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Table of Contents: Volume 11 Number 22, 2 June, 2016

ARTICLES

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Review

Trichoderma: A significant fungus for agriculture and environment

Rajesh R. Waghunde[1]*, Rahul M. Shelake[2] and Ambalal N. Sabalpara

[1]Department of Plant Pathology, College of Agriculture, N.A.U.., Bharuch, India.
[2]Proteo-Science Center, Faculty of Science, Ehime University, Matsuyama, Japan.
[3]Director of Research and Dean P. G. Studies, Navsari Agricultural University, Navsari, India.

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The novel technologies in all areas of agriculture have improved agricultural production, but some modern practices affect the environment. The recent challenge faced by advanced farming is to achieve higher yields in environment-friendly manner. Thus, there is an immediate need to find eco-friendly solutions such as wider application of biocontrol agents. Among various types of species being used as biocontrol agents, including fungi and bacteria, fungal genus *Trichoderma* produces different kinds of enzymes which play a major role in biocontrol activity like degradation of cell wall, tolerance to biotic or abiotic stresses, hyphal growth etc. The understanding of filamentous fungi belonging to the genus *Trichoderma* has continuously evolved since last two decades, from the simple concepts of biocontrol agents to their recently established role as symbionts with different beneficial effects to the plants. Recent findings from structural and functional genomics approaches suggest the additional use of these microbes as model to study mechanisms involved in multiple player interactions that is, microbes-microbes-plant-environment. In this work, historical development of *Trichoderma* spp., mode of action against different biological agents, potential applications and recent mass production techniques are summarized and discussed in detail with updated advances with their application in the agriculture and sustainable environment.

Key words: Biocontrol agent, mycoparasitism, induced resistance, endophyte, mass production, bioremediation, bioreactors, agrochemicals.

INTRODUCTION

Trichoderma - a multifaceted fungus

Fungi in genus *Trichoderma* (Division - Ascomycota, Subdivision - Pezizomycotina, Class - Sordariomycetes, Order - Hypocreales, Family - *Hypocreaceae*) have been known since 1920s for their capability to function as biocontrol agents (BCA) against plant pathogens (Samuels, 1996). They can be used either to improve

*Corresponding author. E-mail: rajeshpathology191@gmail.com.

health of crop plant or to increase the natural ability to degrade toxic compounds by some plants in soil and water. Some species of *Trichoderma* have the multiple interactions (mainly *Trichoderma harzianum* strain T22 and *Trichoderma atroviride* strain P1) with crop plants and soil borne fungal pathogens (Woo et al., 2006). The different species of this genus have long been known not only for the control of plant disease but also for their capability to enhance plant growth and development, elevated reproductive ability, capacity to modify the rhizosphere, capability to grow under adverse conditions, competence in the use of nutrients, strong aggressiveness against phytopathogenic fungi and efficacy in supporting plant growth and enhanced defense mechanisms (Harman et al., 2004; Schuster and Schmoll, 2010; Pandya et al., 2011; Tripathi et al., 2013; Dagurere et al., 2014; Keswani et al., 2014). These properties have made *Trichoderma* a omnipresent genus able to grow in wider habitats and at high population densities (Chet et al., 1997; Chaverri et al., 2011). There is a numerous literature available on *Trichoderma* research but recent updates in cooperation with long-established facts are not summarized in past few years (Gal-Hemed et al., 2011; Sujatha et al., 2013). This review focuses on the occurrence of *Trichoderma* spp., their mode of action, commercial production techniques with applications in agriculture and use in sustainable environmental practices.

The fungus *Trichoderma* has a long history and it was first reported and described in 1794 (Persoon, 1794) and later suggested to have a link with the sexual state of a *Hypocrea* species. However, it was difficult to assign the genus *Trichoderma*/*hypocrea* morphologically. It was even proposed to have only one species, that is *Trichoderma viride*. The first move on development of a particular protocol for species identification was made in 1969 (Rifai, 1969; Samuels, 2006). *Trichoderma* spp. has been known from last 70 years for their ability to produce antibiotics that inhibit growth of pathogenic organisms and used as a biocontrol agents (Harman, 2006). Subsequently, many novel species of *Trichoderma* were revealed and by 2013, the genus already consists of more than 200 phylogenetically defined species based on rpb2 sequence (Atanasova et al., 2013).

PHYLOGENIC EVOLUTION

The genus name *Trichoderma* was first proposed on the basis of macroscopic similarity (Persoon, 1794). The four species categorized in this genus were *T. viride*, *T. nigrscens*, *T. aureum* and *T. roseum* collected in Germany. These species were described as appearing like mealy powder and enclosed by a hairy covering further distinguished from each other by their different colored conditions (Persoon, 1794). However, these four species are now considered to be unrelated to each other and presently known as *Trichoderma viride* (Pers. Ex. Fr.), *Xylohypha nigrescens* (Pers. Ex. Fr.) mason, *Sporotrichum aureum* Pers. Ex. Fr. and *Trichothecium roseum* (Pers.) link ex S.F. Gray. The name *Trichoderma* is now applied to be the most frequently encountered green forms typified by the original *T. viride* species described by Persoon, 1794. The first real generic description of *Trichoderma* was proposed based on colony growth rate and microscopic characters by Rifai, 1969. The genus was sub-divided into nine species, distinguished from each other primarily on the basis of conidiophore branching patterns and conidium morphology. The nine species-aggregates proposed were (1) *T. piluliferum*, Webster and Rifai, (2) *T. polysporum* (link ex Pers.) (3) *T. hamatum* (Bon.) Bain. (4) *T. koningii* Oudemans (5) *T. aureoviride* Rifai (6) *T. harzianum* Rifai (7) *T. longibrachyatum* Rifai (8) *T. pseudokoningii* Rifai and (9) *T. viride* (Pers. Ex. Fr.). However, problem associated with Rifai's key was significant variation which remained to be defined within each of the nine aggregate taxa. During the last couple of decades of the twentieth century, several groups revised and rearranged the *Trichoderma* genus mainly on the basis of morphological characteristics (Bissett, 1984; Bissett, 1991a; Manczinger et al., 2012; Bissett, 1991b; Gams and Bissett,1998; Doi et al., 1987, Samuels, et al., 1998).

There were some earlier reports about false identification of certain species using morphological characteristics, for example name *Trichoderma harzianum* was used for many different species (Kullnig-Gradinger et al., 2002) Recently, methods for safe identification of new species are significantly facilitated by development of and a customized similarity search tool (TrichoBLAST) and an oligonucleotide barcode (TrichOKEY), both available online at http://www.isth.info/ (Druzhinina et al., 2005; Kopchinskiy et al., 2005). Additionally, phenotype microarrays are useful for classification of new species which allow analysis of carbon utilization patters for 96 carbon sources (Bochner et al., 2001). Chaverri and Samuels, 2013 analyzed endophytic species on the basis of their habitat preference and nutrition mode to understand species radiations in diverse groups, and its potential use in development of novel biological control strategies. Several species could be characterized with well-defined isoenzyme patterns during cellulose-acetate electrophoresis, suggesting that this method can be used for the analysis of biochemical diversity between and within particular species of the genus *Trichoderma* (Manczinger et al., 2012). The persistent efforts to clarify variety and geographical incidence of *T. hypocrea* promoted thorough documentations of the genus in many parts of the world (Samuels et al., 2012; Chaverri and Samuels, 2003; Jaklitsch, 2009). Presently, the International Sub commission on *T. hypocrea* assigned 104 species characterized at molecular level

(http://www.isth.info/biodiversity/index.php). A different member of this genus produces a broad array of pigments from bright greenish-yellow to reddish in color but some are colorless.

Trichoderma spp. is ubiquitous in environment

Trichoderma is an asexually reproducing fungal genus most frequently found in soil; nearly all temperate and tropical soils contain 101 to 103 propagules per gram which can be grown in standard laboratory conditions. These species can colonize woody as well as herbaceous plants, in which the sexual teleomorph (genus *Hypocrea*) has observed. Nevertheless, there are many *Trichoderma* strains, including most biocontrol strains with no sexual stages. In nature, vegetative forms of the fungi persist as clonal, often heterokaryotic, individually and in populations that most likely evolve separately in the asexual stage. *Trichoderma* are strong opportunistic invaders, fast growing, prolific producers of spores and also powerful antibiotic producers even under highly competitive environment for space, nutrients, and light (Schuster and Schmoll, 2010; Herrera-Estrella and Chet, 2004; Montero-Barrientos et al., 2011). These properties make *Trichoderma* ecologically very dominant and ubiquitous strains able to grow in native prairie, agricultural, marsh, forest, salt and desert soils of all climatic zones (including Antarctic, tundra, and tropical regions) also found in lake, air, plant biomass, in the vicinity of virtually all types of live plant species, and seeds (Montero-Barrientos et al., 2011; Mukherjee et al., 2013). Recently, marine *Trichoderma* isolates were characterized to evaluate their potential use as halotolerant biocontrol agents and found effective against *Rhizoctonia solani* inducing systemic defense responses in plants (Gal-Hemed et al., 2011).

Trichoderma as a biopesticide in modern agriculture

Trichoderma-based biofungicides are booming in an agricultural market with more than 50 formulations registered products worldwide. Nowadays, there are more than 50 different *Trichoderma*-based agricultural products being produced in different countries and are sold to farmers to get better yields in different crops (Woo et al., 2006). Presently, *Trichoderma* spp. based products are considered as relatively novel type of biocontrol agents (BCAs). The size of current biopesticide market is vague and only scattered information could be obtained based on registered as well as non-registered biofungicides. Recently, *Trichoderma* based BCAs share about 60% of all fungal based BCAs and an increasing number of *Trichoderma* spp. based BCAs products are registered regularly. *T. harzianum* as an active agent in a range of commercially available biofertilizers and biopesticides is being used recently (Lorito et al., 2010; Vinale et al., 2006). The inherent qualities of *Trichoderma* based BCAs are driving factors for their steadily cumulating success (Verma et al., 2007).

There are numerous reports on the ability of *Trichoderma* spp. to antagonize a wide range of soil borne plant pathogens combined with their ability to reduce the incidence of diseases caused by these pathogens in a wide range of crops (Monte, 2001). The mechanisms that *Trichoderma* uses to antagonize phytopathogenic fungi include competition, colonization, antibiosis and direct mycoparasitism (Howell, 2003). This antagonistic potential serves as the basis for effective biological control applications of different *Trichoderma* strains as an alternative method to chemicals for the control of a wide spectrum of plant pathogens (Chet, 1987).

MODE OF ACTION

Trichoderma can work as biocontrol agents in several ways (Figure 1):

1. It may grow faster or use its food source more efficiently than the pathogen, thereby crowding out the pathogen and taking over, known as nutrient competition.
2. A biocontrol agent may excrete a compound that slows down or completely inhibit the growth of pathogens in the surrounding area of such a compound called antibiosis.
3. It may feed on or in a pathogenic species directly known as parasitism.
4. It may promote a plant to produce a chemical that protects it from the pathogen, which is induced resistance.
5. They can grow in an endophytic way in other species and supports plant growth.

Competition

The most common reason for the death of many microorganisms growing in the vicinity of *Trichoderma* strains is the starvation and scarcity of limiting nutrients. This can be effectively used in biological control of fungal phytopathogens. Carbon and iron are two essential elements in most of the filamentous fungi, required for viability. Competition for carbon is effective mode not only in *Trichoderma* but also some other fungi such as strains of *F. oxysporum* (Sarrocco, et al., 2009; Alabouvette et al., 2009). Under iron starving conditions; most fungi produces small size ferric-iron specific chelators to mobilize iron from surrounding environment. *T. harzianum* T35 also controls *Fusarium oxysporum* by competing for both rhizosphere colonization and nutrients (Tjamos et al., 1922). Siderophores produced by some *Trichoderma* isolates are highly efficient chelators for iron

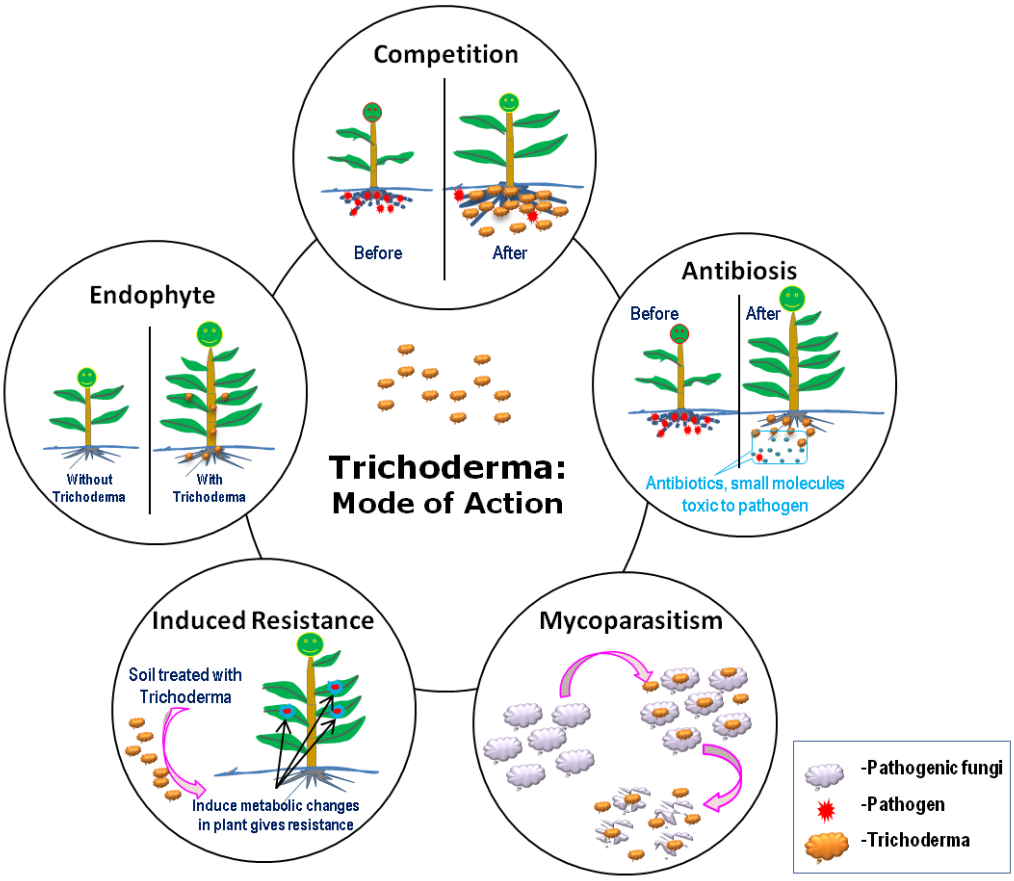

Figure 1. Model depicting mode of action of *Trichoderma* spp. against pathogen and plant growth improvement.

and inhibit the growth of other fungi (Chet and Inbar, 1994). Hence, *Trichoderma* spp. outcompetes with *Pythium* for available iron in soil and effectively controls its growth. There are many more examples about effective application of competition for the biocontrol of pathogens such as *B. cinerea*, which is involved in pre- and post-harvest loss in many countries around the world (Latorre et al., 2001). These reports suggest that the molecular and proteomic assembly of *Trichoderma* is more efficient to mobilize and take up soil nutrients as compared to many other studied pathogens and other organisms.

The proficient utilization of accessible nutrients is resulting from the capability of *Trichoderma* to acquire ATP from the diverse types of sugars, such as those derived from polymers widely available in fungal environments: cellulose, glucan and chitin and others, all of them turning into glucose (Chet et al., 1997). Recently the antifungal properties of filtrates of *Trichoderma* strains were used to control *Ceratocystis paradoxa* responsible for pineapple disease of sugarcane (Rahman et al., 2009). Productions of proteins playing pivotal role in root colonization by *Trichoderma* are also found to be crucial in competition with other root colonizers (Saloheimo et al., 2002; Viterbo et al., 2004; Brotman et al., 2008) and some of them help to establish symbiotic relationship with host plants (Samolski et al., 2012).

