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Biological control of *Striga hermonthica* (Del.) Benth. using formulated mycoherbicides under Sudan field conditions

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Dedication

To the soul of my father and sisters: Khalda and Magda

To all who contribute to the success of this work

Table of content

1 Introduction

1.1 Sorghum as an important food crop

Sorghum (*Sorghum bicolor* (L.) Moench, Poaceae) is an important food crop in Africa, South Asia and Central America. It is the fifth major cereal crop in the world after maize (*Zea mays* L.), wheat (*Triticum vulgare* L.), rice (*Oryza sativa* L.), and barley (*Hordeum vulgare* L.). Worldwide, the area under sorghum is estimated to about 44 million hectares in 99 countries in Africa, Asia, Oceania, and the Americas. Sorghum main producers (in million metric tons) are: USA (11.5), Nigeria (8.0), India (7.5), Mexico (6.3), China (3.1) and Sudan (2.6) (FAO, 2006).

Sorghum is the second most important cereal crop after maize in Sub-Saharan Africa (Haussmann *et al*., 2000). It is the main staple food for about 300 million people who live in the semi-arid tropics (Chantereau and Nicou, 1994).

In many parts of the world sorghum is traditionally being used in food production and various food items are made from this versatile cereal: kisra, injera, porridge, unleavened bread, cookies, cakes, couscous, and malted beverages. Sorghum grains are also major components of feed in livestock and poultry production (Doggett, 1988). Sorghum stover is an important source of dry season maintenance rations for livestock especially in India and Sudan (Doggett, 1988). In many African countries sorghum stems are used for fencing, construction of temporary buildings in the villages, as well as an important source of energy for cooking.

In developed countries sorghum is mainly used for animal feed. Recent work has shown that sorghum and millet (*Pennisetum glaucum* (L.) R. Br) are rich in antioxidants and gluten-free, which make them an attractive alternative for wheat allergy sufferers (Dahlbert *et al*., 2004).

In Sudan, sorghum is the most important cereal crop in terms of production and consumption (Ibrahim *et al,* 1995). It is cultivated all over the country, either under rainfed or under supplementary irrigation. Despite the crop's importance and the long experience in its cultivation, sorghum yield is very low (0.4 t/ha, FAO, 2006) compared to its potential. The low productivity can be attributed mainly to the use of traditional low-yielding varieties, limited or no use of fertilizers, and poor management practices (Ibrahim *et al.*, 1995).

1.2 *Striga* problem

Witchweeds (*Striga* spp.), root-parasitic plants belonging to the family Orobanchaceae (Olmstead *et al*., 2001), are considered the most serious biotic factor that threatens cereal (sorghum, maize, pearl millet and rice) production in the rainfed agriculture of the semi-arid tropics (SAT) including Sudan (Doggett, 1988; Obilana and Ramaiah, 1992, Parker and Richer, 1993). About 21 million hectares of the cereal production area in Africa are estimated to be infested by *Striga*, causing an annual grain loss of about 4.1 million tons (Sauerborn, 1991). Losses in grain yield due to *Striga* infestation vary from 5 to 75%, depending on the level of infestation, susceptibility of the crop, climatic conditions and nature of the soil (Lagoke *et al*. 1991; Sallè *et al.* 1987). Grain yield losses can reach 100% in susceptible cultivars under a high infestation level and drought conditions (Haussmann *et al*., 2000).

In Sudan, more than 500,000 hectares under rainfed cultivation are heavily infested with *Striga*, which commonly results in significant yield losses of 70-100% (Babiker, 2002). Severe infestation by *Striga* may force farmers to shift to less economic crops such as millet, to abandon the land when infestation is too heavy (Sallè *et al*. 1987) or even to migrate from their location to other locations (Obilana and Ramaiah, 1992). *Striga hermonthica* (Del.) Benth. and *S. asiatica* (L.) Kuntze are the major biotic constrains to crop production, especially in the non fertile semi-arid region of Africa, whereas *S*. *aspera* (Willd.) Benth. and *S. forbesii* Benth. are of lower economic importance (Haussmann *et al*., 2000). *S. hermonthica* is the most serious biotic problem to cereal production, it attacks sorghum, maize, pearl millet and rice (Abbasher *et al.* 1998).

Striga is completely dependent on the host for its survival, and its life cycle is closely linked with that of the host plant (Haussmann *et al*., 2000). After maturity, *Striga* seeds remain dormant for several months (after ripening), which may be an evolutionary adaptation to prevent germination during the last rains of the season, or when there are no hosts present (Berner *et al.*, 1997). After this period, *Striga* seeds germinate only after being exposed to favorable moisture and temperature conditions for several days (preconditioning) in the presence of a stimulant, which is usually secreted by the host's roots and some non-host plants. Following germination, the radicles grow towards the host roots, perhaps in response to the concentration gradient of the germination stimulant (Dubé and Olivier, 2001). Attachment to the host root, formation of a special physiological bridge (called the haustorium), and the

establishment of a xylem connection is also initiated and guided by host-derived secondary metabolites (Bouwmeester *et al*., 2003). Once connected, the parasite withdraws water, mineral nutrients, carbohydrates and aminoacides, consequently causing stunted shoot growth, leaf chlorosis and reduced photosynthesis in the host (Ejeta and Butler, 2000; Frost *et al*., 1997). After several weeks of underground development the *Striga* shoots emerge above the soil surface and start to flower and produce an extremely high number of seeds (up to 100,000 seeds/plant) that can remain viable for as long as 20 years (Doggett, 1988; Kroschel and Müller-Stöver, 2004).

This complex biology limited the development of successful control methods that can be accepted and practiced by the subsistence farmers. Nevertheless, several control measures for *Striga* have been developed including cultural, physical, mechanical and chemical methods, in addition to the development of resistant and tolerant host plant varieties. These control measures have been well reviewed by Parker and Riches (1993), Hess and Haussmann (1999) and Elzein and Kroschel (2003). Ogborn (1984) observed that in Africa and Asia, where *Striga* spp. are endemic, reinfestation from wild hosts may make it very difficult to eradicate the weed. It has become obvious that there is no simple, fast and inexpensive solution to the *Striga* problem in Africa. Therefore, the use of integrated control methods and the development of new techniques are required to avoid the expansion of witchweed and to reduce its impact in the infected area.

Biological control is considered as a potential cost-effective and environmentally safe means for reducing weed populations in crops, forests, or rangelands where low profit margins prevent large herbicide expenditure (TeBeest *et al*., 1992; Charudattan, 2001).

Fusarium species appear as effective antagonists for root parasitic plants (Bedi and Donchev, 1991; Abbasher and Sauerborn, 1992; Kroschel *et al.,* 1996; Berner *et al.*, 2003; Kroschel and Müller-Stöver, 2004; Sauerborn *et al*., 2007). All developmental stages of the parasite can be infected by *Fusarium* species, from the seed up to the flowering stage. This makes *Fusarium* species very effective in destructing the soil seed bank and preventing seed reproduction. Therefore Biological control measures using fungi (especially *Fusarium* species) could be integrated with other non-costly control methods to achieve an affordable control of *Striga* that can be adopted by the subsistence farmers.

1.3 Objectives of the Study

Biological control using microorganisms showed a high efficacy in controlling *S. hermonthica* under controlled and field conditions (Abbasher and Sauerborn 1992; Abbasher 1994; Citola *et al*., 1996; Sauerborn *et al*. 1996b; Mohamed 2002; Marley and Shebayan, 2005). However, so far it did not come to practical field application. This could be attributed to a lack of efficacy under field conditions due to environmental obstacles or due to the lack of appropriate delivery systems (Baker and Henis, 1990; Rhodes, 1993).

The pathogenicity of *Fusarium nygamai* and *F*. Abuharaz isolate against *Striga* was studied using infected sorghum grains or a spore suspension as inoculum (Abbasher, 1994; Abbasher and Sauerborn, 1992; Mohamed, 2002; Sauerborn *et al*., 1996b). These formulations were very effective in controlling *Striga* under controlled and natural conditions, however, a high level of fungal inoculum (approximately 800 kg ha^{-1} for the grain inoculum) would be required for effective control, which arises a lot of problems, e.g. concerning sterilization and transportation. Such problems can be overcome by adopting an appropriate formulation technology.

