DAP, no significant differences were found between GM400, GM200 and MN but were statistically higher than GM100 and N0 treatments. At the last sampling event, GM400 presented the highest accumulated N within tubers with no statistical difference from MN.

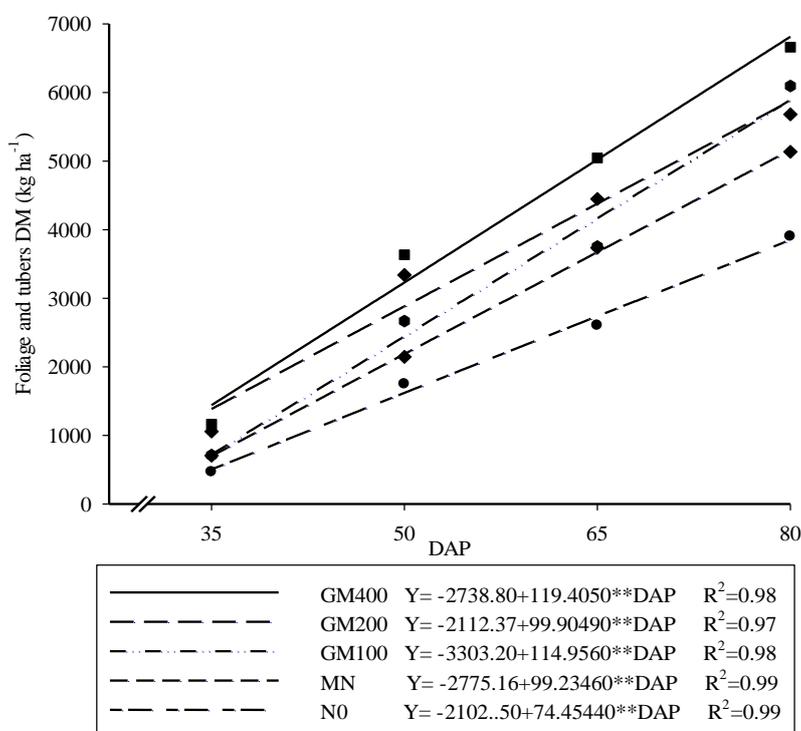
The rest of GM treatments were similar to MN treatment. Additionally, N absence resulted in the lowest N accumulation within tubers before senescence.

**Table 8.** Potato tuber accumulated N ( $\text{kg ha}^{-1}$ ) under different N treatments at four sampling dates after planting (DAP)

Treatment	Accumulated tuber N ( $\text{kg ha}^{-1}$ )			
	35 DAP	50 DAP	65 DAP	80 DAP
GM400	6.27 <sup>a</sup>	26.98 <sup>a</sup>	43.16 <sup>a</sup>	71.62 <sup>a</sup>
GM200	6.02 <sup>a</sup>	25.49 <sup>a</sup>	41.13 <sup>a</sup>	55.16 <sup>b</sup>
GM100	4.11 <sup>b</sup>	21.35 <sup>b</sup>	27.16 <sup>b</sup>	58.83 <sup>b</sup>
MN	2.81 <sup>c</sup>	18.29 <sup>b</sup>	40.90 <sup>a</sup>	62.01 <sup>ab</sup>
N0	1.82 <sup>d</sup>	11.38 <sup>c</sup>	22.22 <sup>b</sup>	28.12 <sup>c</sup>
LSD	0.63	3.96	10.3	11.8
CV (%)	6.68	8.48	13.09	9.49

Means followed by the same letter in the column are not statistically different by Tukey test ( $p \geq 0.05$ ). GM; green manure, MN; recommended mineral N fertilization, N0; zero N treatment and numerical values refer to N dose ( $\text{kg ha}^{-1}$ ).

Total dry matter accumulation of the whole plant was best expressed in linear fit regression models (Figure 7) for all treatments. GM treatments presented higher estimated rates of dry matter accumulation (from  $99.9 \text{ kg ha}^{-1} \text{ day}^{-1}$  to  $119.4 \text{ kg ha}^{-1} \text{ day}^{-1}$ ) than MN fertilization ( $99.74 \text{ kg ha}^{-1} \text{ day}^{-1}$ ) as well as absence of N fertilization ( $74.5 \text{ kg ha}^{-1} \text{ day}^{-1}$ ). Furthermore, the estimated rate increased by increasing GM nitrogen dose. .



**Figure 7.** Foliage and tubers dry matter accumulation ( $\text{kg ha}^{-1}$ ) in potato plants as a function of days after planting (DAP) under different fertilization treatments.

Comparison of total dry matter yield in each sampling date (Table 9) showed significant differences between treatments. Generally, GM treatments resulted in similar or higher DM accumulation than MN fertilization over sampling. The treatment GM100 resulted in similar whole plant dry matter to MN treatment at 35, 65 and 80 DAP. In addition, N0 treatment resulted in dry matter accumulation smaller than all treatments over sampling.

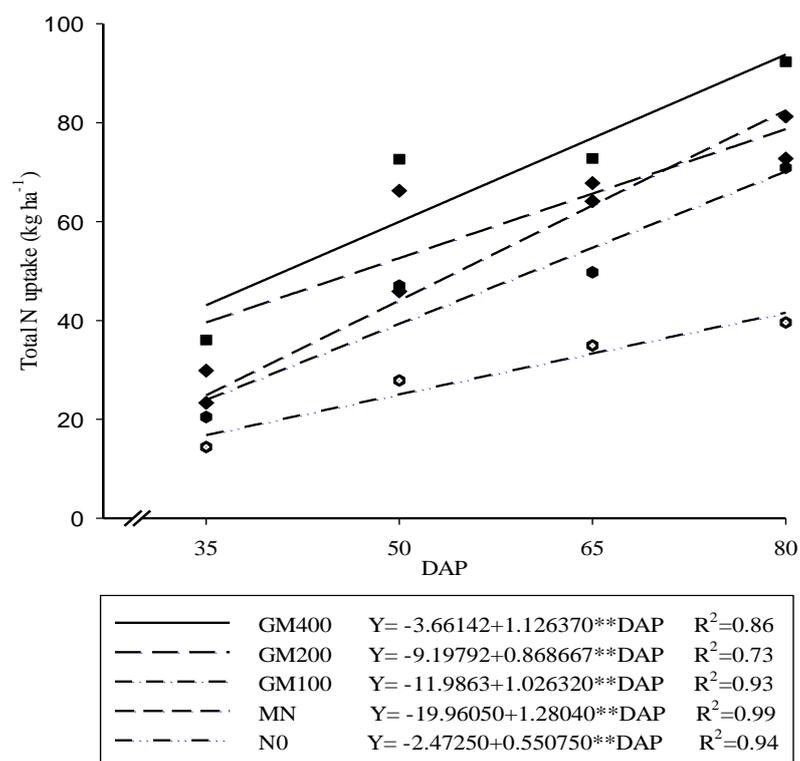
**Table 9.** Potato dry matter accumulation of the whole plant ( $\text{kg ha}^{-1}$ ) under different N treatments at four sampling dates after planting (DAP)