Antibiosis

The mechanism of antibiosis is commonly reported among many species including microorganisms and plants. In case of *Trichoderma*, small size diffusible compounds or antibiotics produced by these species inhibit the growth of other microorganisms (Benitez et al., 2004). Production of volatile compounds was not detected in case of four isolates of *T. harzianum* that were tested *in vitro* against *Rhizoctonia solani* (Cumagun and Ilag, 1997). Strains of *T. virens* able to produce gliovirin involved in antibiosis making it efficient biocontrol agent (Howell, 1998). A mutant of *T. harzianum* strain 2413 with elevated levels of extracellular enzymes and of α-pyrone increased resistance than the wild type against *R. solani* and in assays of grape protection against *B. cinerea* under different controlled environmental

conditions (Rey et al., 2001). In tobacco plants, exogenous application of peptaibols activated defense responsive genes and showed reduced susceptibility to *Tobacco mosaic virus* (Wiest et al., 2002). Coconut smell is typical of *T. viride* isolates suggesting the presence of volatile compounds that are inhibitory to pathogen growth. These metabolites include harzianic acid, alamethicins, tricholin, peptaibols, antibiotics, 6-penthyl-α-pyrone, massoilactone, viridin, gliovirin, glisoprenins, heptelidic acid (Vey et al., 2001; Raaijmakers et al., 2009). The different pathways producing secondary metabolites are illustrated and summarized recently by Daguerre et al., 2014, including pyrone biosynthesis pathway, polyketide biosynthesis pathway, peptaibol biosynthesis pathway, flocculosin terpenoid/steroid biosynthesis pathway, gliotoxin and gliovirin biosynthesis pathways.

Mycoparasitism

Mycoparasitism is one of the main mechanisms involved in the antagonisms of *Trichoderma* as a biocontrol agent. The process apparently include, chemotropic growth of *Trichoderma*, recognition of the host by the mycoparasites, secretion of extra cellular enzymes, penetrations of the hyphae and lysis of the host (Zeilinger et al., 1999). *Trichoderma* recognizes signals from the host fungus, triggering coiling and host penetrations. The process of mycoparasitism involves direct attack of one fungal species on another one. This complex process includes sequential events, involving cycle of recognition of fungal strain by *Trichoderma* spp., attack on cellular machinery, and subsequent penetration inside the host and finally killing of the host. *Trichoderma* spp. even can grow towards fungal host by recognizing them. Such remote sensing activity is partially because of the sequential production of pathogenesis related proteins, mostly glucanase proteases, and chitinase (Harman et al., 2004). The response of different *Trichoderma* strains is not similar in the process of mycoparasitism. Constitutive secretion of exochitinases at low level which degrade fungal cell-walls releasing oligomers plays a central role in growth inhibition of pathogenic fungal strains (Gajera et al., 2013). In some cases, the morphological changes like coiling and formation of appressorium containing higher amount of osmotic solutes such as glycerol induces penetration in host cells. *Trichoderma* attached to the pathogen, coils around the pathogen and formed appresoria releases its content. It results in the production of pathogenesis related peptides which helps in both the entry of *Trichoderma* hyphae and the digestion of the cell wall content (Howell, 2003). The cell wall degradation of target fungus by these produced chemical compounds results in the parasitism. There are many factors affecting this process and at least 20 to 30 proteins and other metabolites are directly involved in this interaction. The functions of different glucanases and chitinases in the process of mycoparasitism are well studied from *Trichoderma* spp. using gene-for-gene experiments and future studies will definitely help us to understand this complex process (Daguerre et al., 2014).

Induced resistance

The major focus of *Trichoderma* research was to understand the direct effects on other fungal species, especially mycoparasitism and antibiosis. The first clear demonstration of induced resistance with *T. harzianum* strain T-39 showed that treated soil made leaves of bean plants resistant to diseases caused by the fungal pathogens such as *B. cinerea* and *C. lindemuthianum*, even though T-39 was applied only on the roots and without any on the foliage (Bigirimana et al., 1997). Induced resistance was found to be beneficial in more than 10 different dicots and monocots, to infection by fungi (*B. cinerea*, *R. solani*, *Colletotrichum* spp., *Phytophthora* spp., *Alternaria* spp., *Magnaporthe grisea*, etc.), bacteria (*Xanthomonas* spp., *Pseudomonas syringae*, etc.), and even some viruses like CMV. The soil treated with *T. harzianum* strain T-39 was also effective against fungal pathogens *B. cinerea* and *Colletotrichum lindemuthianum* in bean plants. Similar findings were reported from *B. cinerea* to other dicots (De Mayer et al., 1998).

Similar studies have been conducted with different *Trichoderma* species and strains on different plant species, including both monocots and dicots. *T. harzianum* strain T-22 is the only microbe reported to induce systemic resistance to pathogens in model plants (Contreras-Cornejo et al., 2011; Salas-Marina et al., 2011; Yoshioka et al., 2012) and also in maize indicative of its unique ability (Harman et al. 2012). Induced systemic resistance is believed to be one of the most important mechanisms of biocontrol effects of *Trichoderma* (Harman, 2006). A variety of strains of *T. virens*, *T. asperellum*, *T. harzianum*, and *T. atroviride* stimulate metabolic changes that enhance higher tolerance to many plant-pathogenic microbes including viruses (Table 1). Likewise, this response appears to be broadly useful for many crops; for example, *T harzianum* strain T-22 induces resistance in plants as diverse as tomatoes and maize, suggesting a little or no plant specificity.

Saksirirat et al., 2009 reported that isolate of *T. harzianum* (T9) induced resistance in tomato plant (cv. Sida cultivar) with reducing 69.32% bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*) after 14 days post inoculation. Similarly, on gray leaf spot (*Stemphylium solani*), isolate *T. asperellum* (T18) induced resistance and showed significant reduction in number of spots by 19.23% after 10 days post inoculation. The elicitor filtrate of *T. harzianum* (PDBCTh10 isolate) was found effective against root rot (*Phytophthora capsici*) in pepper plant and induced

Table 1. Induced systemic resistance elicited by *Trichoderma* spp.

Species Plant and strain	Plant species	Pathogens	Outcome	References
T. virens G-6, G-6-5 and G-11	Cotton	*Rhizoctonia solani*	Protected plant by inducing terpenoid phytoalexins toxic to fungi	Howell et al., 2009
T. harzianum T-39	Bean	*Colletotrichum lindemuthianum, Botrytis cinerea*	No infection on leaves when T-39 was applied only on roots	Bigirimana et al., 1997
	Tomato, pepper, tobacco, lettuce, bean	*B. cinerea*	No infection on leaves when T-39 was applied only on roots	De Meyer, 1998
	A. thaliana (L.) Heynh.	*Botrytis cinerea Pers.*	Ecotype Colombia-0 (Col-0) showed resistance leading to reduced grey mold symptoms	Korolev et al., 2008
	Vitis vinifera	*Plasmopara viticola*	Activation of defense related mechanisms	Perazzoli et al., 2012
	Tomato	*Botrytis cinerea*	0.4% T39 drench showed 84% decline in disease severity	Meller et al., 2013
	Cucumber, bean, tomato, and strawberry	*Botrytis cinerea* and *Podosphaera xanthii*	Protected from foliar diseases by direct or indirect effect via stimulation of beneficial microorganisms in the rhizosphere	Levy et al., 2015
T. harzianum T-22; *T. atroviride* P1	Bean	*B. cinerea* and *Xanthomonas campestris* pv. phaseoli	Activation of pathways related to antifungal compounds in leaves when present on roots	Harman et al., 2004
T. harzianum T-1 & T22; *T. virens* T3	Cucumber	*Green-mottle, mosaic virus*	No infection on leaves when strains were present only on roots	Lo et al., 2000
T. harzianum T-22	Tomato	*Alternaria solani*	No infection on leaves when T-22 was applied only on roots	Seaman, 2003
Trichoderma GT3-2	Cucumber	*C. orbiculare*, *P. syringae* pv. lachrymans	Induction of defense related genes related to lignifications and superoxide generation	Koike et al., 2001
T. harzianum	Pepper	*Phytophthora capsici*	Improved production of the phytoalexins capsidiol toxic to pathogen	Ahmed et al., 2009
T. asperellum (T203)	Cucumber	*Pseudomonas syringae* pv. *lachrymans*	Modulated the expression of proteins related to jasmonic acid/ethylene signaling	Shoresh et al., 2005
T. asperellum SKT-1	*A. thaliana* (L.) Heynh.	*Pseudomonas syringae* pv. *tomato* DC3000	Induced systemic resistance to colonization by SKT-1 and its cell-free culture filtrate	Yoshioka et al, 2012
	A. thaliana	*Cucumber mosaic virus*	Improved defense mechanism against infection of CMV	Elsharkawy et al., 2013
T. harzianum Tr6, and *Pseudomonas* sp. Ps14	cucumber and *A. thaliana*	In cucumber- *Fusarium oxysporum* f. sp. *radicis cucumerinum* and in *A. thaliana* against *B. cinerea.*	Ps14 and Tr6 activated the set of defense-related genes	Alizadeha et al., 2013
T. virens and T. atroviride	Tomato	*Alternaria solani, B. cinerea,* and *Pseudomonas syringae* pv. *tomato* (Pst DC3000)	Secreted proteins- Sm1 and Epl1 both induced systemic acquired resistance	Salas-Marina et al., 2015

resistance resulting with 23% less infection (Sriram et al., 2009). At a molecular level, resistance to different pathogens is due to increase in the activity of defensive mechanisms producing higher concentration of related metabolites and enzymes, such as chalcone synthase (CHS) and phenylalanine ammonio lyase (PAL), chitinase, glucanase and some proteins from cerato-platanin (CP) family and

phytoalexins (HR response) synthesizing enzymes such as PKS/NRPS hybrid enzyme (Djonovic et al., 2006; Seidi et al., 2006; Mukherjee et al., 2012). These comprise pathogenesis related proteins (PR) and enzymes involved in the response to oxidative stress (Gajera et al., 2013).

Endophytes

Endophytic activity of many microorganisms (growth inside plant tissue without any harm) may useful to host plant by stimulating of plant growth, a postponement to the beginning of drought stress and the obstruction to pathogens (Piotrowski and Volmer, 2006). Endosymbiotic species are capable of establishing colonies in plant roots and triggers the expression of many plant genes affecting stress responses. Recently, there are reports showing *Trichoderma* isolates acting as endophytic plant symbionts in some woody plants (Gazis and Chaverri, 2010; Chaverri and Gazis, 2011). Interestingly, strains forming association with roots are altering the gene expression pattern in shoots. These changes are the key points in altering plant physiology and this can be exploited in the improvement of many important traits like uptake of nitrogen fertilizer, abiotic/biotic stress resistance, and photosynthetic efficiency leading to higher yields (Chaverri and Samuels, 2013; Harman et al, 2012). Phylogenetic analysis classifies all known endophytic species as a separate taxa with the exception of *T. koningiopsis, T. stilbohypoxyli* and *T. stromaticum* within their clades at terminal position suggesting endophytism is not an old trait but recently evolved in *Trichoderma* species (Chaverri et al., 2011; Samuels et al., 2006; Samuels and Ismiel, 2009; Druzhinina et al., 2011).

MASS PRODUCTION

Due to increasing interest in the biocontrol, awareness about pesticide hazards, commercial production and use of biocontrol agents has now come into a reality and there are several reports of successful use of formulations of *Trichoderma* in the green house as well as in the field for control of various diseases, particularly for the soil borne pathogens. For mass introduction of *Trichoderma* in the fields, *Trichoderma* spp. is to be multiplied on some suitable and cheap media which can provide a food base for the initiation of the growth. *T. harzianum* and *T. viride* are the two most commonly used species and have been found effective when applied on about 87 different crops in India (Sharma et al., 2014). Available literature reveals that researchers have attempted for use of varied substrates and techniques for multiplication and introduction of *Trichoderma* into the soil (Sabalpara, 2014). One of the greatest impediments to biological control by *Trichoderma* has been the scarcity of methods for mass culturing and delivering the biocontrol agents. The problem in developing biopesticides, a living system, is during the process of formulation and short shelf life. The most widely used fungal antagonists, *Trichoderma* spp. have been grown on solid substrate like wheat straw, sorghum grains, wheat bran, coffee husk, wheat bran-saw dust, diatomaceous earth granules impregnated with molasses and so forth for their mass multiplication (Table 2).

Papavizas et al. (1984) produced biomass of fungal antagonists by liquid fermentation consisting of molasses and brewer's yeast. Montealegre et al. (1993) proposed liquid fermentation method consisting of molasses, wheat bran and yeast on large scale production of *T. harzianum*. Since *Trichoderma* sporulates relatively poorly in liquid media and sporulates well on various solid substrates, solid substrate fermentation (SSF) process was preferred over the other due to some inherent advantages under Indian conditions. These include utilization of large number of agro wastes as substrate for the en mass production of *Trichoderma*, use of a wide variety of matrices, low capital investment, low energy expenditure, less expensive downstream processing, less water usage and lower waste water output, potential higher volumetric productivity, high reproducibility, lesser fermentation space and easier control of contamination. Fermented biomass of *Trichoderma* consisted mainly of chlamydospores and conidia with some amount of mycelia fragments. The controlled physiological parameters are crucial in production of viable spores suggesting carbon to nitrogen ratio in medium or substrate, pH, and cultivation time are important (Agosin et al., 1997).

Solid state fermentation

Among the grains, sorghum proved very useful and cheaper for the production of nucleus culture while among the organic matter farm yard manure and seasoned pressmud proved superior. Pressmud proved very useful and more applicable source especially in sugar factory area. From the agro wastes tested wheat bran and paddy straw suggested as the most promising source for the mass multiplication of *Trichoderma* (Table 2).

Liquid state fermentation

Liquid state fermentation is generally used to produce spores from fungal strains. Among the liquid media, *Trichoderma* Selective Medium (TSM) along with mannitol, molasses and potato jaggery media were found very effective and suggested for the mass multiplication of *Trichoderma* spp. by many workers. Mass multiplication of *T. viride*, *T. harzianum* and *T. longibrachiatum* using decomposed pressmud was found

Table 2. Substrates successfully used for *Trichoderma* production.

S/N	Species	Substrates	References
Solid based			
I. Grains			
1	*T. harzianum* and *T. viride*	Sorghum	Rini and Sulochana, 2007
2	*T. viride*	Sorghum, wheat	Bhagat et al., 2010
3	*T. harzianum*	Rice, sorghum, pearl millet	Parab et al., 2008
4	*T. harzianum*	Maize	Pramod and Palakshappa, 2009
5	*T. harzianum*	Sorghum	Upadhyay and Mukhopadhay, 2009
II. Organic matters			
6	*T. harzienum* P26	Neem cake, coircompost, FYM, Gliricida leaves	Saju et al., 2002
7	*T. harzianum* (T5), *T. viride, T. hamatum* (T16)	Cotton cake	Sharma and Trivedi, 2005
8	*T. harzianum*	FYM, Local cow dung, Jersey cow dung	Pramod and Palakshappa, 2009
9	*T. harzianum and T. viride*	Cow dung with neem cake, coir pith, coir pith in combination with neem cake	Rini and Sulochana, 2007
10	*T. harzianum* Rifai	Tapioca waste Pigeonpea husk and press mud	Jayraj and Ramabadran, 1996
11	*T. viride*	FYM, vermicompost, poultry manure, goat manure, decomposed coconut, coir pith	Palanna et al., 2007
12	*T. harzianum*	FYM, spent compost	Tewari and Bhanu, 2004
13	*T. harzianum*	FYM, compost	Parab et al., 2008
14	*T. viride*	FYM, Peat	Bhagat et al., 2010
15	*T. harizianum*	Jatropha cake and neem cake	Tomer et al., 2015
III. Agricultural wastes			
16	*T. harzianum*	Rice bran, paddy straw, groundnut shells	Parab et al., 2008
17	*T. harzianum, T. viride* and *T. virens*	Spent Malt	Gopalkrishnan et al., 2003

Table 2. Contd.