Granular formulations such as “Pesta” (Connick *et al*., 1991) and alginate pellets (Walker and Connick, 1983) were found to be suitable delivery systems for controlling soilborne pathogens. “Pesta” granules are made by encapsulating bioagents in a gluten matrix (Connick *et al.,* 1991).

Alginate formulations are prepared by incorporating the biocontrol agent’s propagules in a sodium alginate solution, which is dripped to a calcium chloride or calcium gluconate solution. Alginate pellets are then formed due to a mechanism known as ionotrophic gelation (Connick, 1988).

These formulations can be used pre-planting or post emergence and could provide protection against environmental extremes. “Pesta” formulations showed a promising level of *Striga* control under controlled conditions (Elzein, 2003). The simplicity and cost-effectiveness of this technology made testing its suitability under field conditions seem worthwhile. Therefore the main objectives of this study were to:

- Study the efficacy of formulated *Fusarium* species originating from Sudan (*Fusarium nygamai* and *F*. Abuharaz isolate) in controlling *Striga* under field conditions using “Pesta” and alginate granules as formulations and Evaluate their effects on sorghum yield.

- Determine the optimum dose of the granules to be used.
- Study the persistence of the formulated fungi in the soil
- Study the efficacy of a seed treatment as an alternative delivery system for controlling *Striga* under field conditions.
- Identify potential toxins produced by *Fusarium* Abuharaz isolate or translocated to harvested sorghum seeds after mycoherbicides application for environmental safety.

2 Literature review

2.1 Biological control of weeds

Biological control is generally defined as the deliberate use of living organisms to suppress, reduce, or eradicate a pest population (Boyetchko, 1999). The objective of weed biological control is not the eradication of weeds but the reduction and establishment of a weed population to a level below the economic threshold (Rajni and Mukerji, 2000). Means of biological control of weeds comprise herbivorous insects, microorganisms (especially fungi), and smother plants (Sauerborn and Kroschel, 1996). Biological control is considered as a potential cost-effective, safe and environmentally beneficial alternative mean of reducing weed populations in crops, forests or rangelands (TeBeest *et al*., 1992; Charudattan, 2001).

The biological control of weeds gained attention and momentum since the 1970s (Charudattan and Dinoor, 2000). This is because the intensive use of chemical herbicides came under scrutiny due to several areas of concern, which include the development of herbicide resistant or tolerant weeds and environmental contaminations, comprehending effects on non-target organisms as well as the pollution of soil, underground water and food. Strong public criticism due to health concerns arose from such contaminations (Boyette *et al*., 1991a; Jobidon, 1991; Auld and Morin, 1995; Green *et al.,* 1998). These limitations of chemical herbicides encouraged researchers to look for alternative systems of weed control. Ideally such systems would control the target weeds with identical or nearly identical efficacy as chemical herbicides and would at the same time be safe to the environment and to non-target organisms (Boyette *et al.,* 1991a).

There are two broad approaches for biological weed control: 1) the classical or inoculative approach which involves the importation and release of one or more natural enemies that attack the target weed in its native range into areas where the weed is introduced and became a problem because of the absence of its natural enemies. The most successful example of this approach when using plant pathogens as bioagents is the control of rush skeleton weed (*Chondrilla juncea*) in Australia by the introduction of *Puccinia chondrillina* from Eurasia (Watson, 1991). 2) The inundative biological control or bioherbicide approach which involves the periodical application of the (mostly) native agent in a high concentration to control the target weed in a manner similar to a chemical herbicide. When the bioagent is a fungus this

is known as mycoherbicide approach (Daniel *et al.*, 1973; Hasan and Ayres, 1990; Boyette *et al*. 1991a; Charudattan, 1991).

The classical approach differs from the inundative or bioherbicidal approach primarily in its ecological rather than technological response to a weed problem (Watson, 1991). In the classic approach the control of the target host is dependent upon self-perpetuation and natural dispersal of the bioagent (Templeton *et al*., 1979). This is why the bioherbicidal approach works faster than the classical approach due to the avoidance of the lag period for inoculum build-up and pathogen distribution (Charudattan, 1991).

Since 1980, eight bioherbicides have been registered worldwide, and at least 15 new introductions of classical biocontrol agents (one nematode species (*Subanguina picridis*) and 14 fungal species belonging to 9 different genera) have occurred (Charudattan, 2001). The first bioherbicide registered was Devine® which contains chlamydospores of *Phytophthora palmivora* for the control of strangler vine (*Morrenia odorata*) in citrus in Florida. It results in 90-100% control of strangler vine with just one application and the control lasts for 2 years following the treatment (Kenny, 1986). Collego®, the second registered bioherbicide (in 1981), bases on the fungus *Colletotrichum gloeosporioides* f. sp. *aeschynomene* to control northern jointvetch (*Aeschynomene virginica*), a leguminous weed in rice and soybean (*Glycine max* (L.) Merr.) crops in USA. In nearly two decades of combined experimental and commercial use Collego yielded 90-100% weed control without an apparent adverse environmental impact (Charudattan, 2000). BioMal was the third registered mycoherbicide in Canada, containing *Colletotrichum gloeosporioides* f. sp*. malva* against round-leaved mallow (*Malva pusilla*) in several crops (Makowski, 1992; Mortensen, 1988). Due to technical difficulties in production and economical considerations it has not been commercially produced since its registration, however, a different formulation of Biomal is currently under development under the commercial name Mallet WP and is effective against round–leaved mallow and small flowered mallows (*Malva* spp., Charudattan, 2001). Dr. BioSedge®, based on the rust fungus *Puccinia canaliculata,* was registered in 1993 for the control of yellow and purple nutsedge (*Cyperus esculentus* and *C. rotundus*) in the United States (Charudattan, 2001). The dissemination of a small amount of the uredospores (5mg/ha) in the spring and early summer can lead to severe epidemics (Phatak *et al*., 1987), however, the obligate parasitism of the fungus is the main constraint towards

its commercialization (Charudattan, 2001). CAMPERICO®, a bacterial bioherbicide (*Xanthomonas campestris* pv. *poae*) was developed and registered in Japan to control annual bluegrass (*Poa annua*) (Imaizumi *et al*., 1997). BioChon®, a natural decay-promoter and stump-treatment product based on *Chondrostereum purpureum,* a wound-invading basidiomycete, has been approved to prevent resprouting of black cherry (*Prunus serotina*) and to control this weedy, broad-leaved tree in conifer forests in the Netherlands (de Jong *et al*., 1990; Morris *et al*., 1999). A strain of this fungus (HQ1) was registered in Canada in 2002 under the name of Myco-Tech™ Paste to inhibit regrowth on cut stumps of deciduous tree species, in right's-of-way and conifer release management situations (Shamoun, 2003). In 2004, Mycologic Inc. received temporary registration of a different strain (PFC2139) of the same fungal species for use throughout all forest regions in Canada and the United States (Bailey, 2004). Hakatak®, a granular mycoherbicide based on *Colletotrichum gloeosporioides* (Penz.) Sacc. was registered in 1990 in South Africa for the control of *Hakea sericea*. Stumpout®, another mycoherbicide developed in South Africa, contains a local isolate of *Cylindrobasidium laeve* (Pers.: Fr.) Chamuris to prevent the resprouting of cut stumps of *Acacia mearnsii* and *A. pycnantha* Benth. (Zimmermann and Olckers, 2003).

Many other prospective candidates, although successful in research trials, failed to gain registration and commercialization due to one or more of the following factors: 1) lack of acceptable level of efficacy; 2) technical difficulties in production and marketing; 3) competition from chemical herbicides; 4) unprofitable market; 5) regulatory requirements and 6) environmental constraints (Charudattan; 1991; Auld *et al.*, 2003; Ghosheh, 2005).

2.2 Achievements on biological control of *Striga*

Parasitic weeds including *Striga* are an ideal target for biological control since they are very aggressive with regional occurrence and cannot be easily controlled by chemical herbicides. Biological control using herbivores and fungal antagonists has received considerable attention in recent years and appears to be a promising additional tool for integrated weed management (Abbasher *et al*., 1999; Elzein and Kroschel, 2003).