Treatment	Total DM ( $\text{kg ha}^{-1}$ )			
	35 DAP	50 DAP	65 DAP	80 DAP
GM400	1162.72 <sup>a</sup>	3635.78 <sup>a</sup>	5046.86 <sup>a</sup>	6662.61 <sup>a</sup>
GM200	1056.30 <sup>b</sup>	3341.22 <sup>a</sup>	4448.80 <sup>ab</sup>	5682.35 <sup>ab</sup>
GM100	710.680 <sup>c</sup>	2665.98 <sup>b</sup>	3755.03 <sup>b</sup>	6095.47 <sup>ab</sup>
MN	705.270 <sup>c</sup>	2144.31 <sup>c</sup>	3737.94 <sup>b</sup>	5135.79 <sup>b</sup>
N0	464.490 <sup>d</sup>	1746.18 <sup>d</sup>	2601.80 <sup>c</sup>	3902.00 <sup>c</sup>
LSD	67.18	310.6	814.45	1022.53
CV (%)	3.63	5.89	9.22	8.25

Means followed by the same letter in the column are not statistically different by Tukey test ( $p \geq 0.05$ ). GM: green manure, MN: mineral N fertilization  $250 \text{ kg ha}^{-1} \text{ N}$ , N0: Zero N treatment. Numerical values refer to N dose ( $\text{kg ha}^{-1}$ ).

Whole plant N uptake presented a similar regression fit (Figure 8) of dry matter accumulation for all treatments. However, the estimated rate of N uptake for MN fertilization ( $1.28 \text{ kg ha}^{-1} \text{ day}^{-1}$ ) was higher than for GM treatments which ranged between  $0.87$  to  $1.12 \text{ kg ha}^{-1} \text{ day}^{-1}$ . Furthermore, the lowest estimated rate was for N0 treatment ( $0.55 \text{ kg ha}^{-1} \text{ day}^{-1}$ ).

Comparison of whole plant N uptake in each sampling date (Table 10) showed significant differences between treatments. At 35 DAP, the elevated GM nitrogen doses resulted in higher N uptake than MN control as well as the other GM doses. Meanwhile, the N uptake under GM100 treatment was similar to the uptake under MN treatment. The same trend was observed at 50 DAP but with no significant difference between GM100 and MN. At 65 DAP, the trend changed to some extent whereas MN treatment resulted in values similar to GM200 and higher than GM 100. However, values of N uptake under MN treatment remained smaller than GM400. Additionally, Zero N treatment presented inferior N uptake to all other treatments in all sampling dates.



**Figure 8.** Potato total N uptake as a function of days after planting (DAP) under different N fertilization treatments

**Table 10.** Potato total N uptake ( $\text{kg ha}^{-1}$ ) under different N treatments at four sampling dates after planting (DAP)

Treatment	Total N ( $\text{kg ha}^{-1}$ )			
	35 DAP	50 DAP	65 DAP	80 DAP
GM400	36.05 <sup>a</sup>	72.60 <sup>a</sup>	72.76 <sup>a</sup>	92.31 <sup>a</sup>
GM200	29.84 <sup>b</sup>	66.22 <sup>a</sup>	67.78 <sup>ab</sup>	72.75 <sup>b</sup>
GM100	20.46 <sup>c</sup>	47.03 <sup>b</sup>	49.75 <sup>c</sup>	70.87 <sup>b</sup>
MN	23.33 <sup>c</sup>	45.90 <sup>b</sup>	64.15 <sup>b</sup>	81.27 <sup>ab</sup>
N0	14.41 <sup>d</sup>	27.85 <sup>c</sup>	34.93 <sup>d</sup>	39.59 <sup>c</sup>
LSD	3.69	9.66	8.17	13.11
CV (%)	6.59	8.25	6.26	8.15

Means followed by the same letter in the column are not statistically different by Tukey test ( $p \geq 0.05$ ). GM; green manure, MN; recommended mineral N fertilization, N0; zero N treatment and numerical values refer to N dose ( $\text{kg ha}^{-1}$ ).

#### 4.5.3- Harvest index, Nitrogen harvest index, N recovery efficiency and N apparent recovery at 80 DAP

Harvest index (HI) was not affected by treatments (Table 11) whereas no significant differences were detected by Tukey test ( $p \geq 0.05$ ). However, there is a decline in harvest index by increasing N dose, particularly under GM.

Nitrogen harvest index (NHI), which represents the proportion of recovered N within tubers, was influenced by nitrogen doses and sources (Table 11). The treatment

GM100 resulted in the highest NHI followed by GM400 and GM200 which were similar to MN grown plants. Zero N treatment resulted in the lowest NHI.