18	*T. harzianum*	Wheat straw, paddy straw, shelled maize cob, paper waste, saw dust, sugarcane bagasse, spent straw, wheat bran, rice bran	Tewari and Bhanu, 2004
19	*T. viride* and *T. harzianum*	Tapioca rind, tapioca refuse, mushroom spent straw, paddy chaff, wheat bran, groundnut shell, rice bran, sugarcane baggase, wheat straw, shelled maize cob, paddy straw, chickpea husk	Gangadharan and Jeyrajan, 1990
20	*T. harzianum* and *T. viride*	Saw dust, rice bran	Rini and Sulochana, 2007
21	*T. harzianum*	Shelled maize cobs, paddy straw, paddy husk, wheat bran, baggase, sawdust, groundnut shell	Pramod and Palakshappa, 2009
22	*Trichoderma harzianum, T. virens* and *T. atroviride*	Onion rind (dry onion skin), apple and strawberry pomace, rapeseed meal	Smolinska, et al., 2014
23	*T. harzianum* (T5), *T. viride, T. hamatum* (T16)	Tea waste, sorghum straw, wheat straw, wheat bran	Sharma and Trivedi, 2005
Liquid based			
24	*T. hamatum, T. harzianum*, *T. viride*	Molasses and Brewers yeast	Papavizas, 1984
25	*T. harzianum* strain P1,	Defined basal culture medium with mineral solution	Agosin, 1997
26	*T. harzianum*	RM8	Jin, 1991
27	*T. harzianum* strain 1295-22	Modified RM8	Jin, 1991
28	*T. harzianum*	Czapeck's Dox Broth and V8 Broth	Harman, 1991
29	*T. harzianum* Rifai	Potato Dextrose Broth, V8 juice and molasses yeast medium	Prasad, 2002
30	*T. harzianum* Rifai	Potato Dextrose Broth, Czapeck's Dox Broth and Modified Richards' Broth	Das, 2006
31	*T. harzianum*	Local cow urine, Jersey cow urine, Butter milk, Vermiwash	Parab et al., 2008

most effective as compared to the rest of the substrates tested (Gohil, 1993). In addition, several techniques for the mass production of *Trichoderma* spp. were established and proposed by our group and other researchers based on local conditions and availability of substrates (Pandya et al., 2007; Sabalpara, 2014; Pandya et al., 2012; Sabalpara et al., 2009). A novel technique using talc mixed proportionately with FYM (1:10) was developed for direct soil and nursery bed applications (Ramanujam and Sriram, 2009).

Commercial level production

Bacterial based BCAs are being produced and marketed by many commercial firms and available in global market (Velivelli et al., 2014). In India, there are more than 250 BCA products available in the market. Formulization of commercial BCA for agricultural application should possess several desirable characters and need to have substantial proof in order to convince farmers. These include satisfactory market potential, easy preparation, unfussy application, high stability during transportation as well as storage, abundant viable propagules with good shelf life, sustained efficacy and accepted cost. Various carrier materials proved useful for the preparation of formulation of *Trichoderma* based BCAs because it works as a food base (Table 3). Talc is the most common carrier material suggested for commercial production of *Trichoderma* worldwide.

POTENTIAL APPLICATIONS IN MODERN AGRICULTURE AND SUSTAINABLE ENVIRONMENT

The *Trichoderma* genus can grow in a wide range of habitats and this is achieved by evolved diversified metabolic pathways leading to the production of various enzymes and secondary metabolites. Production of commercially important enzymes such as amylases, cellulases, 1-3 beta glucanases, and chitinases were extensively studied and this technology is continuously being updated (Harman et al., 2004; Ahamed and Vermette, 2008; Sandhya et al. 2004). Recently, they have been found useful in the production of silver nanoparticles (Maliszewska et al., 2009; Vahabi et al., 2011).

Table 3. Various formulations of *Trichoderma* spp.

S/N	Formulations	Ingredients
1	Talc based	Trichoderma culture biomass along with medium: 1 liter, Talc (300 mesh, white colour): 2 kg and CMC: 10 g
2	Vermiculite-wheat bran based	Vermiculite: 100 g, Wheat bran: 33 g, Wet fermentor biomass: 20 g and 0.05N HCL: 175 ml
3	Wheat bran based	Wheat flour: 100 g, Fermentor biomass: 52 ml and Sterile water: sufficient enough to form a dough
4	Wheat flour-kaolin	Wheat flour: 80 g, Kaolin: 20 g and Fermentor biomass: 52 ml
5	Wheat flour-bentomite	Wheat flour: 80 g, Bentomite: 20 g and Fermentor biomass: 52 ml
6	Alginate prills	Sodium alginate: 25 g and Wheat flour: 50 g and Fermentor biomass: 200 ml

Adapted from Pandya, 2012.

Bioremediation technology

Investigations on bioremediation of environmental toxicants are entering in a new era with the application of genetic engineering. However, majority of the studies related to bioremediation have been conducted under the laboratory conditions. The concept of utilizing fungi for bioremediation of soil contaminated with certain pollutants is relatively older. There is liberal evidence of various *Trichoderma* spp. contributing to polycyclic aromatic hydrocarbons (PAHs) degradation, even as affecting native mycorrhizal fungi both positively and/or, negatively (Azcbn-Aguilar and Barea, 1997). Degradation potential of rhizosphere-competent *Trichoderma* strains against several synthetic dyes, pentachlorophenol, endosulfan and dichlorodiphenyl trichloroethane (DDT) were demonstrated previously (Katayama and Matsumura, 1993). Hydrolyses, peroxidase, lactases and other lytic enzymes produced by *Trichoderma* spp. are probable factors aiding indegradation of these contaminants. Therefore, application of some detoxifying agents along with *Trichoderma* spp. would provide healthy soil and environment (Table 4). It may help to improve not only the health of soil and plant, but also a sustained crop yield protection. *Trichoderma* spp. inoculated in the soil can grow rapidly because of naturally resistant ability to many toxic compounds, such as fungicides, herbicides, insecticides and phenolic compounds (Chet et al., 1997).

Trichoderma strains may play an important role in the bioremediation of soil contaminated with pesticides and possess the ability to degrade a wide range of insecticides: organochlorines, organophosphates and carbonates. ABC transporter protein systems in *Trichoderma* strains may be involved in resistance mechanisms against tested noxious compounds (Harman et al., 2004).

Biotic and abiotic stress tolerance

Trichoderma species are good source of natural proteins that may facilitate the plant to survive in the biotic as well as abiotic stress conditions. The *hsp*70 gene from *T. harzianum* T34 was cloned and characterized (Mantero-Barrientos et al., 2008) and encoding protein expression in Arabidopsis showed higher tolerance to heat and other abiotic stresses (Mantero-Barrientos, et al., 2008). The encoding protein product of this gene facilitates higher level of fungal resistance to heat and other stresses such as osmotic, salt and oxidative tolerances. Putative kelch-repeat protein coding gene *Thkel1* isolated from *T. harzianum* regulating the glucosidase activity was able to induce improved tolerance to salt and osmotic stresses in *Arabidopsis thaliana* plants (Hermosa et al., 2011). Number of proteins, for example mitogen-activated protein kinase, Sm1 (Small Protein 1), 4-phosphopantetheinyl transferase, and PKS/NRPS hybrid enzyme from *T. virens* were confirmed and involved in conferring resistance against several soil born and foliar pathogens (Howell et al., 2000; Perazzoli et al., 2012; Viterbo et al., 2005).

Wood preservation

Trichoderma spp. displayed a killing action against these fungi in *in vitro* tests, but *in situ* action was ineffective. Ejechi investigated the ability of *T. viride* to inhibit the decay of obeche (*Triplochiton sceleroxylon*) wood by the decay fungi *Gloeophyllum* sp. and *G. sepiarium* under field conditions under dry and wet season in tropical environment for 11 months. *T. viride* exhibited total inhibition of the decay fungi by means of mycoparasitism and competition for nutrients (Ejechi, 1997).

Industrial bioreactors

Biofuel production is one of the eco-friendly ways to reduce expenditure on energy sector and tackle the global warming effects on environment and human health (Rubin, 1997). *T. reesei*, a non-biological agent is one the

most important genus for industrial purposes as a factory for the production of secreted cellulase in biotechnology and a model for basic studies on protein secretion (Ahamed and Vermette, 2009; Li et al., 2013). Molecular insights into the mechanism of the cellulose degrading pathways and genome sequencing of *T. reesei* provide a platform to explore novel ways of metabolic engineering (Kubicek et al., 2009). *T. reesei* contains the smallest number of genes encoding enzymes responsible for plant cell wall degradation within Sordariomycetes (Martinez et al., 2008). An alternative strategy to the first generation energy sources includes manufacturing of biofuels using agricultural waste products with the help of cellulases andhemicellulases produced by *T. reesei* or other strains and further fermentation by other microbes such as yeast (Schuster and Schmoll, 2010). Nonetheless, the efficiency of this process needs to improve several folds of magnitude to reach final goal of equally compatible energy sources like fossil fuels. Additionally, genus *Trichoderma* is a good source of many secondary metabolites useful in application against phytopathogens, which Keswani and co-workers have recently summarized Keswani et al. (2014) Secondary metabolites inhibiting growth of pathogens can be used irrespective of geographic location and such formulations can be produced with longer shelf life.

Sensitivity against agrochemicals

The efficiency of the bioagents is hampered due to poisonous nature of fungicides which are used simultaneously in crop production technology. Therefore, the sensitivity and tolerance of *Trichoderma* have been tested by our group and many others (Sawant and Mukhopadhay, 1990; Pandey and Upadhyay, 1998; Sharma, et al., 1999; Nallathambi et al., 2001; Sushir and Pandey, 2001; Bhatt and Sabalpara, 2001; Patibanda et al., 2002; Lal and Maharshi, 2007, Madhusudan et al., 2010). The effect of different fungicides together with *Trichoderma* spp. has been studied for integrated disease management. *Trichoderma* spp. have shown greater tolerance for broad spectrum fungicides than many other soil microbes as it has the capacity to colonize the pesticides treated soil more rapidly (Oros et al., 2011). *Trichoderma* alone or their combinations with bacteria or their immobilized formulations can have great potential, as more than a few unusual contaminants can be treated at the same time and will have wider applicability, hence improving the overall cost effectiveness of the technology.

CONCLUSIONS

Trichoderma spp. possess many qualities and they have great potential use in agriculture such as amend abiotic stresses, improving physiological response to stresses, alleviating uptake of nutrients in plants, enhancing nitrogen-use efficiency in different crops, and assisting to improve photosynthetic efficiency. The use of this genus has expanded worldwide as general plant protectants and growth enhancers, besides their application in a variety of industrial processes. The genome of *Trichoderma* spp. has been extensively investigated and has proven to contain many useful genes, along with the ability to produce a great variety of expression patterns, which allows these fungi to adapt to many different environments (soil, water, dead tissues, inside the plants, *etc*.). The metabolomics of *Trichoderma* spp. are incredibly complex, especially in terms of secondary metabolites production but with the help of advanced molecular and proteomic approaches, it is possible to explore new pathways, novel functions of compounds produced by this genus and their potential applications. The proteome of *Trichoderma* spp. growing in a variety of conditions and interactions has been mapped, and the information has been used to develop new products based on synergistic combinations of the living fungus with its secreted metabolites. These new formulations, which combine biocontrol with biofertilization, are considered to be more effective than older products and active on a wider range of pathogens.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Toxicity and sublethal effects of insecticides on *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae)

Eliane Carneiro*, Luciana Barboza Silva, Alexandre Faria Silva, Vilmar Bueno Santos, Mayra Layra Santos Almeida, Gabriel Santos Carvalho and Maisa Veras

Graduate Program in Agronomy Crop Science, Federal University of Piauí, UFPI, Bom Jesus, PI, Brazil.

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Exposure of *Helicoverpa armigera* to sublethal concentrations of insecticides can cause physiological deficiencies manifested by reduced longevity, development, fertility and fecundity. Research on the sub-lethal effects, to identify the non-lethal negative impacts of insecticides on pests can provide practical information for integrated management. The objective of this study was to determine the lethal concentrations and sublethal effects of insecticides on larval development and reproduction of *H. armigera*. The insecticides were diluted in water and applied via immersion of soybean leaf discs directly in the solution for three second, subsequently provided to the larvae maintained under controlled conditions for a period of 48 h. For surviving larvae, artificial diet was provided with daily evaluation until pupation. The pupae obtained were weighed after 24 h and transferred to Petri dishes, covered with filter paper until the adult phase, to evaluate longevity and pupal viability. For the assessments related to oviposition, couples were separated in polyvinyl chloride (PVC) cages and fed with 10% honey solution; the counting of eggs was done every two days, until the end of the oviposition period. All of the insecticides tested presented lethal and sublethal effects on the parameters weight, mortality and pupal viability and reduced oviposition and can be used in pest management, representing an alternative in the product rotation for the control of the third instar of *H. armigera*.

Key words: Management, chemical control, lethal concentration.

INTRODUCTION

Brazil is one of the largest global producers of grains, with production of 206.33 million tons in the 2014/2015 crop (Conab, 2015). The Brazilian grain production systems are characterized by being intensive, while cultural practices are extensive in relation to planted areas. These facts, coupled with inadequate farming practices, characterized by the successive planting of host plant species in contiguous areas and inappropriate handling of pesticides, have made agricultural ecosystems susceptible to attack by insect pests, due to the constant availability of food, shelter and breeding sites (Embrapa, 2013).

*Corresponding author. E-mail: elian.cbs@hotmail.com.

Among the major pests causing damage to crops are the stink bugs (Hemiptera: Pentatomidae) and the complex of defoliating caterpillars (*Spodoptera* spp ex, *Heliothis virescens* (Fabricius), *Anticarsia gemmatalis* Hubner, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae). Recently this situation has been aggravated by the introduction of *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae), a pest that has great potential for damage to various crops (Kotkar et al., 2009; Czepak et al., 2013).

The *H. armigera* caterpillar is a major pest of cultivated plants, having as hosts more than 181 plant species belonging to 45 families (Rajapakse and Walter, 2007). It feeds on leaves and stems, but preferably shoots, inflorescences, fruits and pods, being able to adapt to various cropping systems. Besides high polyphagia, it has a broad geographical range, potential migratory mobility, diapause (facultative), high fertility and a propensity to develop resistance to insecticides (Fitt, 1989; Mccaffery, 1998; Moral Garcia, 2006). Beginning with the 2012/2013 crop, *H. armigera* has caused losses to producers, especially in the North, Northeast and Mid-South, by intensely attacking different crops of economic importance in these regions, such as soybeans, cotton, corn, beans, sorghum, etc. (Fathipour and Sedaratian, 2013; Embrapa, 2013). In Bahia, the costs of infestation of the pest were estimated at about US $ 2 billion in crops such as soybeans, corn and cotton due to the increase in the number of applications of insecticides and reduced productivity (Adab, 2013).

The main control method used for *H. armigera* management has been almost exclusively the use of chemical insecticides. However, due to the impacts and the development of strains resistant to the products, it has become necessary to use in rotation insecticides with different modes of action, as well as techniques that can assist in integrated pest management (IPM) (Jacobson et al., 2009; Gunning and Moores, 2010; Perry et al., 2011; Shind et al., 2011; Yang et al., 2013).

Studies of the deleterious effects of insecticides on insect pests can corroborate IPM, as this combines diverse knowledge about the environment and population dynamics of the pest, using rational methods and techniques, in order to keep the pest population below the level of economic damage. Knowledge of the sublethal effects of insecticides on populations of insect pests is still very incipient. However, the few studies available have shown significant results (Desneux et al., 2007; Junior et al., 2009).