2.2.1 Biological control using insects

Many insects, indigenous to India and Africa, have been reported to attack *Striga* spp. The genus of greatest interest concerning biological control is *Smicronyx* of which several species are highly specific to *Striga* while others are specific to dodder (*Cuscuta* spp.). Other potentially important species are *Ophiomyia strigalis* (an agromyzid fly) in Africa and *Eulocastra argentisspara* and *E. undulata* (moths) in India (Parker and Riches, 1993). The insects that attack *Striga* can be classified according to their damage as defoliators such as *Junonia* spp., gall forming as *Smicronyx* spp., shoot borers as *Apanteles* sp., miners as *Ophiomyia strigalis*, inflorescence feeders as *Stenoptilodes taprobanes* and fruit feeders as *Eulocastra spp*. (Bashir, 1987; Kroschel *et al*., 1999).

The first attempt to adopt classical biological control for *Striga* was accomplished in 1974 in Ethiopia. *Smicronyx albovariegatus* and *Eulocastra argentisspara* were imported from India and released at Humera at the Setit River close to the border of Sudan. A second release of *S. albovariegatus* occurred in 1978, however, the establishment of the insects in their new habitat was not confirmed for political unrests in the region (Parker and Riches, 1993; Kroschel *et al.*, 1999). An effort has been made in Sudan to re-distribute *S. umbrinus* from one locality to another by introducing the adult insects from Kadogli in western Sudan to Shambat area in central Sudan, but it did not succeed (Bashir, 1987).

In the 1990s, studies in Burkina Faso and Northern Ghana have been carried-out by Kroschel *et al*. (1995), Jost *et al.* (1996) and Traoré *et al.* (1996) to investigate the potential of the weevils *S. umbrinus* and *S. guineanus* and the butterfly *Junonia orithya* as biocontrol agents for *Striga.* As a result of *Smicronyx* infestation the *Striga* seed production was reduced by 17.4 % on the average (Kroschel *et al*., 1999). However, the potentiality of *S. umbrinus* as biocontrol agent for *Striga* has been evaluated by Smith *et al*. in 1993, utilizing a population model of *S. hermonthica.* The authors proved that when *S. umbrinus* was used as the only control agent, it would have to destroy 95% of the *Striga* seeds each year in order to reduce the density of emerged *Striga* plants by 50%. The model has been corrected by Smith and Webb (1996) and they stated that, at least 70 to 80% of the seed production has to be reduced to have the same impact on *Striga* infestation density. It has been concluded that the use of herbivorous insects could play a role in an integrated control package, lowering the *Striga* population by reducing its reproduction capabilities and spread

(Kroschel *et al*., 1999). However, the augmentation of native insect populations through inundative releases is not applicable in the third world, mainly due to the infeasibility of mass rearing (Bashir, 1987, Kroschel *et al*., 1999).

2.2.2 Biological control using microorganisms

Biological control of *Striga* using microorganisms received less attention than using insects, although since the 1930s several fungi were found to be associated with *Striga* seeds and plants. Naj Raja (1966) reported the occurrence of *Alternaria* sp., *Cercospora* sp., *Neottiospora* sp. and *Phoma* sp. on *Striga asiatica* and *S*. *densiflora* in India. In 1971, five fungi were isolated and found to be pathogenic to *Striga* in North Carolina, including *Curvularia geniculata, Fusarium roseum, F. solani, Rhizoctonia solani, and Sclerotium rolfsii* (Meister and Eplee, 1971). Zummo (1977) noticed diseases of *S. hermonthica* in West Africa such as leaf spot caused by an unknown species of *Cercospora,* a vascular wilt caused by *F*. *equiseti* and a stem lesion induced by *Phoma* sp.

In 1989, twenty-eight fungi and two bacteria were found to be associated with *S. hermonthica* in Sudan. Among them, only *F. nygamai* and *F. semitectum* var. *majus* showed potential to be used as bioagents for the control of *Striga*. They proved to reduce the emergence of *Striga* plants up to 97 and 82%, respectively, when mixed with soil preplanting at a rate of 20 g kg^{-1} soil, and the sorghum performance was significantly improved (Abbasher and Sauerborn, 1995). Both of the *Fusarium* species were able to control *Striga* in sterilized and non-sterilized soil, indicating their ability to compete with other soilborne pathogens (Abbasher and Sauerborn, 1992). In root chamber experiments, *F. nygamai* and *F*. *semitectum* were found to inhibit *Striga* germination by 100% and 93%, respectively, when 10 ml of inoculum ($8x10^6$ spores ml^{-1}) was applied. Using half of this concentration, *F. nygamai* still reduced *Striga* seed germination, prevented the attachment of germinated *Striga* to sorghum roots and reduced the total number of *Striga* shoots by 88, 96, and 89%, respectively. *F*. *semitectum* was comparatively effective. In addition, *F. nygamai* was able to kill 74% of the germinated *Striga* by attacking their germ tube, other *Striga* shoots found to be killed after their attachment (Abbasher, 1994; Abbasher and Sauerborn, 1995; Abbasher *et al*., 1996). Moreover, *F. nygamai* mycelium was noticed to penetrate the seeds of *Striga* and parasitize inside (Sauerborn *et al*.,

1996a). *F. nygamai* was found to be host specific, i.e. it did not attack sorghum or any of the 18 other plant species tested (Abbasher and Sauerborn, 1992).

F. nygamai was tested for its ability to control *Striga* in maize under Ivory Coast's field conditions (crop season 1993 and 1994) using liquid and solid medium (fermented sorghum grains) and adopting two methods of application (foliar application and soil treatment) in the first season. Both liquid and solid inoculums were also applied in maize as pre- and post-emergence application. In the second season, *F. nygamai* was formulated as dry powder or as granules applied into the planting hole which was found to be the most effective application method. No reduction in the number of *Striga* plants was observed between the different treatments, however, emerged *Striga* plants showed symptoms of *F. nygamai* infection. The application of *F. nygamai* on fermented sorghum grains into planting holes was found to improve maize vigor and yield insignificantly (Sauerborn *et al*., 1996b). In the second season, a 10-50% reduction in *Striga* biomass was recorded resulting in a corresponding increase in maize vigor and yield. In addition, when the first season experiment was repeated without reapplication of *F. nygamai* inoculum to evaluate the persistence of the bioagent, the *Striga* population was lowered in the treated plots compared to the control, indicating that *F. nygamai* is able to persist in the soil for at least one year (Sauerborn *et al*., 1996b).

Surveys in Ghana reported that *F. equiseti* and *F. oxysporum* showed a potential to be used for biological control of *S. hermonthica* (Kirk, 1993; Abbasher *et al.,* 1995). *F. equiseti* could cause 50% mortality in a *Striga* population (Kirk, 1993). 13 fungal species that belong to four genera including *Fusarium*, *Curvularia*, *Macrophomina* and *Sclerotium* were isolated from diseased *Striga* plants in Ghana (Abbasher *et al.,* 1995). From them, two isolates of *F. oxysporum* and one isolate of *F. solani* reduced the emergence of *S. hermonthica* by 88%, 98% and 76%, respectively, and consequently improved sorghum yield by 26% compared to the control without *Striga* (the control treated with *Striga* only showed a complete loss of sorghum yield) (Kroschel *et al*., 1996), since mycorrhization between sorghum plants and *Fusarium* isolates may lead to an increase in sorghum yield (Lendzemo and Kuyper 2001). Furthermore, a germination test indicated that *F. oxysporum* isolates were highly pathogenic to *Striga* seeds (inhibit germination or prevent attachment of germinated seeds) (Kroschel *et al*., 1996). Extensive studies have been carried out for developing a granular formulation for one of the isolates, *F. oxysporum* isolate 2

(Foxy 2) (Elzein, 2003; Elzein *et al*., 2004; Elzein and Kroschel, 2004a), including the optimization of inoculum production utilizing agriculture by-products, the development of a method for chlamydospores production (the preferred inoculum for long-term storage) and the optimization of storage conditions.