**Table 11.** Harvest Index (HI), Nitrogen Harvest Efficiency (NHI), Nitrogen Recovery Efficiency and Nitrogen Apparent Recovery at 80 days after planting

Treatment	HI	NHI	NR (kg ha <sup>-1</sup> )	NRE (%)	NAR (%)
AV400	0.846 <sup>ns</sup>	0.775 <sup>b</sup>	92.31 <sup>a</sup>	22.33	13.28
AV200	0.862	0.758 <sup>b</sup>	72.75 <sup>b</sup>	36.38	16.72
AV100	0.871	0.878 <sup>a</sup>	70.87 <sup>b</sup>	70.88	31.30
MN	0.857	0.758 <sup>b</sup>	81.27 <sup>ab</sup>	23.51	16.68
N0	0.825	0.713 <sup>c</sup>	39.59 <sup>c</sup>	-	-
LSD	0.117	0.0763	13.11	-	-
CV (%)	2.84	4.41	8.15	-	-

Means followed by the same letter in the column are not statistically different by Tukey test ( $p \geq 0.05$ ). GM; green manure, MN; recommended mineral N fertilization, N0; zero N treatment and numerical values refer to N dose (kg ha<sup>-1</sup>).

Potato N recovery showed significant differences between treatments (Table 11). The elevated GM dose resulted in a recovery of 92.3 kg N ha<sup>-1</sup> which was higher than the obtained in other GM doses. Meanwhile, it was statistically similar to MN grown plants. Potato under N0 treatment recovered approximately 40 kg N ha<sup>-1</sup> and was smaller than all treatments.

Nitrogen recovery efficiency and nitrogen apparent recovery under GM treatments (Table 11) presented an inverse trend to N doses whereas an increasing N Dose resulted in decline N recovery efficiency and N apparent recovery. Furthermore, the low GM doses resulted in higher N efficiencies than MN fertilizer. Meanwhile, the high GM nitrogen dose (GM400) resulted in NRE similar to MN treatment.

#### 4.5.4. Yield components

GM treatments produced similar and higher marketable and total yields than MN (Table 12). The highest marketable and total yield was recorded under GM400 followed by GM200 which was superior to MN treatment. The treatment GM100 resulted in similar yield to MN treatment ( $p \geq 0.05$ ). Furthermore, zero N treatment presented the lowest marketable and total yield. Yield for GM nitrogen doses, 100, 200 and 400 was greater by 3.89, 15.4 and 31.2 % and by 4.65, 13.65 and 29.72 % than MN treatment for marketable and total yield, respectively.

Tuber dry matter content at harvest (Table 12) was not affected by treatments. Meanwhile, dry matter yield presented significant differences between treatments ( $p < 0.05$ ). The highest DM yield was recorded under GM400 which was statistically

higher than MN treatment but similar to GM200 and GM100. Zero N treatment resulted in the lowest DM yield but was statistically similar to MN and GM100 treatments

**Table 12.** Marketable yield (MY), total yield (TY), tuber dry matter content (%), dry matter yield (kg ha<sup>-1</sup>) and nitrogen use efficiency (NUE) on potato plants under different N treatments

Treatment	MY (kg ha <sup>-1</sup> )	TY (kg ha <sup>-1</sup> )	DM (%)	DM (kg ha <sup>-1</sup> )	NUE (Kg tuber kg N <sup>-1</sup> )
GM400	41615.00 <sup>a</sup>	42887.25 <sup>a</sup>	14.68 ns	6310.40 <sup>a</sup>	107.22
GM200	36617.50 <sup>b</sup>	37575.00 <sup>b</sup>	15.57	5878.60 <sup>ab</sup>	187.88
GM100	32952.50 <sup>bc</sup>	34602.50 <sup>bc</sup>	15.22	5185.50 <sup>abc</sup>	346.03
MN	31717.50 <sup>c</sup>	33062.50 <sup>c</sup>	15.51	5119.90 <sup>bc</sup>	132.25
N0	25522.50 <sup>d</sup>	26555.00 <sup>d</sup>	15.22	4042.010 <sup>c</sup>	-
LSD	3722.57	3691.8	5.44	1160.66	-
CV (%)	4.9	4.69	4.58	9.7	-

Means followed by the same letter in the column are not statistically different by Tukey test ( $p \geq 0.05$ ) and ns; not significant. GM; green manure, MN; recommended mineral N fertilization, N0; zero N treatment and numerical values refer to N dose (kg ha<sup>-1</sup>).

Nitrogen use efficiency (NUE) presented an inverse response to N doses (Table 12) under GM treatments. The lower the N dose the higher the NUE. The GM200 dose increased nitrogen productivity and was higher than MN fertilizer. Meanwhile, a decline in NUE was recorded under GM400 and was smaller than MN.

## 4.6. DISCUSSION

### 4.6.1. Green manure decomposition and nitrogen mineralization

The GM sunhemp presented high decomposition and nitrogen mineralization rates due to its tissue high quality, particularly the low C/N ratio, since it was harvested in early stages. Chaves *et al.* (2004) reported that C/N ratio was the best predictor for N mineralization potential from incorporated organic residues. Furthermore, he found that N mineralization rate after clover and Lucerne green manures (12 and 10 C/N ratio) incorporation was higher than after vetch and oat mixture (31 C/N ratio). Additionally, high quality residues which contain easily degradable components tend to increase soil microbial biomass and activity leading to net N mineralization (Stark *et al.*, 2007). The GM sunhemp residue showed an accelerated decomposition rate particularly early after incorporation whereas approximately 70 % of the initial N content was released during the first six weeks. These results are in accordance with that obtained by Perin *et al.* (2006); Odhiambo, (2010) and Araujo, (2012). Furthermore, nitrogen presented short half life in this experiment and was similar to that reported by Perin *et al.* (2006) and

Araujo, (2012) in the same region and same specie which were 15 and 31.9, respectively.