Exposure of *H. armigera* to sublethal concentrations of insecticides may cause physiological deficiencies, which may manifest as a reduction in longevity, development, fertility or fecundity. Research on these sub-lethal effects can provide practical information for integrated pest management. Therefore, we determined the lethal concentrations and sublethal effects of insecticides on larval development and reproduction of *H. armigera*.

MATERIALS AND METHODS

This experiment was carried out in the laboratory of Plant Science - Professor Cinobelina Elvas Campus - UFPI, Bom Jesus, PI, during the period between January to March 2014.

Breeding and maintenance *H. armigera*

The population of *H. armigera* was obtained from the insect rearing laboratory where they were maintained on artificial diet adapted from Kasten Jr. et al. (1978). Neonate larvae (<24 h old) were isolated and transferred to 100 ml plastic containers with lids, containing artificial diet and remaining until they reached the pupal stage. The adults were transferred to polyvinyl chloride (PVC) cages (40 cm H x 30 cm Ø) lined with bond paper sheets for oviposition, fed a honey-based solution (10%) and kept under controlled conditions (25 ± 2°C, 60 ± 5% RH, 12:12 LD). Eggs were collected and stored in plastic bags and kept in laboratory conditions until the hatching of caterpillars. After hatching the larvae were transferred to pots with diet until they reached the third instar and after this period, one part was kept for maintenance, and the others used in bioassays.

Lethal concentration curves

The test consisted of four treatments: spinosad (Tracer®), chlorantraniliprole (Premio®), *Bacillus thuringiensis* (Dipel®), and control (water). The insecticides were diluted in water, then soybean leaf discs (diameter 5 cm) were immersed for three seconds. After 30 min they were offered to 80 third instar larvae by concentration, which were kept under controlled conditions (25 ± 2°C, 60 ± 5% RH, 12:12 LD) for a period of 48 h. Subsequently, assessment of larval mortality was performed, considering dead individuals being touched with tweezers in the last abdominal segments not responding with coordinated movements. Treatments and concentrations are shown in Table 1. The determination of the concentrations corresponds to 0, 10, 25, 50, 75 and 100% of the recommended concentrations. The insecticides were diluted with distilled water to prepare each dose.Mortality was assessed daily for five days, after was corrected by the mortality in control (Abbott's formula). The mortality results were submitted to Probit analysis (Finey, 1971) through the PROC PROBIT the Statistical program (Sas Institute, 2002) generating the concentration-mortality curve (LC) LC_{25}, LC_{50} and LC_{95}.

Sublethal effects of the insecticides on *H. armigera*

Here, the lethal concentrations LC_{25} and LC_{50} were used to evaluate the effect of the insecticides on the surviving larvae of *H. armigera.* The insecticides were diluted in distilled water and soybean leaf discs (diameter 5 cm) were subsequently immersed into the solutions during three seconds. Then the leaf discs were then dried at room temperature for 30 min and offered to individual caterpillars in 100 mL plastic pots with lids. The plastic pots were kept under controlled conditions (25 ± 2°C, 60 ± 5 RH, 12:12 LD) during 48 h. After this period assessment of larval mortality was performed, considering as dead individuals when touched with tweezers in the last abdominal segments that did not respond with coordinated movements. For surviving larvae, artificial diet was supplied and survival was assessed daily until pupation. The pupae obtained

Table 1. Products, active ingredients and doses used to determine the dose response curve in *H. armigera.*

Active ingredient	Chemical group	a.i. g.L^{-1}	Doses in L.ha^{-1}					
Chlorantraniliprole	Diamide	200	0	0.01	0.025	0.05	0.075	0.1
Spinosad	Spinosyn	400	0	0.008	0.02	0.04	0.06	0.08
B. thuringiensis	*B. thuringiensis*	33.6	0	0.07	0.0150	0.35	0.52	0.7

a.i.: active ingredient.

Table 2. Lethal concentration (LC_{25}, LC_{50} and LC_{95} - 5 days) of insecticides tested on *H. armigera.*

Treatment	Lethal concentrations in L.ha^{-1}			Slope ± SEM	X^2	P
	LC_{25} (CI 95%)	LC_{50} (CI 95%)	LC_{95} (CI 95%)			
Chlorantraniliprole	0.02 (0.000-0.06)	0.04 (0.01-0.7)	0.36 (0.14-2.21)	0.66±0.30	18.33	0.14
Spinosad	0.006(0.000-0.015)	0.01 (0.000-0.02)	0.05 (0.02-5.86)	0.77±0.33	42.79	0.04
B. thuringiensis	0.10 (0.05-0.15)	0.22 (0.15-0.30)	1.50 (0.85-5.12)	1.54±0.32	10.89	0.62

SEM = standard error of the mean; LC = lethal concentration; X^2 = chi-square.

were weighed after 24 h and transferred to Petri dishes covered with filter paper, separated by sex and evaluated daily until adulthood, to evaluate longevity and pupal viability.

The evaluation related to oviposition was performed using 10 replicates per concentration of each treatment, the couples being separated in PVC cages (15 cm diameter by 15 cm high) lined with bond paper and closed at the lower end with cardboard and at the upper end with "tulle" type tissue, secured with elastic. The couples were established with individuals with a maximum of two days of age and fed a 10% solution of honey furnished in coffee cups with cotton. The food was replaced every two days to prevent fermentation. The cages were randomly distributed on shelves in the laboratory; eggs were counted every two days using a stereoscopic microscope until the end of the oviposition period. The experimental design was completely randomized. Eighty third instar larvae were used per concentration, considering each individual (caterpillar, pupa), a repeat. Data were subjected to analysis of variance (ANOVA), with a significance level of 5% probability of error. The means were compared by Tukey test 5% (Sas Institute, 2002).

RESULTS

Lethal concentration curves

Based on the results of the toxicity bioassay there was difference between the treatments in the mortality of *H. armigera*. Spinosad was the product that had greater toxicity, requiring concentrations of only 0.01 and 0.05 L.ha^{-1} to cause mortality of 50 and 95% of subjects, respectively, compared with chlorantraniliprole and *B. thuringiensis*, causing LC_{50} mortality at higher concentrations of 0.04 and 0.22 L.ha^{-1} and LC_{95} at 0.36 and 1.5 L.ha^{-1}, respectively (Table 2).

In assessing the lethal effects, there was no difference between treatments for LC_{25}. The treatments spinosad, chlorantraniliprole and *B. thuringiensis*, showed satisfactory results in concentrations of 0.006; 0.02 and 0.10 L.ha^{-1}, causing mortality of 30, 22 and 20% of the larvae, respectively. For $LC_{50,}$ the best result was obtained for the spinosad treatment that resulted in 36.25% mortality followed by chlorantraniliprole with 25% (Table 3).

Sublethal effects on the development of *H. armigera*

In the evaluation of sublethal effects on pupal weight, there was a difference between the treatments. Treatments that showed the best results were *B. thuringiensis* and spinosad in two concentrations tested, with 168 mg and 169 mg for LC_{25} and for LC_{50} 167 and 173 mg, respectively (Table 4).

For the longevity of pupae, there were differences among the treatments. *B. thuringiensis* caused the greatest longevity in the two concentrations tested, presenting for LC_{25} longevity of 21.21 days and 24.09 days for LC_{50}, an increase of 7 and 10 days vs. control, respectively (Table 4). As for assessing pupal viability, treatment with *B. thuringiensis* differed significantly compared to control. The LC_{25} and LC_{50} treatments with *B. thuringiensis* reduced the viability of pupae by 59.8 and 69.5%, respectively, compared to the control treatment, which showed viability of 97 and 95% (Figure 1).

Sublethal effect on oviposition of *H. armigera*

The evaluation of sublethal effects on oviposition showed significant differences between treatments. For the LC_{25}

Table 3. Percentage mortality of *H. armigera* larvae exposed to LC_{25} and LC_{50} of insecticides.

Treatment	Commercial Name	N[a]	% Mortality	
			LC_{25} ± SEM	LC_{50}± SEM
Chlorantraniliprole	Premio	80	22.00 ± 4.4 [a]	25.00 ± 3.2 [ab]
Spinosad	Tracer	80	30.00 ± 4.2 [a]	36.25 ± 4.6 [a*]
B. thuringiensis	Dipel	80	20.00 ± 3.2 [a]	13.75 ± 4.9 [bc]
Control	-	80	0 ± 0 [B]	0 ± 0 [C]

SEM = standard error of the mean, [a]number of individuals tested, *Means followed by same letter in the column do not differ by Tukey test at 5% error probability.

Table 4. Effect of LC_{25} and LC_{50} on weight and pupal longevity (Mean ± SEM).

Treatment	Weight of Pupae (mg)	Longevity of Pupae (days)	Weight of Pupae (mg)	Longevity of Pupae (days)
	LC_{25}		LC_{50}	
Chlorantraniliprole	252 ± 0.07 [b]	18.23 ± 0.88 [b]	242 ± 0.07 [b]	19.92 ± 2.93 [c]
Spinosad	169 ± 0.07 [a]	18.63 ± 2.78 [b]	173 ± 0.07 [a]	21.21 ± 1.70 [b]
B. thuringiensis	168 ± 0.05 [a]	21.21 ± 1.46 [a]	167 ± 0.06 [a]	24.09 ± 3.78 [a]
Control	308 ± 0.04 [b]	14.11 ± 0.71[c]	321 ± 0.04 [c]	13.97 ± 0.71 [d]

SEM = standard error of the mean. Means followed by same letter in the column do not differ by Tukey test at 5% error probability.

treatment it was *B. thuringiensis* that provided the lowest oviposition, averaging 496 eggs. For LC_{50} oviposition was reduced for all products tested compared with the control (Table 5).

DISCUSSION

Lethal concentration curves

The greatest toxicity was seen with spinosad at the concentrations of 0.006, 0.01 and 0.05 $L.ha^{-1}$ for LC_{25} and LC_{50} and LC_{95}, respectively, showing high toxicity in *H. armigera* (Table 2). The high toxicity of spinosad to *H. armigera* shows the susceptibility of this species to the product due it being the latest in use and having high insecticidal activity. High toxicity of spinosad also was reported by Wang et al. (2009) who studied the toxicity of spinosad in *H. armigera* and obtained an LC_{50} of 0.41 $mg.kg^{-1}$.

For lethal effects spinosad at LC_{50} showed a significant difference compared to the other treatments with 36.25% mortality (Table 3). Yin et al. (2008), studying the sublethal effects of spinosad in *Plutella xylostella* (Lepidoptera: Yponomeutidae), found for LC_{25} (0.12 $mg.L^{-1}$) and LC_{50} (0.28 $mg.L^{-1}$) mortality of 24 and 51% respectively. The difference in percentage mortality of only 32.25% shown in LC_{50} can be explained as a function of the evaluation period. The toxicity evaluation test time was five days, while the mortality assessment was based on product exposure for only two days.

Sublethal effects on the development of *H. armigera*

The treatments presented sublethal effects at the different concentrations tested. Significant effects were found on pupal weight and viability in all treatments. Spinosad and *B. thuringiensis* were the products that most reduced the weight of the pupae (Table 4). For *B. thuringiensis* at LC_{50}, the reduction in weight of the pupae was approximately 48% compared to control. Similar results were found by Lomate and Hivrale (2013), where *B. thuringiensis* inhibited the size and the weight of *H. armigera* by 56 and 54%, respectively. The weight of the pupae found with spinosad at LC_{25} and LC_{50} was 169 and 173 g, representing a decrease of approximately 46%. Yin et al. (2008), working with spinosad at LC_{25} and LC_{50}, found a reduction in the weight of pupae of *P. xylostella* of 381 and 352 mg corresponding to 74 and 69%, respectively.

Reduced pupal weight is a consequence of reduced food intake in the larval period, or of the high metabolic cost required for detoxification which results in low weight of adults. Therefore, these factors can result in reduced fertility and may negatively affect the population growth of the next generation of *H. armigera*.

The longevity of the pupae differed among treatments.

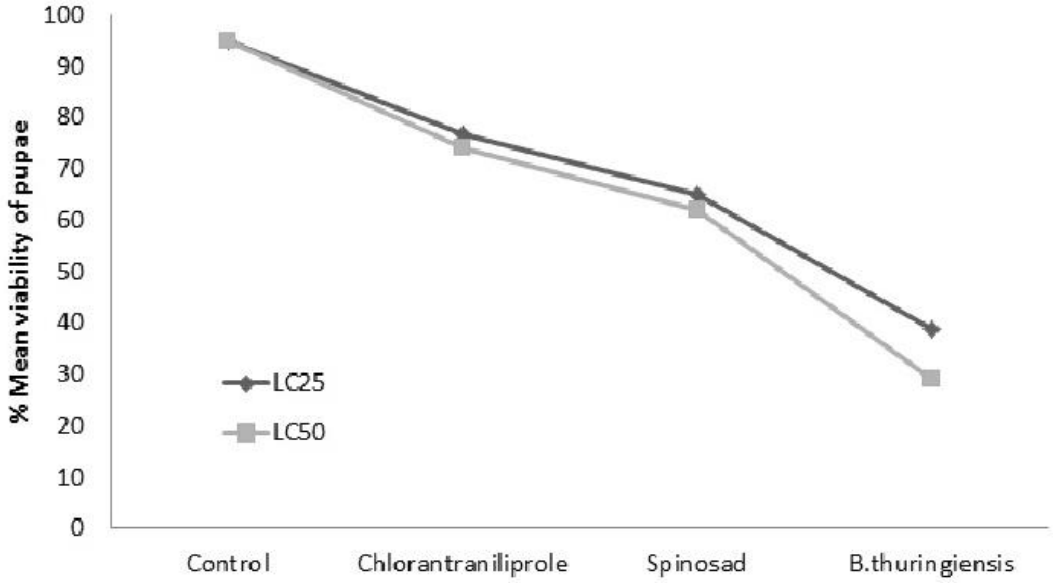

Figure 1. Mean percentage viability of pupae of *H. armigera* in the LC_{25} and LC_{50} treatments.

Table 5. Mean oviposition of *Helicoverpa armigera* exposed to LC_{25} and LC_{50} of different insecticides.

Treatment	Number of eggs	
	LC_{25} ± SEM	**LC_{50} ± SEM**
Chlorantraniliprole	817 ± 56.3[b]	410 ± 43.27 [a]
Spinosad	683 ± 45.3[ab]	398 ± 61.75 [a]
B. thuringiensis	496 ± 39.83[a]	463 ± 40.61 [a]
Control	828 ± 37.2[b]	813 ± 87.13 [b]

SEM= standard error of the mean. Means followed by same letter in the column do not differ by Tukey test at 5% error probability.

For both LC_{25} and LC_{50} the treatment with the greatest longevity was *B. thuringiensis* with an increase of 66 and 58% respectively (Table 4). The increase in longevity of pupae found in treatments is a major factor in the management of *H. armigera*, promoting greater time of exposure of the insect pest to biotic and abiotic factors, attenuating control by natural enemies, mechanical and physical.

A longer development period may imply a longer time of exposure to predators and parasites/parasitoids (Williams, 1999; Thaler et al., 2012.). According to Wang et al. (2009), studying the sublethal effects of spinosad on the survival, development and reproduction of *H. armigera* at a concentration of 0.16 $mg.kg^{-1}$ found an increase of 71% in pupal mortality when compared to the control.

In assessing the viability of pupae, the best results were found for the treatment *B. thuringiensis* for LC_{25} and $LC_{50,}$ with 39 and 29% viability. Despite having shown a low mortality of larvae 13.75 and 20%, respectively. *B. thuringiensis* took longer to cause death than the neurotoxic insecticides spinosad and chlorantraniliprole. This delayed effect is due to the mode of action of the product, since the bacterial spores have to be ingested and only after reaching the mid-intestine and being solubilized, toxins are released that cause the rupture of the tissues, causing death itself. Barbosa et al. (2011), studying the effects of various insecticides on *S. frugiperda,* also observed a slow action of *B. thuringiensis* in relation to chemical products.