Surveys of plant pathogens associated with *Striga* were carried out also in Burkina Faso, Mali and Niger in 1991. About 250 fungal isolates were obtained from these areas, the most common genera were *Alternaria, Cercospora, Drechslera, Macrophomina*, *Phoma and Fusarium*. Eighty-five of them were screened for their pathogencity to *Striga hermonthica* by applying the inoculum before emergence, which is incorporated into the potting material together with *Striga* and sorghum seeds**.** Among these candidates, *F. oxysporum* M12-4A (originating from Mali) consistently suppressed the attachment of germ tubes and the emergence of *Striga* shoots in root chamber experiments. In pot experiments this isolate was recorded to inhibit *Striga* emergence throughout the experiment, indicating complete pre-emergence control by the fungus (Ciotola *et al*., 1995). The efficacy and the host range of this isolate was evaluated under field conditions using freshly fermented sorghum straw or sorghum glumes as growth substrate for the fungus. *F. oxysporum* M12-4A showed a high degree of selectivity and efficacy for the control of *Striga hermonthica*: it *was* able to reduce the number of emerged *Striga* up to 60% as well as the number of *Striga* plants reaching the flowering stage by 45% and consequently cereal grain yield increased up to 75% compared to the control. All developmental stages of the parasite could be infected (Diarra, 1999). Further investigations proved that *F. oxysporum* M12-4A tolerated a wide range of temperature (20-42°C) and survived and remained viable for at least 12 months when grown on sorghum straw or glumes and stored at room temperature (Diarra *et al.*, 1996). Recently in field trials, chlamydospore-rich powder of *F. oxysporum* M12-4A harvested from small-scale fermentor using 1% (w/v) sorghum straw as growth substrate reduced *S. hermonthica* emergence by 92%. Complete inhibition of *S. hermonthica* emergence occurred when the chlamydospore-rich powder was added to the soil at sowing or when sorghum seeds had been coated with the inoculum using Arabic gum as adhesive material (Ciotola *et al*., 2000).

In the Nigerian Savannah, four *Fusarium* species were isolated from wilted *Striga* plants, among them *F. oxysporum* PSM-197 which was identified as a potential candidate for *Striga* control. This isolate completely inhibited *Striga* growth when

grown on sorghum grain and incorporated in the soil as pre-emergence treatment under controlled conditions (Marley *et al*., 1999). Further experiments showed the efficacy of *F. oxysporum* PSM-197 in controlling *Striga* under field conditions (Marley and Shebayan, 2005). The biocontrol agent fermented on gritted sorghum grains applied in four different methods was evaluated, these included: placement in a 15cm wide band at a rate of 0.5 kg over a 10 m ridge, broadcast application of 2.5 kg per plot, spot application of 5 -10 g per planting hole and surface placement of 10g over the sown seed covered with soil. Overall, spot application gave the best results in reducing *Striga* emergence with about 90%, while the broadcast application provided the lowest reduction of *Striga* emergence with 75%, which was still significant compared to the control. The application of the biocontrol agent significantly increased the stand count at both 3 weeks after sowing and at harvesting and increased crop yield of both improved and local sorghum varieties compared with their respective untreated controls (Marley and Shebayan, 2005).

Recently in Sudan a *Fusarium* isolate - named Abuharaz isolate - was obtained from diseased *Striga* plants and proved to be effective in controlling *S. hermonthica* (Mohamed, 2002). In a field experiment this biocontrol candidate propagated on autoclaved sorghum grains and incorporated at the rates of 5, 10, and 15 g $hole^{-1}$ was found to reduce *Striga* parasitism by 44 to 64% and by 22 to 46% in the cropping seasons of 2000/2001 and 2001/2002, respectively. Combining the application of the fungal inoculum with urea at 190 kg ha^{-1} showed an increased control efficacy, *Striga* emergence and *Striga* biomass were significantly reduced by 42 to 68% and 43 to 80% in both seasons respectively, and consequently sorghum grain yield improved significantly 1-3 and 6-9 fold compared to the untreated control, respectively (Mohamed, 2002).

Very recently two green house experiments were conducted in Burkina Faso to study the effect of growth media (compost and sorghum straw), *Striga* seed burial distance and depth (5 and 10 cm vertical and horizontal) on the efficacy of 14 *Fusarium* isolates to control *S. hermonthica*. This study concluded that all *Fusarium* isolates tested were able to reduce the number of emerged *Striga* shoots, *Striga* vigor and dry biomass. As a result, sorghum dry biomass and grain yield were improved. *Fusarium* isolates, growth substrate and their interaction significantly influenced the germination of *Striga* seeds at both 35 and 50 days after sowing. Isolates grown on compost were more effective than those grown on chopped sorghum straw. The

authors suggested that inoculum should be incorporated in the top 5 cm of the soil and not below 10 cm depth because the first *Striga* shoot to emerge in the field are observed within the 10 cm radius (Yonli *et al*., 2006).

2.3 Mycoherbicide formulation

Formulation of a bioherbicide is the key for successful biological control and can be defined as the mixing of the biologically active propagule with inert carriers and other adjuvants, to give a product, which can be effectively delivered to the target weed (Connick *et al*., 1990; Boyette *et al*., 1991a; Rhodes, 1993). The ideal formulation is the one that is produced economically, environmentally safe, stable in the environment, easily applicable using existing agricultural machinery, and storable for at least 18 months at 40°C (Kenney, 1986; Connick *et al*., 1990; Auld *et al*., 2003). The most significant difference between chemical and biological herbicides is that in the latter the active ingredients are living organisms, which are capable to replicate in the environment and frequently require time to multiply after application in order to control the target weed or pest (Rhodes, 1993). Therefore, the formulation must provide conditions that retain viability during preparation, storage and application and favor the survival of the agent in the environment. In order to function as biocontrol agents, the microorganisms must exert one or more specific effects directly or indirectly. The mode of action through which a bioagent suppresses its target includes production of toxins, parasitism or competition. Each mode of action requires a different set of formulation (Rhodes, 1993). The selection of an appropriate formulation that can improve stability and viability may reduce inconsistency of field performance of several potential biocontrol agents (Auld and Morin, 1995; Boyetchko *et al*., 1999).

A mycoherbicide formulation may contain the viable agent in a dormant or low metabolically active state, e.g. dry spores or mycelia. Such formulations seem to have a longer shelf life and are more tolerant to environmental stresses such as temperature fluctuation and humidity extremes. In contrast, formulations with metabolically active propagules have a shorter shelf life and need specific packing that enables gas and moisture exchange and prevents contamination of the product (Green *et al*., 1998).

2.3.1 Inoculum production

Inoculum production is a prerequisite for bioherbicide formulation. Generally three methods have been evaluated for the production of bioherbicides: 1) the use of living host plants (for obligate parasites), 2) solid substrate fermentation and 3) liquid culture fermentation which is considered the most economical method of production (Jackson *et al.,* 1996). The first step of inoculum production of biocontrol agents is the development of a suitable growth medium utilizing cheap, readily available agriculture by-products with the appropriate nutrient balance (Latgè *et al.,* 1977). It is economically unfeasible to use synthetic media or plant extracts for commercial production (Connick *et al.,* 1990).

For a successful formulation, sufficient biomass should be obtained which contains an adequate amount of effective propagules (Jackson *et al.,* 1996). For instance, it was observed that formulations of *Gliocladium* and *Trichoderma* containing chlamydospores were more effective in preventing various diseases and allowed a better proliferation of the fungus in soil than preparations containing conidia (Lewis and Papavizas, 1984; Papavizas *et al*., 1984). One of the advantages of working with *Trichoderma* and *Gliocladium* is their apparent lack of specific nutritional or cultural requirements during fermentation. Nevertheless, in many situations media components as well as growth conditions such as aeration, pH, and temperature may affect the quality and quantity of the effective propagules of the bioagent, therefore, such conditions must be optimized during inoculum fermentation (Churchill, 1982). Other factors that need to be considered for developing a mass production system include: the rate at which an effective biomass is produced, the risk of contamination, as well as the time need for fermentation. A shorter time is preferable since an increased fermentation time reduced viability of some fungi and increased the risk of contamination (Connick *et al*., 1990) as well as the cost of production. However, some fungi such as *Trichoderma harzianum* and *T. viride* require longer fermentation periods of 10-15 days, but this disadvantage is compensated by the increased concentration of mature chlamydospores in the biomass (Churchill, 1982; Papavizas *et al*. 1984). In experiments with *F. oxysporum* (Foxy 2), a potential agent for the biological control of *Striga,* inoculum production has been optimized utilizing agricultural by-products. It was found that abundant chlamydospores (4.3×10^7 ml^{-1}) produced by Foxy 2 within 12 days of incubation in a single-step liquid fermentation through the combination of 0.5% (w/v) maize stover plus 20% (v/v) wheat based

stillage and an agitation level of 200 rpm (Elzein and Kroschel, 2004b). After successful liquid fermentation of the bioagent, biomass can be separated from the medium by different types of filtration and centrifugation, and then it can be dried and milled before formulation (Connick *et al*. 1990; Churchill, 1982).