#### **4.6.2. Dry matter and N accumulation**

Incorporating sunhemp preceding potato stimulated vegetative growth and canopy establishment early in the season which was reflected in higher dry matter accumulation. Therefore, nitrogen was not a growth-limiting under GM treatments. The obtained results are persistent with Alva, (2004). He reported that sub-optimal N supply substantially reduces potato vegetation development. This observation was evident within zero N treatment whereas maximum dry matter accumulation delayed by around 9 days. Furthermore, maximum N accumulation was obtained before maximum dry matter and was dependent on nitrogen dose. These results are in accordance with that obtained by Alva *et al.* (2002) and Macqueen, (2007). They reported that N accumulation precedes dry matter. Additionally, the crescent rates of N uptake and dry matter accumulation by increasing the GM nitrogen doses can be attributed to the interference of improved soil water retention and the high N release after GM incorporation leading to enhanced potato N uptake. These results are in accordance with Li *et al.* (2009). They reported that adequate soil water content enhances nutrient's availability in soil, uptake by plants and improved nutrient status and consequently yield.

Foliage dry matter and nitrogen accumulation presented quadratic patterns over time reaching maximum values early in the growing season in the current experiment. Kleinkopf *et al.* (1981); Alva *et al.* (2002); Macqueen, (2007) and Fernandes *et al.* (2011) reported sigmoid functions for dry matter and nitrogen accumulation with maximum values before senescence which differ from our results. These differences can be seen as site-specific and varieties difference. This speculation is consistent with that reported by Zebarth *et al.* (2005) since they found different dry matter and nitrogen accumulation patterns between Shepody and Russet Burbank potato varieties grown under the same conditions. Taking into account the results obtained Fernandes *et al.* (2010) the undertaken treatments presented comparable results for MN fertilization. They reported maximum DM accumulation (882 kg ha<sup>-1</sup>) between 78 and 85 DAP. Moreover, the decline of foliage N and dry matter content before senescence can be attributed to the stimulus effect of the experimental conditions to augment nitrogen compounds translocation from foliage to tubers as explained by Zebarth *et al.* (2004a). Furthermore, it can be seen as accelerated early defoliation under green manuring (Fernandes *et al.*, 2010).

Fresh tuber yield was reported to be responsive to N fertilization rates whereas increasing N rate increase tuber mass and average tuber weight (Belanger *et al.*, 2002). Studies on fresh and dry tuber yield accumulation mentioned different models of yield increase over time. Kleinkopf *et al.* (1981); Vos, (1999) and Macqueen, (2007) reported linear increases of fresh and dry tuber yield with different daily estimated rates for potato from tuber set until senescence. Fresh tuber yield accumulation in the present study presented linear increase and is consistent with the above mentioned studies. Additionally, the estimated rates of fresh yield and N accumulation are similar to that mentioned by Macqueen, (2007) as he obtained estimated fresh tuber accumulation rates between 70 to 100 kg ha<sup>-1</sup> day<sup>-1</sup> and estimated rates for N from 1.2 to 2 kg ha<sup>-1</sup> day<sup>-1</sup> for different varieties and different organically managed sites. Meanwhile, the estimated rates of dry matter accumulation in the current study are inferior to that mentioned in the previous study which was 150 to 200 kg ha<sup>-1</sup> day<sup>-1</sup> in intensive farms. This Difference can be regarded as variety-specific characteristic whereas the used variety in our experiment is not for processing and has medium maturity. On the other hand, Fernandes *et al.* (2010) and Fernandes *et al.* (2011) reported sigmoid functions for potato fresh tuber, dry matter and nitrogen accumulation for the same variety under Brazilian conditions. Our data did not fit into that model and this difference in plant performance could be attributed to management or site-specific influence.

#### **4.6.3. Harvest index, Nitrogen harvest index, Nitrogen recovery, N recovery efficiency and N apparent recovery at 80 DAP**

The results of the current study presented comparable harvest index to that reported by Alva *et al.* (2002). They reported HI for ‘Russet Burbank’ and ‘Hilite Russet’ of 0.78 and 0.87 at full maturity, respectively. Moreover, Macqueen, (2007) obtained HI from 0.53 to 0.83 for different varieties in different organically managed sites. Additionally, Fernandes *et al.* (2010) found that tubers DM accounted for 0.87 of the total dry matter yield at 97 DAP. Additionally, yield at maturity was 38 t ha<sup>-1</sup> of the same variety in Brazil. Therefore, it can be speculated that GM treatments provided potato with adequate N supply and was not excessive. In addition, potatoes grown under green manuring can be harvested early without yield loss risks due to the accelerated accumulation rates in the early stages.

Tubers nitrogen content (NHI) of the total accumulated N was not affected by green manure treatments when compared with MN. The obtained NHI ranges are in accordance with that reported by Li *et al.* (2003) and Zebarth *et al.* (2004a). They re-

ported that N present in tubers ranges from 0.7 to 0.85 with low values occurring at high fertilizer rates or in immature crops.

Potato N recovery from MN fertilizer varies according to various factors, e.g. location, season, applied N amount and variety. Nitrogen recovery efficiency was reported to range from 29-77% (Zebarth *et al.*, 2009). In addition, Shrestha *et al.* (2010) reported that N recovery from MN ranged between 110 to 250 kg ha<sup>-1</sup> and N recovery efficiency up to 50%. Furthermore, potato N recovery in the absence of external N supply recorded wide variations according to site, SOM level and years. Porter *et al.* (2000) reported 48 to 88 kg N ha<sup>-1</sup>, Nyiraneza and Snapp, (2007) reported 22 kg N ha<sup>-1</sup>, Zebarth *et al.* (2008) mentioned 24 to 114 kg ha<sup>-1</sup> depending on SOM level and climatic conditions. In this experiment, potato grown in zero N treatment recovered approximately 40 kg ha<sup>-1</sup> which is comparable to the cited ranges. Meanwhile, potatoes under MN control presented low N recovery as well as N recovery efficiency but still in the mentioned rates.

The increasing N recovery and recovery efficiency with GM nitrogen doses can be attributed the fast N mineralization from sunhemp biomass after incorporation. This result is consistent with Honeycutt, (1997) who reported increased response of potato N uptake to elevated GM nitrogen rates. This increase was attributed to the growing N supply and improved soil water retention which enhanced potato N acquisition N recovery and N recovery efficiency as well as N apparent recovery by crops from organic fertilizers were reported to be lower than mineral nitrogen fertilizers (Rodrigues *et al.*, 2006). The authors reported 40% decline in N recovery by triticale and maize supplied with commercial organic fertilizers compared to ammonium nitrate during three years. The decline in N recovery efficiency and apparent recovery by increasing N GM doses in this experiment is in accordance with that obtained by Gholipouri *et al.* (2012). They reported higher agronomic N efficiencies when applying 100 kg N ha<sup>-1</sup> than applying 200 kg ha<sup>-1</sup> in two potato varieties.