Spinosad presented pupal viability of 65 and 62% for the LC_{25} and LC_{50}, respectively. These results are in agreement with results reported by Wang et al. (2009) when third instar larvae of *H. armigera* were exposed to 0.04 $mg.kg^{-1}$ of spinosad, the viability of pupae found was 68.26% and control was 98.97%. Sayed and Sheikh (2014) in studies with *Spodoptera littoralis* (Lepidoptera: Noctuidae) found that the viability of pupae subjected to

LC_{50} (40 µg.m^{-1}) of spinosad was 55%. Information on the toxicity of products is important because it helps in the selection of insecticide, as some products provide longer lasting control, which reduces the number of applications and consequently entails lower costs for pest control and impacts on the environment. Effects of insecticides on the biotic potential of a pest are also relevant, especially when it comes to IPM, which aims to provide more efficient product rotation, decreased resistance and maintenance of populations for longer periods below the damage level of the crop in question, along with other control methods.

Sublethal effects on the oviposition of *H. armigera*

Comparing the effect of the insecticide treatments on oviposition, it was observed that *B. thuringiensis* and spinosad interfere with the potential for oviposition by *H. armigera*. The treatment with *B. thuringiensis* at LC_{25} significantly reduced oviposition by 41% compared to control (Table 4).

For the treatment spinosad at LC_{50} the reduction in oviposition was 51% compared to control. Similar results were found by Storch et al. (2007), studying the effect of spinosad on oviposition in *Anticarsia gemmatalis*, with a reduction in the number of eggs of 44.55%. Yin et al. (2008), studying the sublethal effects of spinosad on the oviposition of *P. xylostella*, showed a reduction of 50.4% in the number of eggs. The reduction in oviposition for chlorantraniliprole at LC_{50} was of approximately 50%. Zhang et al. (2013) found similar results studying the sublethal effects of the LC_{40} of chlorantraniliprole at a concentration of 21.57 µg.L^{-1} in *H. armigera*, obtaining a reduction in oviposition of 56% when the results were compared to control.

The results of lethal and sublethal effects are of fundamental importance, since an insecticide can act directly, causing mortality after application, as well as exerting adverse effects on development and reproduction, impacting the population dynamics of the next generation. Information on the adverse effects of the products serves as a parameter in IPM, enabling better decision-making for *H. armigera* control in the field.

Conclusions

Spinosad showed the greatest toxicity in the control of *H. armigera.* Spinosad and *B. thuringiensis* decreased in all parameters evaluated, causing negative effects on the pupal stage and oviposition.

Spinosad products chlorantraniliprole and *B. thuringiensis* may be used in pest management, as an alternative to product rotation in *H. armigera* control third instar. Further studies should be conducted to confirm these results in the field.

Conflict of Interest

The authors have not declared any conflict of interest.

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Potassium doses for African mahogany plants growth under two hydric conditions

Bruna Aparecida Pereira Perez, Sérgio Valiengo Valeri*, Mara Cristina Pessôa da Cruz and Rodrigo Tenório de Vasconcelos

Department of Vegetable Production, College of Agriculture, São Paulo State University, Prof. Acess Road Paulo Donato Castellane, s/n, Jaboticabal, SP, Brazil.

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African mahogany (*Khaya senegalensis*) is a good option for the growing demand of the furniture industry since it is resistant to *Hypsipyla grandella* Zeller, a borrowing insect which causes damage to plants of the genus *Swietenia* in the American continent. The objective of this paper was to verify the effects of potassium doses on the growth of young African mahogany plants growing under two hydric conditions. The doses consisted of 0, 50, 100, 150, and 200 mg dm^{-3} of K in combination with hydric conditions of 30 and 70% of the soil maximum water holding capacity in pot, the treatment replications being distributed according with a completely random design and a 5 × 2 factorial scheme with 5 replications. At 180 days, the plants showed higher efficiency of potassium use, stomata conductance, transpiration, photosynthesis, height, diameter, leaf area, root dry matter, stem, and leaves at 70% of moisture. At the moisture of 30%, the potassium doses increased leaf area and leaf dry matter. Maximum water use efficiency was attained at 71 mg dm^{-3} of K. At 70% of soil moisture, increasing doses of K caused the reduction in N concentration in the leaves and a concomitant increment in phosphorus and potassium concentrations. Young African mahogany plants do not demand high doses of K as long as the other mineral necessities are met. Under hydric restriction, potassium increases the efficiency of water use and leaf biomass.

Key words: *Khaya senegalensis,* argisol, hydric restriction, potassium fertilization, photosynthesis.

INTRODUCTION

Khaya senegalensis A.Juss. (African mahogany), a member of the Meliaceae family, is of natural occurrence in Africa, between latitudes of 15 and 18°N, along a line parallel to the equator including mainly Senegal, South Sudan, and the northern regions of Cameroon and Uganda (Lamprecht, 1990). It is found in river and swamp forests and dispersed through high pluvial precipitation forest savannas. Its habitat may be found between altitudes of 0 and 1800 m and rainfall precipitation between 700 and 1750 mm/year. It is the highest drought resistant species of the *Khaya* genus. Humid areas, though, favor its growth. It is the tallest among the savanna trees as well as the most valued noble wood since it is hard dense and long lasting (Orwa et al., 2009).

*Corresponding author. E-mail: sergio.valeri1@gmail.com. Tel: +55(16)992075633.

African mahogany is a good option for the growing demand of the furniture industry since it is resistant to *Hypsipyla grandella* Zeller, a borrowing insect which causes damage to plants of the genus *Swietenia* in the American continent (Pinheiro et al., 2011).

Nowadays, studying growth and development of plants under conditions of restricted water availability has become very important since conclusions from several research works referring to climatic changes in the world suggest that dry periods will increase in intensity, frequency, and duration (Misson et al., 2010). Under those conditions, the species, variety and source options as well as studies concerning physiology and nutrition of plants are of fundamental importance.

Swietenia macrophylla King in Hook. plants, under conditions of water deficiency, showed good control of transpiration and stomata conductance, in addition to having recovered leaf turgor after 12 h, showing a satisfactory tolerance to drought (Cordeiro et al., 2009). *Khaya ivorensis* A.Chev. also showed tolerance to moderate hydric deficiency since, 14 days after irrigation suppression, it recovered its hydric status three days after rehydration and, a little later, the gaseous exchanges (Albuquerque et al., 2013).

Potassium may influence the plant responses to water deficiency. It is related to the osmotic potential of plants since it influences cell expansion and ion transport in addition to being fundamental for the stomata movement. Potassium well-nourished plants use the soil water more efficiently whereas the ones with potassium deficiency have a poorer photosynthetic performance due to the irregular opening of the stomata which causes reduction in the amount of CO_2 taken in (Prado, 2008).

It was verified in *Eucalyptus grandis* plantlets (going through their hardening period) growing under hydric stress, that fertilizing them with potassium brought about a reduction in the amount of water lost by the plant (Silva et al., 2004).

So, the objective of this paper was to evaluate the effect of doses of potassium on the growth of young African mahogany plants growing under two hydric conditions.

MATERIALS AND METHODS

Description of the experimental environment

The experiment took place between August, 2013 and March, 2014. It was carried out in a 30 m long, 4.5 m high, and 5 m wide greenhouse which was covered by a screen capable of intercepting up to 30% of the sun light.

Seed provenance

Seeds of *K. senegalensis* were provided by the Vasconcelos Florestal company, which is found in the municipality of Monte Alto, state of São Paulo, Brazil. The origin of seeds is the Indian settlement of Tiakane, Burkina Faso (Africa), localized at 11° 11' 10'' of N latitude and 1° 12' 14'' of W longitude. Burkina Faso climate is tropical semi-arid with high temperatures with a mean annual temperature of 30°C and potential annual evapotranspiration of approximately 2000 mm. A long dry period (from October to May) and a short rainy one (from July to September) are the most important climatic characteristics of that region (Couteron and Kokou, 1997).

Experiment installation

The plants grew in black polyethylene 6.5 dm^3 vases covered with newspaper to reduce excessive warming by solar radiation. These vases had their bottom covered with a 0.05 mm mesh to reduce soil losses.

A total of 5.5 dm^3 of an Argisol was placed in each vase. Each soil sample was taken from a 20 cm of topsoil in the "São Gabriel" farm, in the municipality of Monte Alto (21° 17' 16'' S and 48° 28' 33'' W), state of São Paulo, Brazil, where, formerly, there were pastures. The soil granulometric analyses showed the following values: sandy texture with 8.6% of clay, 4.9% of silt, 30% of fine sand, and 56.5% of coarse sand. Soil fertility analyses, following Raij et al. (2001) procedures, were of P resin: 2 mg dm^{-3}; OM: 10 g dm^{-3}; pH ($CaCl_2$): 4.7; K^+: 1.0 mmolc dm^{-3}; Ca^{2+}: 9.0 mmolc dm^{-3}; Mg^{2+}: 5 $mmol_c$ dm^{-3}; H + Al: 28 $mmol_c$ dm^{-3}; CEC at the pH of 7: 43 $mmol_c$ dm^{-3} and basis saturation (V): 35%.

Taking into consideration the soil chemical analysis results, calcium carbonate and calcium magnesium were applied following the Ca:Mg proportion of 3 : 1 so as to raise the soil base saturation (V) up to 50%.

Making use of the simple superphosphate in its powder form, 80 mg dm^{-3} of P were applied to the soil and after that the soil was moistened so as to reach 70% of its total water retention capacity. Such condition was kept during the 30 days period of incubation (time required for soil reaction with lime and fertilizers applied). Fifteen days later, the following fertilization doses were applied to each vase: 25 mg dm^{-3} of N (ammonium sulfate), 0.25 mg dm^{-3} of B (boric acid), 0.5 mg dm^{-3} of Zn (zinc sulfate), and 0.5 mg dm^{-3} of Cu (copper sulfate). The following N (alternating the source of N between urea and ammonium sulfate) doses were applied on the soil surface: 25 mg dm^{-3} at 45 days after sowing, 25 mg dm^{-3} at 75 days, 50 mg dm^{-3} at 110 days, and 50 mg dm^{-3} at 130 days, totalizing the application of 225 mg dm^{-3} of N.

Experimental design

The treatment replications were distributed according to a randomized complete block design with five potassium doses (0, 50, 100, 150, and 200 mg dm^{-3} of K_2O) and two levels of water availability: 30 and 70% of the soil total water holding capacity. These treatment combinations resulted in a 5 × 2 factorial scheme with five replications. Each replication consisted of one vase bearing two plants, totaling 100 plants in the experiment.

Conduct and evaluation of the experiment

The potassium to the plants was provided by potassium chloride (60% of K_2O). The potassium dose was split in three times; the first one took place 30 days after period of incubation (20% of the treatment potassium concentration), the second, 60 days after incubation (40% of the treatment potassium concentration), and the third 120 days after incubation (40% of the treatment potassium concentration). Water to the soil was supplied so that the levels of 30 and 70% of the total soil retention capacity were reached.

Soil maximum water retention capacity was determined by weighing 10 vases with mesh in the base with 5.5 dm^3 of soil in

each one. The vases with dry soil were weighed and, after that, water was added so as to soak the soil. After a period of 48 h of drainage, the vases were again weighed. The soil maximum water retention capacity was considered to be the difference between the mass after drainage and the mass of the dry soil.

Four untreated seeds were sown per vase. The vases were covered with a screen which reduced to 50% the sun light reaching the soil surface during seed germination; moisture level in the soil was maintained for 35 days at 70%. At the end of that period, only two plants per vase were left. Starting 40 days after sowing, the procedures to bring about soil moisture variation begun.

In order to guarantee the levels of 30 and 70% of soil moisture during the experiment, the vases were daily weighed and the difference in weight from one day to the next was considered to be the needed amount of irrigation water.

At 180 days after germination, the concentrations of nitrogen, phosphorus, and potassium were measured in the plant leaves. The potassium use efficiency in the plant leaves was also determined. Nitrogen determination made use of the semi-micro Kjeldahl method after sulfuric digestion while the nitro-perchloride method was used to measure P and K contents. Phosphorus and potassium were measured by the metavanadate colorimetry and flame photometry methods, respectively, according to the methodology described by Bataglia et al. (1983).

The nitrogen, phosphorus and potassium contents in the leaves were calculated by multiplying the leaf dry matter amounts by the respective nutrient concentrations. These values were used to calculate the potassium use efficiency (PUE) in the leaves, according to the equation proposed by Siddiqi and Glass (1981). PUE = (leaves total dry matter)^2/potassium concentration (g^2 g^{-1}) in the leaves.

Physiological processes such as stomata conductance, transpiration, and photosynthesis at 135, 142, 152, and 180 days after germination were evaluated. These evaluations were performed between 8 and 11 a.m. in totally expanded leaves in the median part of the third whorl of the composite leaf of a branch of the third verticil with the help of an IRGA (Infra Red Gas Analyzer) model LCpro-SD, a product of ADC BioScientific. Room temperature and relative humidity were measured; CO_2 concentration was fixed at 380 ± 10 µmo m^{-2} s^{-1}. FFFA (photosynthetically active photon flux) of 800 µmol m^{-2} s^{-1}, corresponding to the maximum photosynthesis value found in a preliminary test with 6 plants, was used.

Each variable was represented by the mean value of the four performed evaluations.

Based on the mean photosynthesis and stomata conductance results, water use efficiency (WUE) was calculated: WUE = photosynthesis/stomata conductance.

At 180 days of germination, the chlorophyll index was evaluated with a portable chlorophyllimeter (CCM 200, Opti Science) and evaluations of survival, height, diameter, leaf area, root, stem and leaves dry matter mass, root/aerial part dry mass ratio were made.

Plant aerial part height was considered to be the length between the stem basis in contact with the soil and the apex of the youngest leaf pair. Diameter was measured 5 cm above soil surface.

Leaf area was measured with equipment named Li-Cor 3100. The leaves, the stem, branches and roots were separately placed inside bags and put to drying in a forced ventilation oven at 65 to 70°C untill a constant weight was reached.

Statistical analysis

The data were statistically analyzed with the AgroEstat program (Barbosa and Maldonado, 2011). Analysis of variance and polynomial regression analysis were made for the effects of potassium on measured variables.

RESULTS AND DISCUSSION

African mahogany plants survival was of 100% in all treatments. Higher concentrations of nitrogen (41.67%) and potassium (21.17%) and lower concentration of phosphorus (6.7%) were detected in leaves when soil moisture content was of 30% in comparison with 70% (Table 1), this is probably due to the dilution effect since under the latter condition, the physiological characteristics of stomata conductance, transpirations and photosynthesis were higher (Table 1) with a consequent increase in biometric characteristics (Table 1).

When potassium dose was increased at soil moisture of 70%, a tendency to decrease nitrogen concentration in the leaves was observed, and that variation could be adjusted by a third degree equation ($\hat{Y} = 20.25 - 0.0149x - 0.0006x^2 + 0.000003x^3$; $R^2 = 1$; $p = 0.0352$). This result confirms data found in a study of the interaction between nitrogen, phosphorus and potassium in other species when the highest doses of potassium resulted in the highest production and the highest concentrations of nitrogen were found in the plants that received the lowest doses of potassium (Macleod, 1969).

The data showed that phosphorus foliar concentration was influenced by potassium only at soil moisture of 30% and that the largest dose of potassium resulted in a linear increment in the concentration of phosphorus in the leaves ($\hat{Y} = 1.46 + 0.002x$; $R^2 = 0.8778$; $p = 0.007$).

As to the foliar concentration of potassium, it was linearly increased with potassium doses when soil moisture was of 30% ($\hat{Y} = 9.236 + 0.017x$; $R^2 = 0.7505$; $p = 0.0004$) and at 70% ($\hat{Y} = 7.376 + 0.0164x$; $R^2 = 0.7409$; $p = 0.0005$).