Inoculum production based on solid fermentation is especially useful for small-scale experiments such as laboratory, green house and field tests that require minimal efforts for implementation. Furthermore, it is required for the mass production of fungi, which do not sporulate in liquid culture or cannot survive the liquid fermentation process. In addition, it could also be advantageous for developing countries where no high-tech fermentation facilities are in place, agricultural by-products are available and work is not a limiting factor for production (Lewis, 1991).

2.3.2 Types of mycoherbicide formulations

The type of formulation to be used for a bioherbicide depends on the biology and mode of action of the bioagent and on the available application technology. In foliar applied bioherbicides, the propagules remain on the leave surface after application and are faced to several constrains such as rain wash, UV light and desiccation (Rhodes, 1993; Greaves *et al*., 1998). Many bioherbicide agents required dew periods of 6 to 24 hours for the germination of propagules and penetration of the host (Connick *et al*., 1990; Auld and Morin, 1995). This is probably the most critical period in biological weed control, indicating that a specific timing of application is necessary to coincide with moist conditions (Klein and Auld, 1995). Such problems may be overcome by developing formulations that protect the agent against desiccation until host penetration is complete (Rhodes, 1993; Green *et al.*, 1998). For soil application many of the environmental problems that foliar applications of bioherbicides face are reduced, however, there may be difficulties with the optimum placement of the bioagent in the soil, competition from soil microflora, and the extremely heterogeneous nature of the soil which often cause an unpredicted harsh environment for introduced organisms (Rhodes, 1993; Paau, 1998).

Most formulations of biological control agents are largely based upon techniques developed for formulation of agrochemicals, involving the use of organic solvents, surfactants and drying methods which can be harmful to the bioagent propagules (Connick *et al.,* 1991; Rhodes, 1993). However, processes and ingredients associated with food industry often are suitable also for bioherbicide technology, e.g.

alginate formulations (food gels, Connick, 1988), invert emulsions made with lecithin (mayonnaise, Daigle and Connick, 1990; Quimby *et al.,* 1988) and “Pesta” granules based on wheat flour-kaolin using a hand-operated Pasta machine (Connick *et al*., 1991). Generally, bioherbicide formulations can be divided in two groups: liquid and solid formulations.

2.3.2.1 Liquid formulations

These include aqueous, oil, or polymer-based products, that are mostly used as post-emergence sprays to cause leaf and stem diseases on the target weed (Boyette *et al.,* 1991b, Shabana *et al.,* 1997; Chittick and Auld, 2002). The simplest, most straightforward formulation contains the propagules of the bioagent suspended in water, which is most frequently used in the early stages of the development of a mycoherbicide (Hofmeister and Charudattan, 1987; Connick *et al.,* 1990; Daigle and Connick, 1990). However, under ideal conditions for fungal infection, a simple aqueous suspension can be also used successfully in the field (Auld *et al*., 1990). Recently oil suspension emulsions and invert emulsions have gained attention in bioherbicide formulation (Boyette *et al.,* 1991b). Invert emulsions can buffer the pathogen against environmental extremes and protect against desiccation, but they are complex to prepare and highly viscous so that they cannot be applied by convential spray machinery (Auld, 1993; Boyette, 1994). A commercially successful example of a liquid formulation is ¨DeVine¨ which consists of chlamydospores of *Phytophthora palmivora* formulated as a liquid concentrate with a 6-week shelf life to control stranglervine (*Morrenia odorata*, Kenney, 1986).

The efficacy of liquid suspensions can be enhanced by adding adjuvants and surfactants. Surfactants are the most commonly used additives, usually regarded as essential to ensure a relatively even distribution of inoculum on the leaf, especially if its surface is hydrophobic. Adjuvants are defined as compounds which assist to or modify the action of the active ingredient (Rhodes, 1993). In a bioherbicide formulation several adjuvants can be used to enhance spore germination, improve pathogen stability, and modify the environmental requirements or expand the host range of the bioagents (Boyette *et al*., 1996). For instance, unrefined corn oil was used as an adjuvant to enhance the bioactivity of *Colletotrichum truncatum* to control hemp sesbania (*Sesbania exaltata*), to reduce its dew period requirement from 12 to

2 h and to decrease the necessary spray volume 100-fold, from 500 to 5l/ha (Boyette, 1994; Boyette *et al*., 1996).

A wettable powder formulation is a type of formulation in which the bioagent is mixed with water and a carrier such as kaolin, hydrated silica powder, peptone or starch. The mixture is dried and then resuspended in water as needed for spray application (Mortensen, 1988; Connick *et al.,* 1990). Commercial mycoherbicides formulated as wettable powders are "Collego"(*Colletotrichum gloeosporioides* f. sp. *aeschynomene*) against northern jointvetch (*Aeschynomene virginica*) and "BioMal" (*Colletotrichum gloeosporioides* f. sp. *malvae*) against round-leaved mallow (*Malva pusilla*) (Bowers, 1986; Makowski, 1992).

2.3.2.2 Solid formulations

Solid or granular formulations are quite suitable for microorganisms that infect their target weed at or below the soil level and hence suitable for pre-emergence application (Connick, 1988; Boyette *et al*., 1991a; Daigle and Cotty, 1992). Furthermore, a solid formulation can buffer environmental extremes (Boyette *et al*., 1991a; Connick, 1988; Weidemann, 1988), often more easily than a liquid formulation. In simple solid formulations, the bioagent propagules are firstly produced through fermentation and then mixed with carrier material to make the formulated product (Mortensen, 1988). Grains, peat, charcoal, clay, vermiculate, alginate, bagasse, mineral soil, or filter mud can be used as carrier or substrate material for such a formulation. A carrier is an inert material that influences the performance of the formulation, while a substrate is an organic carrier that acts as a food source for the bioagent (Auld and Morin, 1995; Boyette *et al*., 1991b; Green *et al*., 1998; Paau, 1998). The amended substrate is less likely to be washed away by rains and under favorable conditions the bioagent can reproduce, grow and sporulate, then disperse to the site of action providing a continuous supply of inoculum (Connick, 1988). However, the drawbacks of solid formulations are their bulkiness and the higher costs for material storage and transport (Paau, 1998).

Encapsulating fungal propagules in organic or inorganic matrices such as alginate pellets allow the controlled release of the organism from the formulation (Boyette *et al*., 1991a; Connick, 1988; Weidemann, 1988). Walker and Connick (1983) developed an elegant method of encapsulating bioherbicide fungi in alginate using *Alternaria macrospora, A*. *cassiae*, *Fusarium lateritium, Phyllosticta sorgicola* and

Colletotrichum malvarum to form biodegradable pellets. In this method, the fungal propagules are mixed with a sodium alginate solution that may also contain inert materials such as kaolin and nutrients. This mixture is dripped into a solution of Ca^{+2} ions, where the calcium ions quickly react with the alginate to transform each droplet into a gel bead. The gel beads are removed after a few min, rinsed with water, and left to dry. The granule matrix protects the biocontrol agent during storage and in the environment. The produced granules are uniform in size and can be applied using the existing agricultural equipment (Walker and Connick, 1983; Connick *et al.,* 1990). The main disadvantage of the alginate formulation is its relatively high cost (Connick, 1988).