#### **4.6.4. Yield components**

Sunhemp as N source resulted in similar and higher potato fresh tuber yield and dry matter production when compared with MN despite the lower N recovery. These results are similar to that obtained by Campiglia *et al.* (2009). He reported potato yield of 48.5 t ha<sup>-1</sup> after subclover and hairy vetch incorporation, a yield similar to the obtained with MN fertilization. In addition, different experiments showed yield increases when legumes preceded potato with additional mineral N supply. Carter *et al.* (2003)

reported yield increase by 7.5% when green manure preceded potato compared to mineral nitrogen alone. Sincik *et al.* (2008) reported 12.7 % and 15 % more tuber yield when potato was cropped after incorporation of common vetch and faba bean than when cropped with mineral N fertilizer alone. Yield increase after green manure incorporation was explained and attributed to other factors, besides nitrogen, such as improved water retention, cation exchange capacity and water infiltration, stimulated soil microbial activity and reduced crop pathogens which provide better conditions for crop growth and higher yields (Bath, (2000); Rodrigues *et al.*, (2006); Carter *et al.*, (2003); Curless *et al.*, (2004) and Campiglia *et al.*, (2009)).

Nitrogen use efficiency for the total and marketable yield decreased by increasing GM nitrogen. This result is in accordance with the trend mentioned by Darwish *et al.* (2006), Kumar *et al.* (2007), Goffart *et al.* (2008), Fontes *et al.* (2010) and Irena *et al.* (2011). The decline of NUE was attributed to the low ability of potato to assimilate soil mineral nitrogen. Comparison of NUE for sunhemp treatments with MN treatment showed higher efficiency of sunhemp N than mineral N. This high efficiency can be attributed to the non-nitrogen benefits of sunhemp incorporation as mentioned by Bath (2000) and Campiglia *et al.* (2009). In addition, Fontes *et al.* (2010) reported values of 115 and 99 (kg tubers kg<sup>-1</sup> N) as nitrogen productivity per each applied nitrogen unit (in the same region and variety) for total and marketable yield, respectively when 300 kg N ha<sup>-1</sup> was supplied. In our study, the mean values for NUE of sunhemp nitrogen doses are in that range suggesting that green manure use provides a high NUE in potato

#### 4.7. CONCLUSIONS

Sunhemp incorporation prior to potato provided potato with adequate nitrogen supply during its peak growth stage due to high tissue quality properties and fast nitrogen release.

Sunhemp as a nitrogen source in this experiment stimulated plant growth greater than mineral fertilizer as well as fresh tuber yield accumulation whereas as more than 90% of the fresh tuber harvest was obtained at 80 DAP. This accelerated tuber bulking could be an advantage for early harvest when market opportunities are available or inadequate conditions without significant yield losses.

Green manure, as a nitrogen source in potato production, presented similar harvest index to that of mineral nitrogen fertilization. Furthermore, comparable N doses of

GM increased nitrogen harvest index with potato tubers and resulted in similar nitrogen recovery and higher nitrogen recovery efficiency.

Potato presented higher yields and improved nitrogen productivity under green manuring. Thus, economic yields with less nitrogen supply could be attained by substituting mineral N fertilizer by green manures which seemed to be more sustainable from economic and environmental point of view.

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## 5. GENERAL CONCLUSION

Integration of different organic fertilizers presented a great efficacy to improve potato productivity in the short run since comparable yield levels were obtained despite the low N supply in relation to mineral fertilization. Furthermore, the evaluated organic fertilizers varied in their effect on the potato crop whereas the GM sunhemp presented higher efficiency in enhancing N uptake and recovery over the growing season than compost. However, compost showed better efficiency in yield augmentation as well as N use efficiency. This differential effect indicates the diverse potentials of organic fertilizers to improve soil properties and influence subsequent potato growth and productivity. Furthermore, highlights the response of the potato crop to soil properties and water supply besides N supply.

Non-aerated compost extract in this experiment proved to stimulate potato growth and productivity when integrated with other organic fertilizers. Therefore, it can be applicable as N supplement or for nitrogen deficiency rectification in case of other N forms supply constraints. However, the reasons for its efficiency in this study were not clear. We speculated two possible reasons of its efficacy. Firstly, could be related to its mineral content as extracts presented a reasonable content of nitrate, readily available for assimilation via leaves by potatoes. The second one could be attributed to the contribution of extract nitrogen content in enhancing soil microbial activity to mineralize nitrogen for plant uptake. Thus, other experiments in controlled conditions using the  $^{15}\text{N}$  isotop technique are required to trace N supplied from extract and find out its route and mode of action after application.

The tested nutritional indices, SPAD and leaf N-total presented a convenient opportunity to some extent for in-season N status monitoring of organically fertilized potato. However, different experiments are still required in order to stabilize acceptable thresholds to help farmers in optimizing their nutrient management.

The GM sunhemp (*Crotalaria juncea* L) can be employed to supply potato with adequate N quantities. Our results presented no nitrogen deficiencies and similar and higher yields to mineral N fertilizer. Furthermore, split green manure application proved to be a possible option to improve potato yield. Additionally, dry matter yield was increased by green manuring. Therefore, it could be an opportunity if these results will be persistent with processing cultivars. Meanwhile, experiments in this regard are required to validate this observation.

The GM sunhemp presented greater nitrogen productivity than mineral form. Moreover, the split sunhemp application showed higher N efficiency than full incorporation before planting. Therefore, green manuring can improve potato production systems sustainability by substituting synthetic N sources. In addition, high yield with low N rates supply.