When soil moisture was 70%, potassium was used with efficiency 38.8% higher than that observed when soil moisture was of 30%. Applying potassium to the soil decreased soil efficiency of potassium dose at that soil moisture, the variation being adjusted by a third degree curve ($\hat{Y} = 3425.2 - 27.409x + 0.3811x^2 - 0.0014x^3$; $R^2 = 0.9996$; $p = 0.0153$). This result confirms data found for *Corymbia citriodora* (Hook.) K.D. Hill and L.A.S. Johnson also subjected to different potassium doses and soil moisture levels, when a decreasing linear effect in the efficiency of potassium use by the leave was observed. This fact was supposed to be due to the conservation of the leaves dry matter as the concentration of potassium increased (Sá et al., 2014).

When soil moisture was of 30%, stomata conductance (45.5%), transpiration (35.6%) and photosynthesis rate (32.8%) were lower than at 70% (Figure 1). *Eucalyptus* clones under four irrigation regimes also showed reduction in stomata conductance and photosynthesis rate when water availability was reduced, probably as a consequence of the partial shutting of the stomata soon after the first morning hours (Tatagiba et al., 2007). When under conditions of low water availability, plants activate several mechanisms to avoid dehydration such as the

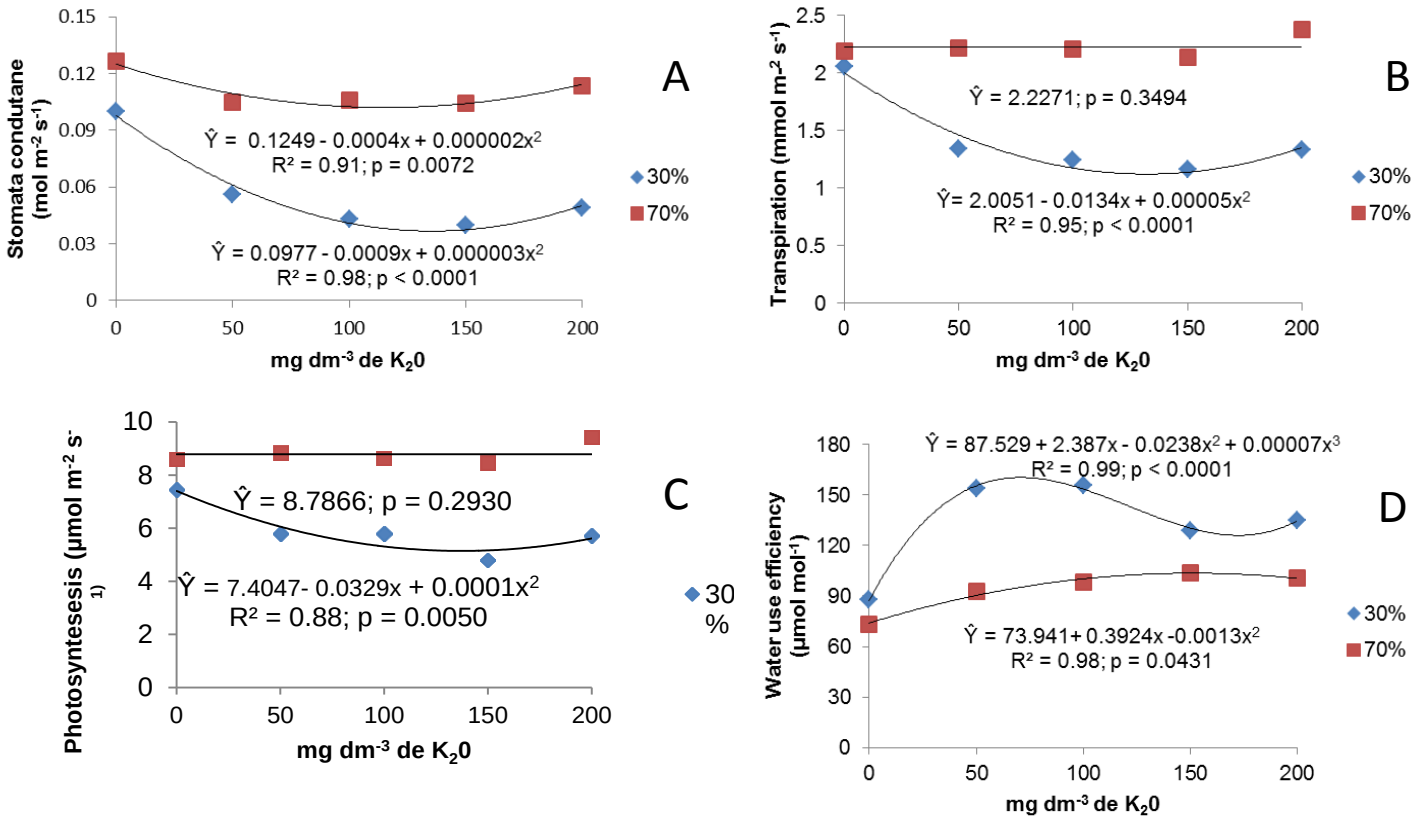

Figure 1. Variations of stomata conductance (A), transpiration (B), photosynthesis (C) and water use efficiency (D) African mahogany seedlings at 180 days of age, subjected to two hydric conditions (30 and 70% of the soil maximum water retention capacity in pot) and five potassium doses (0, 50, 100, 150 and 200 mg dm^{-3}).

reduction in transpiration. This response is achieved by the shutting of the stomata, which limits water vapor loss by reducing stomata pore although the providing of CO_2 to the chloroplasts is also part of the mechanism (Taiz and Zeiger, 2013). The reduction in stomata conductance is, nonetheless, higher than that verified for carbon assimilation and this brings about an increment in the efficiency of water use by the plants. If the low availability of water persists for a long period, photosynthesis rate decreases and, as a consequence, plant growth is reduced (Lima et al., 2003).

Increasing doses of potassium, when soil moisture content was of 30%, caused reductions in stomata conductance, transpiration and photosynthesis rate whereas when soil moisture content was of 70%, only reduction in stomata conductance was observed; these variations being explained by second degree equations (Figure 1), probably due to the participation of potassium in the regulation of the opening and shutting of the stomata, thus reducing the excessive loss of water by the plant under hydric stress (Taiz and Zeiger, 2013). This favorable effect of potassium was observed in *Eucalyptus grandis* W.Hill plantlets under moderate hydric stress promoting reduction of water loss by the plant (Silva et al., 2004).

When soil moisture content was of 30%, the plants showed higher efficiency in the use of water compared to 70% (Table 1). This result may be due to the reduction in stomata conductance being higher than that verified in carbon assimilation when soil moisture was of 30% which increases the efficiency of the instantaneous use of water (Pita et al., 2005). These results are concordant with those reported for six *Eucalyptus* species which were subjected to hydric stress (20% of the soil water holding capacity) for 10 weeks and this resulted in more severe reduction in stomata conductance than in photosynthesis rate (Merchant et al., 2007). Plants of *Eucalyptus* genotypes growing in soils treated with potassium made a more efficient use of water than plants growing in conditions of no restriction of water (Mendes et al., 2013).

Water use efficiency increased with the application of potassium both at soil moisture contents of 70 and 30%. The variation verified when soil moisture was of 70% is explained by a second degree equation and the maximum efficiency is estimated with the application of151 mg dm^{-3} of K. When soil moisture content was of30%, the variation is explained by a third degree equation and the highest efficiency was estimated by the

Table 1. Means, F values of analysis of variance and coefficient of variation (CV%) of the evaluated characteristics African mahogany seedlings at 180 days of age, subjected to two hydric conditions (30 and 70% of the soil maximum water retention capacity in pot) and five doses of potassium (0, 50, 100, 150 and 200 mg dm^{-3}).

Sources of variation	Characteristics													
	N	P	K	PUE	gs	E	A	WUE_{inst}	Height (cm)	Diameter (mm)	Leaf area (cm^2)	Dry matter (g)		
												Root	Stem	Leaves
Means														
Humidity (H)														
30%	22.78[a]	1.66[a]	10.93[a]	1829[b]	0.06[b]	1.43[b]	5.91[b]	132.37[a]	49.51[b]	8.62[b]	2307[b]	39.10[b]	11.71[b]	19.60[b]
70%	16.08[b]	1.78[a]	9.02[b]	2990[a]	0.11 [a]	2.22[a]	8.79[a]	93.68[b]	57.57[a]	11.11[a]	2757[a]	62.07[a]	20.85[a]	26.01[a]
K (mg dm^{-3})														
0	21.26	1.71	8.05	2596	0.11	2.12	8.02	80.49	50.46	9.21	2163	52.29	14.58	19.72
50	20.63	1.69	10.09	2354	0.08	1.78	7.31	123.4	57.00	10.16	2641	53.64	17.60	22.76
100	18.88	1.62	9.37	2528	0.07	1.72	7.20	126.94	49.18	9.57	2444	51.21	14.93	22.86
150	18.26	1.87	10.21	2527	0.07	1.65	6.63	116.38	57.28	10.19	2703	47.04	17.81	24.27
200	18.10	1.73	12.17	2041	0.08	1.85	7.57	117.93	53.87	10.21	2707	48.76	16.49	24.43
Significance F - test														
H	335.1**	2.7 [ns]	19.2**	51.7**	223.7**	163.9**	144.8**	110.5**	14.7**	49.1**	11.2**	19.3**	47.1**	30.0**
K	12.3**	1.3 [ns]	9.4 [ns]	1.5[ns]	17.4**	6.8**	3.7*	20.6**	2.5 [ns]	1.3 [ns]	2.4 [ns]	0.2 [ns]	1.0 [ns]	2.1 [ns]
H x K	17.1**	3.2 [ns]	0.4[ns]	1.7[ns]	4.2**	7.2**	3.8*	6.1**	0.2 [ns]	0.4 [ns]	0.8[ns]	0.2 [ns]	0.8 [ns]	0.9 [ns]
CV%	6.7	14.6	15.5	23.7	15.0	12.1	11.5	11.5	13.9	12.7	18.9	36.5	28.9	18.1

[a,b]Means, in the same column, followed by the same small case letter, are not significantly different by the Tukey test ($p > 0.05$); ns = non-significant; * and ** = Significant differences, respectively, at $p < 0.05$ and $p < 0.01$ for the F test. *gs*, stomata conductance (mol m^{-2} s^{-1}) *E*, transpiration (mmol m^{-2} s^{-1}); *A*, photosynthesis (µmol m^{-2} s^{-1}); WUE_{inst} , instantaneous water use efficiency (µmol mol^{-1}); N, foliar nitrogen concentration (g kg^{-1}); P, foliar phosphorus concentration (g kg^{-1}); K, foliar potassium concentration (g kg^{-1}); PUE, potassium use efficiency (g g^{-1}).

application of 70.7 mg dm^{-3} of K (Figure 1).

Providing the plant with the adequate dose of potassium is of fundamental importance for the efficiency of water use since this nutrient is associated with characteristics that interfere in the efficiency of water use such as turgor regulation, opening and shutting of the stomata and transpiration (Taiz and Zeiger, 2013). Similar results were reported for *Corymbia citriodora* and *Eucalyptus camaldulensis* Dehnh. plants growing under water stress and fertilized with different doses of potassium. The highest doses resulted in an increased water use efficiency and in the leaf relative water content (Teixeira et al., 1995).

Increasing potassium dose, when soil moisture content was of 70%, did not affect significantly plant height, diameter, leaf area and root, stem, and leaves dry matter of the plants (Table 1).

Potassium concentration in plant leaves which did not receive potassium fertilization was of 8.05 g kg^{-1}, this being a value close to 9.0 g kg^{-1}, which is considered adequate for African mahogany (Bevege et al., 2006). This suggests that the content of 1.0 $mmol_c$ dm^{-3} of K present in the soil before the experiment was started was sufficient for the initial phase of plant growth.

S. macrophylla (Brazilian mahogany) plantlets growth was not affected by potassium fertilization either, thus suggesting that this species is a K low-demanding one or that it converts efficiently potassium in biomass (Tucci et al., 2011). Omitting K from sowing fertilization of *Copaifera langsdorffii* (Rashed Tree or Salam Tree) and *Hymenea courbaril* (courbaril) did not interfere in plant height, diameter and aerial part dry matter. The Rashed Tree plants showed a tendency to increased leaf biomass when they did not receive potassium (Duboc, 1994).

This kind of behavior is likely to be common in

species of tropical forests since Rashed Tree, *Platycianus regnellii* ('pau-pereira', in Brazil), and courbaril did not respond to fertilization with potassium, probably because they are slow-growing plants compared to fast-growing species, which do respond to potassium (Silva et al., 1997). So, at a soil moisture content of 70%, the absence of significant responses to potassium by the plants seems to be related to their low demand of that nutrient at their initial growth stages.

When growing in a soil moisture content of 70%, the plants were taller, had a larger diameter, a larger leaf area and plant components dry matter than those growing at 30% (Table 1). Differences in percentage between plant components the ones of plants growing at 70 and 30% were of 43.8% (stem dry matter), 37% (root dry matter), 24.6% (leaf dry matter), 22.4% (diameter), 16.3% (leaf area), and 14% (height). Similar results were reported for eucalyptus genotypes plants which, under various irrigation regimes, grew more than the non-irrigated ones (Mendes, 2011).

Under conditions of hydric stress, plants undergo important modifications in physiological and morphological processes which affect their capacity to withstand environmental adversities. Under such conditions, cell volume is reduced and, consequently, turgor pressure. Thus, activities depending on turgor pressure and cell expansion such as root growth in length and cell expansion are extremely sensitive to hydric stress (Taiz and Zeiger, 2013). Water deficiency may lead to plant growth paralysis or just to a reduction in plant growth (Vellini, 2007). On the other hand, leaf area reduction may be seen as a plant strategy to withstand drought since leaf area reduction leads to a transpiration reduction (Taiz and Zeiger, 2013).

Significant effects of potassium doses on leaf area were observed only when soil moisture content was of 30%. The variations in leaf area were verified to occur according to the linear equation ($\hat{Y} = 1976.2 + 3.309x$; $R^2 = 0.5889$; $p = 0.0184$). The increment in leaf dry matter determined by increasing potassium doses, when soil moisture was of 30%, is described by the linear equation $\hat{Y} = 16.72 + 0.0288x$; $R^2 = 0.675$; $p = 0.0187$.

Similar results were reported for eucalyptus clones growing under two hydric conditions and potassium doses in which only leaf area and leaf dry matter were significantly affected by potassium doses (Mendes et al., 2013). On the other hand, studies on the effects of water management and potassium fertilization on the behavior of eucalyptus plants at the rustification phase, showed no effect of potassium on the aerial part of the plant; only root dry matter was significantly influenced. Therefore, it is suggested more studies.

Conclusions

K. senegalensis plants, at their initial stage of development, demand low amounts of potassium as long the other nutritional elements are provided. Under restricted water conditions, potassium increases the efficiency of water use and leaf biomass.

Conflict of Interests

The authors have not declared any conflict of interests

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Cuscuta campestris yunker, a dangerous obligate parasitic weed observed in cassava crop in the eastern part of the Democratic Republic of Congo

Mushagalusa N. G.[1.2], Achiza B. J.[1], Bisuri B. K.[1], Sinza C. B[2]., Bigirimwami C. L.[1] and Lubobo A. K.[3]

[1]Faculté des Sciences Agronomiqueset de l'Environnement, Université Evangélique en Afrique, 3323 Bukavu, DR Congo.
[2]Institut Supérieur de Développement Rural (ISDR/Bukavu), DR Congo.
[3]CIAT, Harvest-Plus, Bukavu DR Congo.