The alginate formulation has been adopted to formulate soilborne pathogens to control weeds as well as soilborne plant diseases. Boyette and Walker (1985) applied *F. lateritium* formulated in alginate granules pre-emergence in the field to control velvetleaf (*Abutilon theophrasti* Medic). and prickly sida (*Sida spinosa*). The authors reported 59% and 46% mortality of *S. spinosa* and *A*. *theophrasti,* respectively. The relatively low level of control was attributed to the high summer temperature and drought during the experiment. Weidemann and Templeton (1988) formulated *F. solani* in alginate pellets to control Texas gourd (*Cucurbita texana*). Under field conditions, the alginate formulation of *F. solani* effectively controlled Texas gourd causing more than 80% weed control within 6 weeks when applied as a single pre-emergence treatment. The first commercial biopesticide formulated as alginate granules was "GlioGard", registered by the Environmental Protection Agency (EPA), using *Gliocladium* sp. to control damping-off diseases (Lewis *et al*., 1995). The alginate formulation proved to be very effective for entomophathogenic fungi as well and helped to protect the bioagent from inactivation by UV radiation and high temperatures (Pereira and Roberts, 1991).

The efficacy of alginate formulations was improved by additional nutritional amendments. The addition of soy flour or ground oatmeal (2% w/v) was found to increase spore production on the granules and more conidia disseminated into the soil by rainfall or irrigation (Weidemann and Templeton 1988). Daigle and Cotty (1992) noticed that the spore yield of *Alternaria cassiae* formulated in alginate pellets increased when the kaolin filler was substituted by corncob grits. This increase was attributed mainly to the high nutritive value of the corncob grits. Alginate pellets with wheat bran as food substrate were developed for eleven isolates of *Trichoderma* spp.

and *Gliocladium virens* and used to control *Rhizoctonia solani* in the soil (Lewis and Papavizas, 1987). All isolates significantly reduced the growth of *Rhizoctonia* from infested beet seed. The same authors reported that a small amount of pellets or fungal biomass (3 to 7.5g net weight) were as effective as a large amount (30g) in suppressing the pathogen. A rice alginate prill formulation of *F. oxysporum* f. sp. *erythroxyli* isolate EN-4, a candidate to control coca (*Erythroxylum coca*), was found to increase the pathogen population in the soil and cause disease in coca (Bryan *et al*., 1997).

The alginate technology has also been employed to control parasitic weeds. Dry chlamydospores of *F. oxysporum* (Foxy 2) encapsulated into alginate pellets amended with yeast (1 or 2%, w/v) significantly reduced the total number of *Striga* shoots and the number of healthy *Striga* shoots with an efficacy of 81 to 100% and consequently improved sorghum performance (Elzein, 2003). Also chlamydospores of *F. oxysporum* f. sp. *orthoceras* were formulated in alginate granules amended with wheat bran or brewer's yeast (2%, w/v). Those pellets did not reduce the emergence rate of *O. cumana* shoots in sunflower but increased the proportion of diseased shoots and the formulated propagules remained virulent for at least six months (Müller-Stöver *et al.,* 2004).

Connick *et al.* (1991) developed another simple, cheap and effective way for bioherbicide formulation based on the Pasta making process (the reason why they referred to the final product as "Pesta"). "Pesta" granules are made by mixing the fermented fungal suspension with wheat flour (semolina), sucrose and kaolin to form a dough that is kneaded and rolled into thin layers using a hand-operated Pasta machine. The sheets were dried, ground into granules and sieved to separate granules of different sizes. In this formulation the wheat flour (semolina) provides nutrients for a fast outgrowth and sporulation of the fungus, enhances its biological activity and protects the propagules from desiccation. Additionally, sucrose functions as a stabilizer for the membrane during the drying process by replacing water molecules in the lipid bilayer and also prevents the denaturation of proteins in the dry state by forming hydrogen bonds with the protein when water is removed (Leslie *et al*., 1995; Zidack and Quimby, 1999). This technique was firstly used to encapsulate a variety of phytopathogenic fungi such as *Alternaria cassiae* and *A*. *crassa*, *Colletotrichum truncatum* and *Fusarium lateritium* to control sicklepod (*Senna obtusifolia*), jimsonweed (*Datura stramonium*), hemp sesbania (*Sesbania exaltata*) or

velvetleaf (*A*. *theophrasti*), respectively. The formulated fungi were able to grow and sporulate well on the granules after application to the soil. “Pesta” formulated *A. cassiae* and *A*. *crassa* and *C. truncatum* caused 68-100% mortality of their target weeds while *F. lateritium* was less effective causing only 30% mortality (Connick *et al.* 1991). The authors attributed this low efficacy to the low viability of the isolate or a poor persistence after formulation. Generally the bioagents suffer from losses during this formulation process and these losses varied considerably depending mainly on the type of inoculum used (Müller-Stöver, 2001; Daigle *et al*., 2002; Elzein and Korschel, 2004a). The highest losses when formulating *F. oxyporum* f. sp. *orthoceras* occurred during the drying process (Müller-Stöver, 2001).

In 1993, Boyette *et al*. formulated *F. oxysporum* in “Pesta” granules for the control of sicklepod, coffee senna (*Senna occidentalis*) and hemp sesbania. In greenhouse experiments, the weeds were successfully controlled using the “Pesta” formulation (applied pre-emergence) up to 98%, 95%, and 80%, respectively. Daigle *et al*. (1997) proved that the “Pesta” formulation technique could be scaled-up for the production of large amounts of uniform granules for field application by using twin-screw extrusion instead of a hand-operated pasta maker. The modified formulation technique was used for the biological control of swamp dodder (*Cuscuta gronovii*) using *Alternaria conjuncta/infectoria*, and for the reduction of the aflatoxin level in peanut using an atoxigenic strain of *Aspergillus flavus* as biocompetitor.

Since 2000, the “Pesta” technology has gained attention for the control of parasitic weeds. Broomrape (*O. cumana*) in sunflower (*Helianthus annuus* L.) was controlled effectively when *F. oxysporum* f. sp. *orthoceras* encapsulated in “Pesta” granules was applied pre-emergence under controlled conditions (Kroschel *et al.*, 2000; Müller-Stöver, 2001; Shabana *et al*., 2003; Müller-Stöver *et al*., 2002, 2004). Also the efficacy of this formulation for the use of fungi to control *Striga* spp. comprehensively tested by Elzein (2003) under controlled conditions using an isolate of *Fusarium oxysporum*. The “Pesta”-formulated *F. oxysporum* (Foxy 2) at 2 g per pot (4 kg of soil) was able to control *Striga* under controlled conditions with an efficacy of more than 89% (Elzein, 2003) indicating the benefit of using small amounts of the formulated material compared to the bulky organic solid inoculum used before, to manage *Striga* and improve sorghum yield.

The “Pesta” formulation technique has also be successfully used to encapsulate the entomopathogenic nematodes *Steinernema carpocapsae,* a highly effective biological control agent for numerous insect pests in the soil (Connick *et al*., 1993).

2.3.3 Application of bioherbicides

A delivery system for a bioherbicide must be easy, effective, timely at the appropriate site of action and compatible to the available agriculture machinery (Lewies, 1991; Rhodes, 1993). The formulated product can be applied to the soil, seeds or to aerial parts of the target weed.

2.3.3.1 Soil application

Soil application is suitable for fungal pathogens that infect weeds at or below the soil surface (Connick, 1988; Boyette *et al.,* 1991a). Soil treatment is most effective when the agent is applied as a post-fumigation treatment or at the time of planting to place the biocontrol in the vicinity of the target (Rhodes, 1993; Boyetchko *et al.,* 1999). Solid formulations can be broadcasted or incorporated into the soil, whereas liquid formulations can be delivered in furrow below the seed weed (Lewies, 1991; Daigle *et al*., 2002).

DeVine™, a product based on *Phytophthora palmivora* and sold as aqueous suspension of chlamydospores, is applied to the wet soil as a high-volume spray in order to achieve efficient placement of the biocontrol agent in proximity of the host roots (Kenny, 1986; TeBeest and Templeton, 1985).