The tested nutritional indices in the second experiment presented another possibility to monitor potato N status and confirmed somehow the results of the first experiment. However, no thresholds were established as well.

The GM sunhemp, as nitrogen source, stimulated plant growth and presented great yield accumulation early in the season. This accelerated growth and yield accumulation could be an advantage for early harvest when market opportunities are available or inadequate conditions without significant yield losses.

In this series of experiments, potato presented higher yields and higher nitrogen productivity under organic fertilization. Thus, economic yields with less nitrogen supply could be obtained by substituting mineral N fertilizer by green manures which seemed to be more sustainable from economic and environmental point of view.

From a practical point of view, this work has provided potato farmers, particularly small-scale ones, who do not have access to synthetic nitrogen fertilizers with sustainable alternatives and technical management practices for competitive and profitable yields.

## APPENDICES

### Analysis of variance for chapter 1

Example for combined ANOVA calculation method

**Table 1.** General ANOVA with 9 treatments;

SOV	DF	SS
<b>Treatments</b>	8	88.845
<b>Block</b>	3	1.3600
<b>Error</b>	24	9.4150
<b>Total</b>	35	99.62

**Table 2.** ANOVA in split-plot using the factorial arrangement (2×4);

SOV	DF	SS
<b>Block</b>	3	0.22750
<b>Fertilizers</b>	1	0.03125
<b>Error (a)</b>	6	8.42375
<b>CE</b>	3	22.7275
<b>Fert* CE</b>	3	10.9612
<b>Error (b)</b>	18	2.35125

**Table 3.** Combined ANOVA

SOV	DF	SS	Table
<b>Block</b>	3	1.36	(1)
<b>Control fact</b>	1	55.125	= SQ Ts (table 1)-SQ fert +SQ CE+ SQ fert*CE
<b>Fertilizers</b>	1	0.0312	2
<b>Error (a)</b>	6	8.4237	= SQ Error T (table 1) - SQ Error (B table 2)
<b>CE</b>	3	22.727	2
<b>Fertilizer *CE</b>	3	10.961	2
<b>Error (b)</b>	18	2.3512	2
<b>Total</b>	35		1

**Table 1.1.** Summary of mean squares (MS) of the combined ANOVA and significance for SPAD index reading at three sampling dates after planting (DAP)

SOV	DF	MS		
		45 DAP	55 DAP	65 DAP
<b>Block</b>	3	0.903241	0.147037	0.657315
<b>Cont Vs Fac</b>	1	76.4672	137.78	324.9126
<b>Fertilizer</b>	1	0.36125 <sup>ns</sup>	3.51125 <sup>*</sup>	4.882812 <sup>*</sup>
<b>Error (a)</b>	6	3.489375	0.373125	1.365823
<b>CE</b>	3	3.1525 <sup>*</sup>	13.82583 <sup>**</sup>	34.3778 <sup>**</sup>
<b>Fert*CE</b>	3	4.627083 <sup>**</sup>	1.14375 <sup>ns</sup>	0.147813 <sup>ns</sup>
<b>Error (b)</b>	18	0.690347	0.427292	0.508368
<b>Mean</b>		39.353	38.72	37.453
<b>CV1 (%)</b>		4.75	1.58	3.12
<b>CV2 (%)</b>		2.11	1.69	1.90

Cont; MN control, fac; factorial combinations, CE; compost extract concentrations and Fert; organic fertilizers. \*\*, \* and ns; significant at 1% and 5% probability and insignificant by F test, respectively

**Table 1.2.** Summary of mean squares (MS) of the combined ANOVA and significance for leaf N-total content at three sampling dates after planting (DAP)

SOV	DF	MS		
		45 DAP	55 DAP	65 DAP
<b>Block</b>	3	0.002884	0.061796	0.009793
<b>Cont Vs Fac</b>	1	0.012013	0.015022	1.393198
<b>Fertilizer</b>	1	1.25E-05 <sup>ns</sup>	0.04205 <sup>ns</sup>	0.000248 <sup>ns</sup>
<b>Error (a)</b>	6	0.031883	0.010385	0.031654
<b>CE</b>	3	0.227321 <sup>**</sup>	0.125792 <sup>**</sup>	0.115764 <sup>**</sup>
<b>Fert*CE</b>	3	0.017204 <sup>ns</sup>	0.019875 <sup>ns</sup>	0.014033 <sup>ns</sup>
<b>Error (b)</b>	18	0.00834	0.012797	0.011851
<b>Mean</b>		4.84	3.85	3.04
<b>CV1 (%)</b>		3.69	2.65	5.85
<b>CV2 (%)</b>		1.89	2.94	3.58

Cont; MN control, fac; factorial combinations, CE; compost extract concentrations and Fert; organic fertilizers. \*\*, \* and ns; significant at 1% and 5% probability and insignificant by F test, respectively

**Table 1.3.** Summary of mean squares (MS) of the combined ANOVA for dry matter production (DM), foliage nitrogen content (FN), tubers N content (TN), tubers N proportion (TNP) and recovery at 85 DAP.

SOV	DF	MS				
		DM	FN	TN	TNP	NR
<b>Blocks</b>	3	3794067	50.57103	463.5833	0.004338	62.9478
<b>Cont Vs Fct</b>	1	107133	110.0387	763.1559	0.06348	1496.018
<b>Fert</b>	1	404721 <sup>ns</sup>	29.10845 <sup>ns</sup>	1254.967 <sup>ns</sup>	0.000161 <sup>ns</sup>	1499.014 <sup>**</sup>
<b>Error (a)</b>	6	4149705	36.41345	376.9082	0.046889	30.54987
<b>CE</b>	3	56816.67 <sup>ns</sup>	16.83389 <sup>ns</sup>	143.2834 <sup>ns</sup>	0.000507 <sup>ns</sup>	271.9485 <sup>**</sup>
<b>Fert * CE</b>	3	815293.3 <sup>ns</sup>	10.11141 <sup>ns</sup>	32.52425 <sup>ns</sup>	0.001495 <sup>ns</sup>	13.74132 <sup>ns</sup>
<b>Error (b)</b>	18	1683684	18.89412	255.7003	0.001235	38.34253
<b>Mean</b>		7021.1	15.92	75.58	0.826	91.16
<b>CV1 (%)</b>		29.01	37.9	25.67	26.22	6.06
<b>CV2 (%)</b>		18.48	27.3	21.16	4.25	6.79