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***Cuscuta campestris* (field dodder) is an obligate shoot parasite weed causing growth inhibition and yields losses of many agricultural crops worldwide. *C. campestris* is often observed in many crops; among is cassava in the eastern part of the Democratic Republic of Congo. In 2014, subsistence farmers realised that it may be responsible for yield reduction of many crops. This study evaluates the effect of *C. campestris* on the yield of a bio-fortified cassava. Six quadrats were installed in an existing 2 ha cassava field. In each quadrat, cassava plants infected by *C. campestris* were marked while in the same quadrat all other non-infected plants were controls. Yield parameters of 40 infected plants were compared to parameters from 43 uninfected plants. At the harvest time, the number of tubers by plant, fresh weight of tubers, tuber diameter, tuber length, and tuber dry weight were measured. Treatment means were separated using the least significant difference test at 5% probability level and the relative yield loss per cassava plant was estimated. Tuber diameter, tuber length and tuber dry weights were significantly reduced in *C. campestris* infested cassava as compared to controls (pV<0.001). *C. campestris* caused substantial estimated yield losses of 48% per plant. The number of tubers was not reduced (pV=0.281), likely because *C. campestris* appeared six months after the planting date, when the tubers number was already set.**

Key words: *Cuscuta campestris*, cassava, yield, yield loss, field dodder, Democratic Republic of Congo.

INTRODUCTION

Cuscuta campestris Yunker (field dodder), a member of the Convolvulaceae, is an annual obligate stem parasite causing growth inhibition and yield losses in various crops worldwide (Holm et al., 1997; Mishra et al., 2006). The *C. campestris* radicle has no apical meristems and degenerates a few days after germination (Sherman et

*Corresponding author. E-mail: nachigera@yahoo.fr, nachigera@uea.ac.cd.

al., 2008). It is an above ground; obligate parasites that must make contact with a susceptible host in order to survive. Once the weed seedling makes contact with a host, it develops haustoria and connects to the host's vascular bundles, forming a highly efficient absorption system that enables the parasite to divert resources from the host (Dawson et al., 1994; Costea and Stefanovic´, 2009; Vaughn, 2003).

C. campestris is not only an aggressive weed, but can additionally serve as a vector for pathogens, such as Cucumber Mosaic Virus and African Cassava Mosaic Virus (Wisler and Norris, 2005). Current control methods include separation of dodder seed from crop seed, pre-emergent herbicides, and mechanically burning or pulling off dodder from host plants (Wisler and Norris, 2005).

In March 2015 *C. campestris* was observed in Cassava crop at the Kashushatrial station of the Université Evangélique en Afrique where bio-fortified cassava was grown by the International Center for Tropical agriculture (CIAT).

Cassava is the main crop in the Democratic Republic of Congo used as food by the majority of the population (FAO, 2001). Lack of soil fertility and diseases such as African Cassava Mosaic Virus and Bacterial Blight are the greatest production challenges in this region. Cassava is a very hardy crop that can grow reasonably well in areas of infertile or very acid soils, under conditions of low or unpredictable rainfall, and with minimum care and inputs. For that reason, it is the preferred crop by subsistence farmers in this region, who often live in isolated areas with poor soils and sometimes mountainous topography. Cassava yields can be substantially reduced by weed competition (Ekeleme et al., 2003; Howeler, 2014). In Africa, weeds are responsible of 17% of Cassava yield loss and their effects are equivalent to yield loss caused by Bacterial Blight (Howeler, 2014).

In the DRC, Cassava is usually planted in the middle of September and weeded by hand (hoe) two to three times during the first 3 to 4 months. The time Cassava takes to mature depends on varieties and varying from 10 to 24 months. Manual weeding can become insufficient if the cultivated area is bigger. *C. campestris* is challenging to control because it climbs and wraps tightly on the plants, causing breakage of shoots during hand weeding (Melifonwu et al., 2000). It can regrow from stem fragments, so even when it weeded, fragments of dodder can be disseminated in the field and develop new plants if they are attached to the cassava stem (Dawson et al., 1994). Small farmers believe that dodder does not have roots then they believe it is a dangerous specie because it is very difficult to be managed and physical removal of that weed by hand is time-consuming. The weed continues growing after the destruction of basal organs. Also, dodder is cited as a factor disseminating many virus (Roos and Aldrich, 1988), therefore it may contribute to the African Cassava Mosaic Disease dissemination, the most important factor limiting cassava yields in many parts of Africa (Fauquet and Forget, 1990). This study evaluates the effect of the dodder on cassava yield at the Université Evangélique en Afrique' trial station following observations made from farmers' fields.

MATERIALS AND METHODS

An experiment was carried out at Kashusha, the trial station of the Université Évangélique en Afrique located at 02°19'0.02" latitude and 28°47'45,9" longitude and an altitude of 1712 m where a bio-fortified cassava crop variety 01/1661 was planted on September 28, 2014. *C. campestris* was observed in the cassava crop six months after planting date and the assessment of the effect of that weed on the cassava yield undertaken immediately in March 2016.

Considering the distribution of the weed in the field, an area of 2 ha was selected. Six quadrats (2×2 m) were installed using a completely random design. A plastic sheet fixed on cassava stem was used for quadrat demarcation. In each quadrat, cassava plants infected by weed were marked using a coloured plastic bandage while in the same quadrat all other non-infected plants were used as controls. Yield parameters of 40 infected plants were compared to parameters from 43 uninfected plants.

In April 2015, *C. Campestris* plants died and stopped competing with cassava, however, bandages on oldest infected cassava plants were kept as they were clearly identified at the harvest period, which was made on first October, 2015.

At the harvest period, the number of tubers by plant, the fresh weight of tubers, diameter and tuber length were determined. To evaluate the tuber dry matter, samples were oven-dried at 70°C for 96 h until constant weight, and weighed.

All data were subjected to analyses of variance (ANOVA) using GENSTAT 13 statistical package. Treatment means were separated using the least significant difference (LSD) test at 5% probability level. The relative yield loss (YL) per cassava plant due to *C. campestris* infestation was estimated using the equation $YL(\%)=(1-Y_{CW}/Y_{CM})\times100$. Where Y_{CW} and Y_{CM} are crop yields per plant in competition with weeds and in weed-free conditions respectively (Mahajan et al., 2014).

RESULTS

Dodder weed significantly reduced tuber diameter ($p<0.001$), tuber length ($p=0.046$) and tuber dry weight of cassava ($p<0.001$). The number of tubers was not affected.

Tuber diameter decreased from 5.9 cm to 5.1 cm when cassava crop was infected by dodder (Figure 1a; Table 1). The length tuber changed from 17.7 to 15.6 cm because of parasitism effect from the weed (Figure 1b). Finally, the dry weight of each tuber was reduced by 39% leading to an average yield loss of 47% for infected plants (Figure 2).

Tuber dry weight per plant decreased from 922.8 to 483.86 $g.plant^{-1}$ when cassava was infected by *C. campestris*. The coefficient of variation seems to be higher because parameters were taken for each tuber and by plant. Usually there is a big difference between tubers on the same plant, and parameters like diameter and tuber length can change from a tuber to another. This variation should disappear or decrease when the

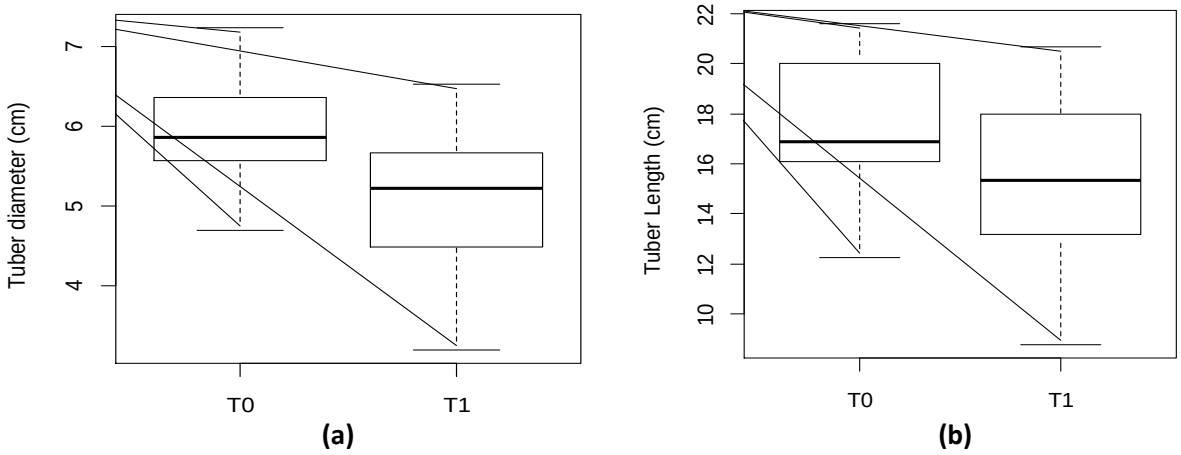

Figure 1. Tuber diameter (a) and Tuber length (b) for uninfected (T0) and infected (T1) cassava plants by *C. campestris*.

Table 1. Effect of field dodder on cassava tuber yield.

Parameter	Uninfected plants	Infected plants	LSD	CV	F-test	P-value
Tuber diameter (cm)	5.899±0.61[a]	5.088±0.90[b]	0.448	14.33	13.26	<0.001
Number of tubers	4.734±1.39[a]	4.224±2.00[a]	0.943	36.94	1.19	0.281
Tuber fresh weight (g)	299.49±94.25[a]	230.29±124.12[b]	62.580	41.45	4.96	0.031
Tuber dry weight (g)	199.86±59.4[a]	121.62±65.63[b]	36.059	39.36	19.1	<0.001
Tuber Length (cm)	17.677±2.63[a]	15.635±3.21[b]	2.006	21.14	4.2	0.046
Tuber fresh weight per plant (g)	1381.6±392.8[a]	916.8±309.4[b]	321.9	49.14	8.46	0.005
Tuber dry weight per plant (g)	922.88±209.5[a]	483.86±251.4[b]	191.707	47.82	21.27	<0.001

Means followed by the same letter within a line are not different at P=0.05 level.

yield per hectare is considered. Also, because the weed infestation was made naturally the density of *Cuscuta* attached to cassava was very different from one plant to another leading to the high variation of effects on the yield.

DISCUSSION

Field dodder is a problematic weed in tropical areas that can substantially reduce the yields of many crops (Holm et al., 1997; Mishra et al., 2006). In this study, cassava yield was reduced by 47% for cassava infected by *C. campestris* six months after the planting date. Peasant farmers usually stop weeding cassava following canopy closure, as the crop canopy suppresses most weeds. Because field doddergets most of its energy from its host rather than through photosynthesis, cassava leaves do not affect the development of dodder, which can thrive under the cassava canopy, causing substantial yield losses.

The effect of *C. campestris* on cassava yield can become more important when the infection begins before the number of root tubers is set. Usually the most weed sensitive stage of Cassava occurs during the third month after planting date, when root tuberisation takes place (Melifonwu, 2000). At that period, some weeds like grasses dominate Cassava crops at the above and the below-ground levels, causing reductions of 50 to 90% yield (Chikoye et al., 2001; Melifonwu, 1994). Different from field dodder, grasses are sensitive to shade; they die when shaded during a long period and they may or not reduce cassava yield if they appear 6 months after cassava planting date.

Effective control of dodder is extremely difficult to achieve since dodder seeds can remain viable in soil for 10 to 30 years or more and continue to germinate and emerge throughout the warm seasons, depending on the crop species and environmental conditions (Mishra et al., 2006). Lanini (2004) found that growing wheat (*Triticum aestivum* L.) followed by corn (*Zea mays* L.) in a field heavily infected by Lespedeza dodder (*C. pentagona* Engelm.) reduced the number of dodder plants infesting tomato by 90%. Thus, control measures that reduce seed

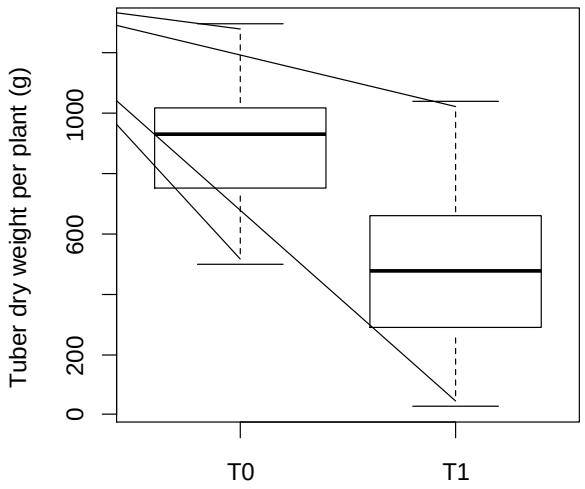

Figure 2. Tuber dry weight per plant for uninfected (T0) and infected (T1) cassava plants by *C. campestris*.

production, can reduce infestations in subsequent years. Once dodder germinates and attaches to a host, the nature of attachment and association between host and parasite excludes mechanical control and requires a highly selective herbicide to destroy the parasite without crop damage (Fer, 1984). In South-Kivu province, Eastern D.R. Congo, small farmers do not have access to herbicide, so it is likely that dodder control will remain a major problem in the region. Field crops are hand weeded and usually farmers destroy only the belowground party of the weed while the aboveground continue growing on the stem and branches (Melifonwu et al., 2000). Several different methods for dodder control in crops were reviewed by Parker and Riches (1993) and they suggest intercropping system as a control method. In South-Kivu cassava is intercropped with many other crops such as legumes (common bean, soya bean), maize and banana. Unfortunately those crops are also potential field dodder hosts.

To control that weed, herbicide like glyphosate are usually recommended, unfortunately *C. campestris* can be resistant to this chemistry product (Nadler-Hassar et al., 2009), depending on environmental conditions. In contrast to Nadler-Hasser et al. (2009) results, Cook et al. (2009) found dodder to be Gylphosate-sentitive when it was applied at a dose of 140 g/ha in a Citrus field crop.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Productivity and economics of medicinal rice Njavara (*Oryza sativa* L.) as influenced by different establishment techniques in lowland condition of Kerala, India

S. Rani[1]* and P. Sukumari[2]

[1]Department of Agronomy, TNAU, Coimbatore, Kerala, India.
[2]College of Agriculture, Vellayani, Trivandrum, Kerala, India.

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Field experiments were conducted at Cropping Systems Research Centre, Karamana, Thiruvananthapuram, Kerala during summer/third crop/puncha season in 2007 and 2008 to find out the effect of nutrient sources under different management systems. The experiment was laid out in split plot design with four replicates. The treatments consisted of four management systems, *viz*., System of Rice Intensification SRI (M_1), Integrated Crop Establishment Method (ICM) (M_2), Package of Practices (PoP) (M_3) of Kerala Agricultural University and Conventional Management Practices (M_4) in main plot. Nutrient sources, such as organic sources (S_1), integrated nutrient sources (S_2) and inorganic sources (S_3) were the sub plot treatments. Conventional management practices (M_4) recorded higher values of growth (height of plants, number of leaves hill^{-1}, leaf area index (LAI) and number of tillers per unit area) and yield attributes (No. of productive tillers m^{-2}, grain and straw yield). Sources of nutrients did not influence growth characters in Njavara. Among the nutrient sources, higher number of grains panicle^{-1}, filled grains panicle^{-1} and crop yield (grain and straw) were recorded under integrated nutrient source than organic and inorganic sources. The maximum net return (30,562 Rs ha^{-1}) and B:C ratio of 2.40 were also noted under the conventional management practices with integrated nutrient source.

Key words: Rice establishment techniques, economics, productivity, Njavara rice.

INTRODUCTION

In India, rice area increased from 36.46 million hectares in 1960s to 42.86 million hectares in 2010 to 2011, production from 39.31 million tonnes in 1964-1965 to 95.98 million tonnes in 2010-2011 and productivity increased from 1078 to 2239 kg ha^{-1} during the same period (CMIE, 2012 Cultivation of specialty rices like medicinal rice which fetches substantially higher prices is more profitable (Swaminathan, 2004).