2.3.3.2 Seed treatment

The application of microorganisms to seeds for use in agriculture, forestry and horticulture has been extensively investigated for many years. A seed treatment with biocontrol agents seems to be an attractive delivery system since it combines specific effects with a limited environmental impact and delivers bioagents at the right amount, right place and right time (McQuilken *et al*., 1998). This method of application has been adopted to control damping-off diseases using antagonistic bacteria. Seeds of different crops are pelletized by treating them with natural stickers such as methylcellulose or synthetic ones such as Nu-film™, which contain bacterial suspensions (Nelson *et al*., 1986; Suslow and Schroth, 1982). The treated seeds are then dried or coated with inert material before drying. An example of a commercially

available seed inoculant is Quantum 4000^{TM} containing *Bacillus subtilis* (McQuilken *et al*., 1998). In contrast to chemical fungicides that are effective only for a limited period (approximately 2 weeks), the antagonistic bacterium colonizes the developing root system and can protect the seedling from disease-causing fungi in the soil throughout the season (Suslow and Schroth, 1982). Fungal biomass and conidia have also been formulated as seed treatments with commercial potential, for instance, powdered biomass of *Pythium oligandrum* containing an abundant number of oospores was mixed with a clay carrier and used for pelleting seeds of cress *(Lepidium sativum*), carrot (*Daucus carota* L) and sugar beet (*Beta vulgaris* L.) to control damping–off (Lutchmeah and Cooke, 1985). Ciotola *et al*. (2000), reported effective control of *S. hermonthica* under field conditions using a dry chlamydospore-rich powder of *F. oxysporum* M12-4A as a seed treatment with Arabic gum as adhesive material. Also Elzein (2003) showed successful control of *S. hermonthica* with dry chlamydospore-rich powder of *F. oxysporum* (Foxy 2) used as a seed treatment for sorghum seeds.

2.3.3.3 Arial application

Formulations of bioagents used as foliar sprays vary according to the crop to be treated, the target pest and the delivery system. A liquid or slurry formulation is most commonly used for aerial application. Emulsifiers, stickers, spreaders and other adjuvants and additives help in application, dispersal and adhesion of the bioagent on plant surface and protect from environmental extremes (Connick *et al*., 1990; Greaves *et al.,* 1998). The aerial application of bioherbicides could be performed utilizing the existing spray application equipments available for chemical pesticides, but often using a considerable greater volume of water (Boyette *et al*., 1991b; Boyetchko *et al.,* 1999; Greaves *et al.,* 1998). Collego and DeVine consist of a single highly effective fungal pathogen applied with or without amendment as an aerial post-emergence spray (TeBeest *et al.*, 1992). Other means of aerial application include painting the bioherbicide over a cut tree stump, applying it through center-pivot irrigation or by the natural wind-borne dissemination from an inoculum source (Charudattan, 2001, Phatak *et al.,* 1987).

2.4 Phytotoxins and mycotoxins production

Phytotoxins are defined as microbial metabolites that are harmful to plants at low concentrations. Frequently, these compounds play a role in pathogenicity and the induction of disease symptoms and the virulence of the pathogen may depend on its capacity to produce one or more toxins. Phytotoxins are characterized by a low molecular weight and belong to a variety of classes of natural products (Evidente and Motta, 2001).

Recently, research has been directed towards isolating phytotoxins produced by some fungi pathogenic to weeds and using the isolated, non-specific compounds as natural herbicides either in their native form or as derivatives and analogues (Strobel *et al*, 1991, Boyetchko, 1999). With the increasing number of herbicide-resistant weeds, phytotoxins provide a new pool of compounds to control problematic weeds.

Fusarium species normally produce a number of phytotoxic compounds such as: fumonisin, enniatin, moniliformin and fusaric acid, responsible for a broad range of biological activity such as growth inhibition, necrosis, chlorosis, wilting and inhibition of seed germination (Wakulinski, 1989). Fusaric, 9, 10-dehydrofusaric acids and their methyl esters were found to be the main phytotoxins produced by *F. nygamai* (Capasso *et al*., 1996). These phytotoxins proved to have a herbicidal activity against *Striga,* causing a dramatic reduction of *Striga* seed germination and inducing large necrotic spots on punctured leaves at low concentrations (10^{-6} M). The compounds are not toxic to arthropods at 10^{-4} M (Zonno *et al*., 1996). Fusaric and dehydrofusaric acid also reported to be produced by a strain of *F. oxysporum* characterized as a potential bioagent for *S. hermonthica* control in West Africa (Savard *et al.,* 1997).

Mycotoxins are toxic secondary metabolites produced by fungi such as *Fusarium* species and can be accumulated in infected grains in the stores. Trichothecenes, zearalenone and fumonisins are the major *Fusarium* mycotoxins occurring on worldwide basis in cereal grains, animal feeds and forage and are often associated with chronic and acute mycotoxicoses in farm animal and man (D'Mello *et al.*, 1999).

Trichothecences are the major class of mycotoxins produced by *Fusarium* moulds. They constitute a large group of fungal metabolites sharing the basic chemical structure, a 12, 13-epoxytrichotec-9-ene ring system (Bennett and Klich, 2003).

Trichothecences noncompetitively inhibit protein biosynthesis in eukaryotic cells with effects on mammals including diarrhea, vomiting and gastro-intestinal inflammation

(Bretz *et al*., 2005). *F. nygamai* was not recorded to produce zearaleone and trichothecene toxins (deoxynivalenol, 3α–acetydeoxynivalenol, nivalenol, T-2 toxin, HT-2 toxin and diacetoxyscirpenol) when propagated on wheat grains (Abbasher, 1994).

Fumonisins are mycotoxins produced by several species of *Fusarium,* e.g. *F. moniliforme*, *F. proliferatum* and *F. nygamai* (Magnoli *et al.*, 1999; Norred *et al.,* 1999). Fumonisins might initially be produced under favorable field conditions but their production can be increased under improper storage conditions (Magnoli *et al*., 1999; D'Mello *et al.*, 1999). Fumonisins affect animals in different ways by interfering with sphingolipid metabolism, they cause leukoencephalomalacia in horses (equines) and rabbits and pulmonary edema and hydrothorax in pigs (Norred *et al.,* 1999). In humans, these mycotoxins are associated with esophageal cancer (Bennett and Klich, 2003).

Very recently, fumonisin B_1 that is naturally produced by *F. nygamai* proved to be an effective bioherbicide for *Striga* control when applied post-emergence at low concentrations (Kroschel and Elzein, 2004), which do not represent any health hazards to mammals (Abbas *et al*., 1991; Nelson *et al*., 1993; Kroschel and Elzein, 2004).

3 Materials and methods

3.1 Laboratory experiments

3.1.1 Fungal cultures

The bioagents used in this study are:

- *Fusarium nygamai* (FN), this species was firstly isolated from *Striga hermonthica* during a survey carried out in 1989 for microorganisms associated with diseased *Striga* plants in different areas in the Sudan (Abbasher, 1992).
- *Fusarium* sp. Abuharaz isolate (FA), isolated in 1999 from diseased *Striga hermonthica* plants growing on a sorghum field at Abuharaz on the premises of the Faculty of Agriculture and Natural Resources, University of Gezira, Sudan. Its identification to the species level is not yet completed (Mohamed, 2002). Therefore the isolate is referred to as *F*. Abuharaz (FA) in the course of this study.

The inoculum of FN was produced using a stock culture provided by the Institute of Plant Production and Agroecology in the Tropics and Subtropics, University of Hohenheim. The stock culture was preserved on Special Nutrient-poor Agar (SNA,) (Nirenberg, 1976) with 5% (v/v) glycerol at -80°C. The inoculum of FA was produced using a preserved culture provided by the Abu Haraz Faculty of Agriculture and Natural Resources, University of Gezira, Sudan. The two fungi were firstly grown on Potato Dextrose Agar (PDA, Sigma, Germany) plates, then transferred to plates containing SNA + glycerin and stored at 4°C in the refrigerator without sub-culturing. For long-term preservation, FA was also stored at –80°C.