Cont; MN control, fac; factorial combinations, CE; compost extract concentrations and Fert; organic fertilizers. \*\*, \* and ns; significant at 1% and 5% probability and insignificant by F test, respectively

**Table 1.4.** Summary of mean squares (MS) of the combined ANOVA for fresh tuber yield classes, marketable yield (MY) and total yield (TY)

SOV	DF	MS				
		Class 4	Class 3	Class 2	MY	TY
<b>Blocks</b>	3	3705.767	9170.253	4940773	4925700	5156450
<b>Cot Vs fact</b>	1	645367.1	17057204	51474	18982545	26628180
<b>Fert</b>	1	357779.7 <sup>**</sup>	1503326 <sup>**</sup>	3011426 <sup>ns</sup>	8770175 <sup>*</sup>	12670720 <sup>*</sup>
<b>Error (a)</b>	6	8280.65	5717.083	1260930	1392180	1520417
<b>CE</b>	3	298147.1 <sup>**</sup>	7411190 <sup>**</sup>	1.03E+08 <sup>**</sup>	1.08E+08 <sup>*</sup>	1.19E+08 <sup>*</sup>
<b>Fert * CE</b>	3	754793.3 <sup>**</sup>	8654597 <sup>**</sup>	38967700 <sup>**</sup>	13201460 <sup>*</sup>	9531967 <sup>*</sup>
<b>Error (b)</b>	18	1394.902	46819.65	2784953	2971524	2955162
<b>Mean</b>		1428.38	6141.52	36747.61	42889.13	44317.51
<b>CV 1 (%)</b>		6.37	1.23	3.06	2.75	2.78
<b>CV2 (%)</b>		2.62	3.52	4.52	4.02	3.88

Cont; MN control, Fac; factorial combinations, CE; compost extract concentrations and Fert; organic fertilizers. \*\*, \* and ns; significant at 1% and 5% probability and insignificant by F test, respectively

**Table 1.5.** Summary of mean squares (MS) of the combined ANOVA for tuber dry matter content (DM%) and dry matter production (DMP)

SOV	DF	MS	
		DM (%)	DMP
<b>Blocks</b>	3	0.358958	6263.35
<b>Cont Vs fat</b>	1	0.114601	317336.6
<b>Fert</b>	1	0.058226 <sup>ns</sup>	339464.3 <sup>ns</sup>
<b>Error (a)</b>	6	0.210475	79231.17
<b>CE</b>	3	1.61314 <sup>*</sup>	3711260 <sup>**</sup>
<b>Fert * CE</b>	3	0.037517 <sup>ns</sup>	231253 <sup>ns</sup>
<b>Error (b)</b>	18	0.327672	132387.9
<b>Mean</b>		13.28	5893.69
<b>CV1(%)</b>		3.45	4.78
<b>CV2 (%)</b>		4.31	6.17

Cont; MN control, Fac; factorial combinations, CE; compost extract concentrations and Fert; organic fertilizers. \*\*, \* and ns; significant at 1% and 5% probability and insignificant by F test, respectively

## Analysis of variance for chapter 2

\* Example for combined ANOVA calculation method

**Table 1.** General ANOVA with all treatments as RCBD

SOV	DF	SS
<b>Treatment</b>	9	130.7122
<b>Block</b>	3	2.62475
<b>Error</b>	27	28.09275
<b>Total</b>	39	141.4297

**Table 2.** Split-plot ANOVA with the factorial treatments (2\*4)

SOV	DF	SS
<b>Block</b>	3	0.9559375
<b>Managment</b>	1	0.0028125
<b>Error (a)</b>	3	1.008438
<b>N doses</b>	3	22.16594
<b>N doses* Mana</b>	3	0.3134375
<b>Error (b)</b>	18	19.45812

**Table 3.** ANOVA for Control treatments

SOV	DF	SS
<b>Treatment</b>	1	13.52
<b>Block</b>	3	6.415
<b>Error</b>	3	2.88

**TABLE 4.** Combined ANOVA

SOV	D F	SS	Table
<b>Block</b>	3	2.62475	1
<b>Control</b>	1	13.52	3
<b>Cont Vs fact</b>	1	94.71001	=SST-(SS Cont+SS Mang+SS Dose+ SS Mang*Dose)
<b>Manag</b>	1	0.002813	2
<b>Error (a)</b>	9	8.637442	= SS Error total (table 1)– SS Error (b) ( Ttable2)
<b>N doseS</b>	3	22.16594	2
<b>Doses</b>	* 3	0.313438	2
<b>Mang</b>			
<b>Error (b)</b>	18	19.45812	2
<b>Total</b>	39	141.4297	

**Table 2.1.** Summary of mean squares (MS) of the combined ANOVA and significance for leaf nutritional indices at 21 day after emergence

SOV	DF	MS		
		SPAD	CHLT	N-total
<b>Block</b>	3	0.874917 <sup>ns</sup>	0.586463 <sup>ns</sup>	9.734383 <sup>ns</sup>
<b>Control</b>	1	13.52000 <sup>**</sup>	0.252050 <sup>ns</sup>	30.88980 <sup>**</sup>
<b>Cont * Fact</b>	1	94.71001	0.23870300	36.8901500
<b>Manag</b>	1	0.002813 <sup>ns</sup>	1.4196120 <sup>ns</sup>	145.30860 <sup>**</sup>
<b>Error (a)</b>	9	0.959716	0.474239	20.30942
<b>N dose</b>	3	7.388647 <sup>**</sup>	0.187413 <sup>ns</sup>	10.472310 <sup>*</sup>
<b>N dose* Mang</b>	3	0.104479 <sup>ns</sup>	0.115513 <sup>ns</sup>	0.8578030 <sup>ns</sup>
<b>Error (b)</b>	18	1.081007	0.104018	2.493638
<b>Mean</b>		38.9	7.22	53.27
<b>CV 1 (%)</b>		2.52	9.54	8.46
<b>CV 2 (%)</b>		2.67	4.47	2.96

<sup>\*\*</sup>, <sup>\*</sup> and <sup>ns</sup>; significant at 1% and 5% probability and insignificant by F test, respectively.  
Cont; control treatment, Fact; factorial treatments and Mang; incorporation timing

**Table 2.2.** Summary of mean squares (MS) of the combined ANOVA and significance for dry matter (DM) production, foliage N content (FN), tuber N content (TN) and accumulated N proportion (TNP).