Njavara (shashtika in Sanskrit), a rice (*Oryza sativa* L.) landrace described in ancient Sanskrit treatises of

*Corresponding author. E-mail: malarrani@rediffmail.com.

Ayurveda for its nutritive and medicinal properties, is traditionally used in Kerala for Ayurveda treatments (Kumar et al., 2010). Njavara is widely used for internal consumption as an efficient health food as well as for external application under Panchakarma treatment. Ayurvedic physicians use Njavara for a wide range of ailments including rheumatism, arthritis, cerebral palsy, muscular dystrophy, blood pressure and also for the relaxation and rejuvenation of weak muscles in aged persons. At present, Njavara cultivation is confined to some pockets mainly in the Northern part of Kerala. No other medicinal rice is used in the world as widely as Njavara is used in Ayurveda (Thomas et al., 2006). Production is not sufficient even to meet the indigenous demand. At present, Njavara is exported in a very small extend. Its importance as a health food offers opportunity to establish niche global market (Balachandran et al., 2006). Based on glume color differences, two types of Njavara are recognized, black and golden yellow glumed types. Seed color of the black glumed variety is red and highly resistant to drought conditions. It matures in about 60 to 90 days and reaches about more than 1 m height (Menon, 1996). Under the conventional management system this short duration landrace is low tillering and low yielding. Increase in the yield of Njavara, even in small increments, is of great relevance in the present rice production scenario. Keeping this in view, the present study was undertaken to study the response of medicinal rice Njavara to nutrient sources under management systems like System of Rice Intensification (SRI), Integrated Crop Establishment Method (ICM), Package of Practices (PoP) of KAU and Conventional Management Practices.

MATERIALS AND METHODS

Site and soil description

Field experiments were conducted for two consecutive years, that is, summer/third crop/puncha season of 2007 and 2008 at Cropping Systems Research Centre, Karamana, Thiruvananthapuram, Kerala. The soil was acidic in reaction (5.5 pH), high in organic carbon content (1.23%), medium in available nitrogen (261.9 kg ha^{-1}), available phosphorus (22.0 kg ha^{-1}) and available potassium (140.2 kg ha^{-1}) status.

Experimental treatments and design

The experiment was laid out in a split plot design with three replicates. The establishment techniques in main plot were SRI (M_1), ICM (M_2), PoP (M_3) (Recommendation of Kerala Agricultural University) as well as the Conventional Management Practices (M_4) while nutrient sources including, organic sources (S_1), integrated nutrient sources (S_2), and inorganic sources (S_3) were used in sub plot.

Crop husbandry

In SRI treatments, single, eight day old seedlings were transplanted at 20 × 20 cm spacing. Four weedings with rotary weeder were given at 10 days interval starting from 10 days after transplanting (DAT) to panicle initiation (45 days). Soil was kept at field capacity up to panicle initiation and then onwards 5 cm standing water was allowed in the field till 10 days before harvesting. In ICM treatments, two seedlings of 12 days age were transplanted at 20 × 20 cm spacing. Two rotary weedings on 10 and 20 DAT were given followed by one hand weeding on 35 DAT. A thin film of water was kept in the field till 10 days before harvesting. In PoP treatments, three seedlings of 18 days age were transplanted at 15 × 10 cm spacing. Weed control was achieved through one manual weeding on 15 DAT and application of 2, 4-D at 1 kg a.i ha^{-1} on 25 DAT. Water level was maintained at about 1.5 cm during transplanting. Thereafter, it was increased gradually to about 5 cm, intermittent draining and reflooding was done. Field was drained 10 days before harvesting. In conventional management practices pre germinated seeds were broadcasted at 80 kg ha^{-1}. A thin film of water was maintained up to 10 DAS after which water level was raised up to 5 cm and field was drained 10 days before harvest. Two manual weedings were done at 15 and 35 DAS.

Data recording

Plant height

Plant height was recorded at panicle initiation and at physiological maturity (at physiological maturity the crop will have maximum dry matter). After physiological maturity, senescence starts and lose of dry matter occurs, using the method described by Gomez (1972). Height was measured from the base of the plant to the tip of the longest leaf or tip of the longest ear head, whichever was longer and the average of five plants was recorded in centimetres.

Leaf number $hill^{-1}$

Leaf count was taken from the five tagged observation hills at panicle initiation and physiological maturity and mean was expressed as number of leaves $hill^{-1}$.

Leaf area index (LAI)

LAI was computed at panicle initiation and physiological maturity, using the method described by Gomez (1972). The maximum width 'w' and length 'l' of all the leaves of the middle tillers of six sample hills were recorded and LAI was calculated using the relationship.

Leaf area of a single leaf = $l \times w \times k$
where k is the adjustment factor (0.75 at maximum tillering, panicle initiation and flowering and 0.67 at harvest stage).

$$LAI = \frac{\text{Sum of leaf area of 6 sample hills } (cm^2)}{\text{Area of land covered by the 6 sample hills } (cm^2)}$$

Number of tillers m^{-2}

Number of tillers m^{-2} was recorded at weekly interval during the period between two to seven weeks of transplanting/sowing (maximum tillering) and at physiological maturity.

Grains $panicle^{-1}$

Grains from five panicles collected randomly from the net plot were counted and the mean value was expressed as the number of

Table 1. Interaction effect of establishment techniques and nutrient sources on yield attributes of Njavara rice (mean over two years).

Interaction effects (Establishment techniques/Nutrient sources)	Grains panicle^{-1}	Filled grains panicle^{-1}	Grain yield (kg ha^{-1})	Straw yield (kg ha^{-1})
M_1S_1(SRI + Organic)	60.05	55.56	842.90	1244.00
M_1S_2 (SRI + INM)	58.68	54.23	833.85	1224.00
M_1S_3 (SRI + Inorganic)	116.82	53.96	816.75	1198.50
M_2S_1 (ICM + Organic)	109.40	47.74	788.05	1167.50
M_2S_2 (ICM+ INM)	114.17	52.88	760.60	1116.00
M_2S_3 (ICM+ Inorganic)	101.35	45.74	732.60	1078.00
M_3S_1 (PoP + Organic)	104.03	44.40	854.25	1257.50
M_3S_2 (PoP + INM)	98.06	44.67	905.85	1390.50
M_3S_3 (PoP + Inorganic)	96.74	44.88	935.25	1345.00
M_4S_1 (CP + Organic)	93.44	41.62	974.80	1431.50
M_4S_2 (CP + INM)	95.81	43.44	1197.95	2251.00
M_4S_3 (CP + Inorganic)	90.81	41.43	947.00	1388.50
SEd	47.61	1.02	18.32	28.11
CD (0.05)	47.24	2.11	44.60	58.02

grains per panicle.

***Number of filled grains panicle*-1**

The central panicle from each sample hill was threshed separately and the numbers of filled and unfilled grains were recorded.

Grain and straw yield

The net plot area was harvested individually, threshed, winnowed, dried, weighed and expressed in kg ha^{-1}. Straw harvested from each net plot was dried in sun and the weight was expressed in kg ha^{-1}.

Economics of cultivation

The economics of cultivation was worked out based on the costs of the various inputs and produce at the time of experimentation. Total cost of cultivation of the treatments was a sum of basic cost of cultivation, cost involved for management systems and cost involved for nutrient sources.

Net income

Net income was computed using the formula:

Net income (Rs. ha^{-1}) = Gross income - Total expenditure

Benefit cost ratio

Benefit cost ratio was computed using the formula:

BCR = Gross income/Total expenditure

Statistical analysis

Data recorded during the field investigation were statistically analyzed with split plot design method as suggested by Gomez and Gomez (1984) for test of significance. Wherever, the treatment differences were found significant, the critical differences were worked out to 5% probability level and the values furnished.

RESULTS AND DISCUSSION

Effect of management systems on growth

Growth parameters like height of plants, number of leaves hill^{-1}, leaf area index (LAI) and number of tillers per unit area were significantly influenced by management systems. Tallest plants were produced in conventional management practices (110.67 cm) which was 13.7% higher than ICM (95.51 cm). Earlier reports were also available on the tendency of broadcast crop to grow taller and accumulate more dry matter compared to transplanted rice (Rathore et al., 1995). Maximum number of leaves hill^{-1} were produced in conventional management practices (25.06) and it was 12% higher than the number of leaves produced in SRI (22.03). Increase in plant height and tiller number in conventional management practices might have contributed to a corresponding increase in the number of leaves (Table 1). Similar result was reported by Thomas (2000). Conventional management practices had significantly higher LAI than all other management systems. LAI in PoP was significantly higher than ICM and SRI. In the early stages of crop growth, tiller production was more in PoP than in conventional management practices. But after 3 weeks of transplanting/sowing, tiller production in conventional management practices increased at a faster rate than in PoP and this trend continued till maximum tillering stage. Tiller count per unit area at physiological

Table 2. Effect of establishment techniques and nutrient sources on growth and yield attributes of Njavara rice (mean over two years).

Treatment	Plant height (cm)	No. of leaves hill^{-1}	LAI	No. of tillers m^{-2}	Grains panicle^{-1}	Filled grains panicle^{-1}	Grain Yield (kg ha^{-1})	Straw yield (kg ha^{-1})
Establishment techniques								
M_1 (SRI)	100.83	22.03	0.82	95.7	58.93	54.58	831.2	1222.5
M_2 (ICM)	95.51	22.98	0.7	146.7	53.12	48.78	760.4	1120.5
M_3 (PoP)	103.87	24.15	1.085	589.4	48.67	44.60	898.5	1331.0
M_4 (CP)	110.67	25.06	1.47	978.9	46.39	42.16	1039.9	1691.0
SEd	1.95	0.41	0.30	3.09	0.56	0.54	9.28	15.55
CD (0.05)	4.52	0.94	0.69	6.99	1.27	1.21	21.14	35.18
Nutrient sources								
S_1 (Organic)	103.02	23.74	1.07	452.7	51.59	47.33	865.0	1275.5
S_2(Integrated)	103.37	23.79	1.05	451.7	53.08	48.80	924.6	1495.0
S_3(Inorganic)	101.76	23.14	0.9	453.3	50.67	46.50	857.4	1252.0
SEd	1.58	0.37	0.24	3.06	0.49	0.45	9.155	24.57
CD (0.05)	NS	NS	NS	NS	1.07	0.93	18.90	29.00

maturity also was the highest in conventional management practices.

Effect of management systems and nutrient source on yield

SRI with organic source (M_1S_1) registered the maximum grains per panicle which was on par with SRI with integrated nutrient source (M_1S_2) and SRI with inorganic source (M_1S_3). Bozorgi et al. (2011) also reported that high plant density resulted in higher percentage of unfilled grains. Wang et al. (2002) observed lower sterility percentage with single seedling per hill than with two seedlings hill^{-1}. Among the interactions, conventional management practices with integrated nutrient source (M_4S_2) recorded the highest grain yield (1197.95 kg ha^{-1}) (Table 1), which was significantly superior to all other interactions. Significantly, higher grain yield realized in conventional management practices may be attributed to the significantly better growth and yield parameters realized in this treatment compared to other management systems. Straw yield also showed the same trend like grain yield in management system, nutrient sources and its interactions.

Higher number of grains and filled grains per panicle were recorded in SRI (M_1) which was followed by ICM, PoP and conventional management practices, respectively. Maximum grain yield recorded in conventional management practices (M_4) (1039.9 kg ha^{-1}) was 13.5% higher than PoP, 20% higher than in SRI and 27% higher than ICM (Table 2). Significantly higher grain yield realized in conventional management practices may be attributed to the significantly better growth and yield parameters realized in this treatment compared to other management systems. Significantly higher growth characters like leaf number per plant, leaf area index, flag leaf area, tiller number per unit area and leaf area duration might have resulted in increased photosynthesis and production of photosynthates which finally transformed into significantly higher number of panicles per unit area and higher relative accumulation of dry matter in the panicles. Among the nutrient sources, integrated nutrient source (S_2) recorded the maximum grains per panicle and filled grains per panicle. It was significantly higher than organic source and inorganic source having the least value. The highest grain yield (924.6 kg ha $^{-1}$) was obtained in integrated nutrient source (S_2) which was followed by organic source (865.0 kg ha^{-1}) and inorganic source (857.4 kg ha^{-1}), respectively.

Economics

Conventional management practices with integrated nutrient source (M_4S_2) recorded the highest gross income (52,423 Rs ha^{-1}), net income (30,562 Rs ha^{-1}) and BCR (2.40) (Table 3). The lowest gross income was recorded in ICM, inorganic source and their interactions (M_2S_3) (Table 3). The combination of wider spacing and intermittent irrigation in SRI provides an ideal environment for weed growth, leading to need for more frequent weeding. The cost of production was higher under SRI practice. SRI required more labour than conventional management practices. Particularly, SRI

Table 3. Effect of establishment techniques, nutrient sources and their interactions on gross income, net income and benefit cost ratio (mean over two years).

Treatment	Total cost of cultivation (Rs ha^{-1})	Gross income (Rs ha^{-1})	Net income (Rs ha^{-1})	BCR
Management systems				
M_1(SRI)	31,774	35,692	3918	1.13
M_2(ICM)	27,689	32,658	4969	1.19
M_3(PoP)	26,659	38,600	11941	1.47
M_4(C.P)	25,925	44,979	19053	1.76
SEd	-	403.880	403.880	0.015
CD (0.05)	-	913.661	913.661	0.034
Organic sources				
S_1(organic)	31,719	37,151	5431	1.18
S_2(integrated)	26,538	39,974	13436	1.53
S_3(inorganic)	25,777	36,821	11044	1.45
SEd	-	393.864	393.864	0.014
CD (0.05)	-	812.898	812.898	0.028
Interaction effects				
M_1S_1(SRI + Organic)	35,246	36,205	959	1.03
M_1S_2 (SRI + INM)	30,486	35,802	5,316	1.17
M_1S_3 (SRI + Inorganic)	29,590	35,068	5,478	1.18
M_2S_1 (ICM + Organic)	31,411	33,858	2,447	1.08
M_2S_2 (ICM+ INM)	26,651	32,657	6,006	1.23
M_2S_3 (ICM+ Inorganic)	25,755	31,461	5,706	1.22
M_3S_1 (PoP + Organic)	31,561	36,684	5,123	1.16
M_3S_2 (PoP + INM)	26,801	39,016	12,215	1.45
M_3S_3 (PoP + Inorganic)	25,905	40,100	14,195	1.55
M_4S_1 (CP + Organic)	27,221	41,857	14,636	1.54
M_4S_2 (CP + INM)	21,861	52,423	30,562	2.40
M_4S_3 (CP + Inorganic)	20,965	40,658	19,693	1.94
SEd	-	787.729	787.728	0.027
CD (0.05)	-	1625.796	1625.796	0.057

required 40% more labor for nursery management, transplanting and kono weeding than conventional practices. Some studies have shown higher labor requirements of more than 25% in SRI practice (Latif et al., 2004; Thakur et al., 2009).

Conclusion

Njavara rice responded to establishment techniques and nutrient sources. Crop growth and yield were the highest in conventional practices followed by PoP, SRI and ICM. Among the nutrient sources, integrated nutrient source was the best followed by organic and inorganic, with respect to crop yield and economics. Sources of nutrients did not influence growth characters in Njavara. Even though Njavara did not respond to SRI and ICM to the same extent in tillering like other varieties, all the tillers produced in SRI were productive tillers and 91% of the tillers produced in ICM bore panicles. It is observed that in transplanted crops (SRI, ICM and PoP), number of productive tillers per hill increased with increase in number of seedlings per hill. Similar results were reported earlier by Obulamma and Reddy (2002). Conventional management practices coupled with integrated nutrient source can be considered as the ideal crop production package for Njavara rice in lowlands.

Conflict of Interests

The authors have not declared any conflict of interests.

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