SOV	DF	MS			
		DM	FN	TN	TNP
<b>Block</b>	3	119759.3	31.93234	162.3837	0.000229
<b>control</b>	1	702812.5 <sup>*</sup>	6.607794 <sup>ns</sup>	3909.49 <sup>**</sup>	0.083593 <sup>*</sup>
<b>con * fact</b>	1	12400889	82.63084	1597.516	0.084617
<b>Manag</b>	1	685035.1 <sup>*</sup>	16.08657 <sup>ns</sup>	0.03125 <sup>ns</sup>	0.001209 <sup>ns</sup>
<b>Error (a)</b>	9	123058.2	38.27958	68.015	0.001665
<b>N dose</b>	3	4491323 <sup>**</sup>	109.9176 <sup>*</sup>	799.948 <sup>**</sup>	0.000905 <sup>ns</sup>
<b>N dose* Mang</b>	3	466997.7 <sup>*</sup>	38.10813 <sup>ns</sup>	58.8646 <sup>ns</sup>	0.004877 <sup>ns</sup>
<b>Error (b)</b>	18	101123.6	16.20143	86.74656	0.001987
<b>mean</b>		7624.21	23.76	62.73	0.718
<b>CV 1(%)</b>		4.6	26.04	13.15	5.68
<b>CV 2(%)</b>		4.17	16.94	14.85	6.21

<sup>\*\*</sup>, <sup>\*</sup> and <sup>ns</sup>; significant at 1% and 5% probability and insignificant by F test, respectively.  
Cont; control treatment, Fact; factorial treatments and Mang; incorporation timing

**Table 2.3.** Summary of mean squares (MS) of the combined ANOVA and significance for nitrogen recovery (NR), total yield (Ty) and marketable yield (MY)

SOV	DF	MS		
		NR	TY	MY
<b>Block</b>	3	316.1373 <sup>ns</sup>	24417553 <sup>ns</sup>	22775243 <sup>ns</sup>
<b>Control</b>	1	3261.089 <sup>**</sup>	2.41E+08 <sup>**</sup>	2.44E+08 <sup>**</sup>
<b>Cont * Fact</b>	1	462.8422	2.74E+08	2.72E+08
<b>Manag</b>	1	0.330078 <sup>ns</sup>	69345920 <sup>*</sup>	61238950 <sup>*</sup>
<b>Error (a)</b>	9	239.6968	12051966	10416544
<b>N dose</b>	3	1427.386 <sup>**</sup>	1.98E+08 <sup>**</sup>	1.86E+08 <sup>**</sup>
<b>N dose* Manag</b>	3	235.9409 <sup>ns</sup>	1233649 <sup>ns</sup>	1801316 <sup>ns</sup>
<b>Error (b)</b>	18	82.146	4420474	4273142
<b>Mean</b>		86.45	42596.45	41011.45
<b>CV1 (%)</b>		17.91	8.15	7.87
<b>CV2 (%)</b>		10.48	4.94	5.04

<sup>\*\*</sup>, <sup>\*</sup> and <sup>ns</sup>; significant at 1% and 5% probability and insignificant by F test, respectively.

Cont; control treatment, Fact; factorial treatments and Mang; incorporation timing

**Table 2.4.** Summary of mean squares (MS) of the combined ANOVA and significance for fresh tuber yield classes, dry matter content (DMC) AND dry matter production (DMP) .

SOV	DF	MS				
		Class 4	Class 3	Class 2	DMC	DMP
<b>Block</b>	3	283773.3 <sup>ns</sup>	2068308 <sup>ns</sup>	28309893 <sup>ns</sup>	2.159833 <sup>ns</sup>	119759.3 <sup>ns</sup>
<b>Control</b>	1	10878.12 <sup>ns</sup>	6426112 <sup>ns</sup>	1.71E+08 <sup>**</sup>	0.720000 <sup>ns</sup>	6101625 <sup>**</sup>
<b>Cont * Fact</b>	1	6566.58	5809490	1.98E+08	12.22756	13150302
<b>Manag</b>	1	197663.3 <sup>ns</sup>	276024.5 <sup>ns</sup>	54132010 <sup>*</sup>	0.045000 <sup>ns</sup>	2519474 <sup>*</sup>
<b>Error (a)</b>	9	489364.4	2685773	8580868	0.490299	401330.3
<b>N dose</b>	3	349084.0 <sup>ns</sup>	3231498 <sup>ns</sup>	1.53E+08 <sup>**</sup>	2.671146 <sup>ns</sup>	7126987 <sup>**</sup>
<b>N dose* Mang</b>	3	378471.7 <sup>ns</sup>	3467180 <sup>ns</sup>	3820140 <sup>ns</sup>	0.375417 <sup>ns</sup>	38993.03 <sup>ns</sup>
<b>Error (b)</b>	18	215507.7	1466194	3321290	1.36592	211660.8
<b>Mean</b>		1585.00	6358.45	34653.00	14.11	6040.42
<b>Cv1 (%)</b>		44.14	25.77	8.45	4.96	10.49
<b>CV2 (%)</b>		29.29	19.04	9.58	8.28	7.62

<sup>\*\*</sup>, <sup>\*</sup> and <sup>ns</sup>; significant at 1% and 5% probability and insignificant by F test, respectively.

Cont; control treatment, Fact; factorial treatments and Mang; incorporation timing