3.1.2 Inoculum production

To determine the best medium for sporulation of the two fungi, they were grown in four different liquid media: Potato Dextrose Broth (PDB, Sigma, Germany), Yeast Extract medium (YE) containing 1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_4$ x $7H_2O$, 0.5 g KCl, 0.2 g glucose, 0.2 g sucrose and 2 g yeast extract in 1l of deionized water, Vegetable Juice Broth (V-8 B) (200ml vegetable juice and 2 g $CaCO_3$ in 1litre of deionized water) and Richard's solution (150 ml vegetable juice, 10 g KNO_3, 5 g KH_2PO_4, 2.5 g $MgSO_4$ x $7H_2O$, 0.02 $FeCl_3$, 50 g sucrose and 850 ml deionized water). Six Erlenmeyer flasks with 100 ml of each medium plus 0.02g

Chloramphenicol were inoculated after autoclaving by adding an agar plug (1 cm dia.) from the fungal culture growing on SNA and incubated at room temperature (21 ±3°C) on a rotary shaker (150 rpm). After 5 days, 3 flasks with each medium were blended for 5 sec in a Warring blender and mycelial fragments were removed by filtration through a double layer of cheesecloth. The number of conidia and chlamydospores was determined using a haemacytometer. The number of spores was further determined every 3 days for two weeks. Richard‘s solution broth was selected as growth medium to obtain inoculum for formulation purposes using either Erlenmeyer flasks as described above or a bench-top fermenter (Labfors, Infors AG, Switzerland). Starter flasks with 100 ml of Richard's solution were inoculated with an agar plug (1 cm dia.) of a fungal stock culture on SNA and incubated on a shaker at 150 rpm for 3 days at room temperature. The growth medium in the fermenter was then inoculated with 100 ml starter culture. The fermenter medium temperature was maintained at 28°C, the agitation speed adjusted to 300 rpm and the aeration rate was 1 l / min. Foam control was provided by means of an automatic addition process of an antifoam silicone agent through a peristaltic pump. The fermenter was run for 2 days. For further processing, the fungal biomass was blended for 5 sec in a Warring blender and filtrated through a double layer of cheesecloth to remove mycelial fragments. To increase conidia concentration, the resulting solution was centrifuged for 5 min at 4000 rpm and the major part of the supernatant was discharged. The number of conidia in the final solution before formulation was approximately 1.0-3.5x10^8 (FN) and 1.3-1.6x10^8 (FA).

3.1.3 Preparation of durum wheat flour–kaolin granules

The two fungi were formulated in wheat flour-kaolin ("Pesta") granules using the technique described by Connick *et al.* (1991). The granules were prepared by blending 32 g of durum wheat flour (Semolina, Divella, Italy), 6 g kaolin, and 2 g sucrose in a dish. Twenty three ml of liquid inoculum were added, the mixture was kneaded well with gloved hands and passed through a small hand operated pasta maker at the widest roller setting. The resulting sheet was folded and extruded again for up to 4 or 5 times. Then the dough sheet was passed through the pasta maker at an intermediate setting and finally through the narrowest setting that was possible without destroying the sheet. The sheets (1 to 1.5 mm thick) were air-dried on aluminium foil over night and ground in a laboratory mill (Grindomix GM200, Retsch,

Germany) at 4000 rpm for 10 sec. The ground material was then sieved to a particle size of 250 µm to 2 mm and stored in the refrigerator at 4°C in sealed plastic bags.

To determine the number of colony forming units (cfu) in the preparations, 100 mg of the formulated granules were added to 10 ml deionized water in a glass tube. The sample was vortexed from time to time together with 3 glass beads until the granules were completely dissolved. Three plates (per each of three replicates) with half-strength PDA were inoculated with 0.1 ml of an appropriate dilution of the disintegrated granules, which was evenly distributed over the surface of the plate using a triangle loop. The plates were incubated at room temperature. After 3 days the number of cfu per gram of formulated material was determined.

3.1.4 Preparation of alginate pellets

The alginate formulation was prepared as described by Walker and Connick (1983) and modified by Müller-Stöver *et al.* (2004) 1.8 g sodium alginate were dissolved in 135 ml sterilized distilled water while stirring on a magnetic stirrer. To the dissolved sodium alginate, 6% (w/v) kaolin and 10% (w/v) durum wheat flour were added. For the pot experiment, sorghum flour and yeast extract (6% (w/v) each) were added as well. Then 15 ml of liquid inoculum containing $1.1x10^8$ conidia ml^{-1} for FA and $1.4x10^8$ conidia ml^{-1} for FN were given to the solution. The pH of the solution was adjusted to 7 by adding 0.01 N NaOH. The preparations were pelletized by dripping the mixture into 0.1 M calcium chloride under continuous stirring using a peristaltic pump. The resulting pellets were collected and washed thoroughly under tap water. Then the pellets were spread on aluminum foil to be air-dried and passed through a 3.15 mm mesh screen to separate adhering ones. The sieved pellets were stored in the refrigerator at 4°C.

The cfu g^{-1} alginate pellets were determined by dissolving 0.1 g of the pellets in 1% sodium carbonate and plating appropriate dilutions on half-strength PDA. Three replicates per preparation were used, inoculating 2 petri dishes per dilution.

3.1.5 Comparison between the stability of alginate and “Pesta” formulations in the soil

The main objective of this experiment was to compare for how long could the bioagents be detected in the soil after their application either as alginate pellets or as “Pesta” granules. For this purpose, petri dishes (9 cm) were filled with 42.5 g dry

loamy soil sieved through a 4.0 mm mesh screen. Twenty mg from each formulation of each fungal isolate were added to each dish (4 replicates per treatment) and carefully mixed with the soil. Untreated petri dishes served as control. At the beginning of the 9 weeks lasting experiment the water content was adjusted to 50% of the maximum water holding capacity of the soil. To prevent evaporation, the petri dishes were sealed with parafilm and incubated at 25°C in the dark. After one week of incubation, the content of each dish was thoroughly mixed and two samples of 0.5 g were taken out of each dish. Each sample was put in a test tube and 10 ml water were added. The content of each tube was vortexed for 30 sec, then left for approximately 30 min. Afterwards, the content was mixed again for another 30 sec, then appropriate dilutions were made, from which two samples of 0.2 ml were taken and spread over plates containing PDA+PCNB agar with chloramphenicol (Fauzi and Paulitz, 1994). The agar plates were incubated at room temperature and cfu were determined after 3 days. Moisture content of the soil in the petri dishes was checked and water was added when necessary. Soil samplings were repeated every two weeks for 9 weeks. Data were corrected for percent soil moisture before analysis. The experiment was not repeated.

3.1.6 Seed treatment experiments

Different methods to directly coating sorghum seeds with the bioagents as an alternative delivery system were tested.

3.1.6.1 Seed soaking

100 ml of Richard's solution containing $5x10^9$ cfu/ml (FN) and $4x10^9$ cfu/ ml (FA), harvested after 7 days of incubation as described above, was taken as stock solution for seed soaking. The number of cfu in the stock solution was determined by using the serial dilution technique and plating a 0.1 ml aliquot of fungal inoculum on half-strength PDA (2 plates per each dilution).

The seed soaking treatments were as follows:

a) 50 surface-sterilized sorghum seeds [sorghum seeds were immersed on 70% ethanol for 10 min then in 10% NaOCl for 20 min and finally rinsed in sterilized water 3 times and left to dry (Kroschel, 2001)], soaked in 30 ml of the stock solution for 30 min without any additives, taken out and left to dry on cheesecloth over night.

b) 150 µl of a superabsorbent polymer (W97766, kindly provided by Stockhausen GmbH, Krefeld, Germany) were added to 30 ml of the stock solution while stirring on a magnetic stirrer. 50 sterilized sorghum seeds were then soaked in the solution for 30 min, taken out and left to dry on cheesecloth over night.

c) 0.45 g sodium alginate was added to 30 ml of the stock solution while stirring to avoid clump formation. The pH was adjusted to 7, then sterilized sorghum seeds were soaked for 30 min. The soaked seeds were suspended into a 0.1M calcium chloride solution for 5 min, rinsed in deionized water, and left to dry on cheesecloth over night.

The germination percentage of the soaked seeds was determined using the blotter test using 20 seeds / dish. To study the colonization of sorghum roots developing out of the *Fusarium*-treated seeds, two large (46 x 57 cm) autoclaved glass microfibre filter papers (GF/A Whatman®) were fastened together using sterilized deionized water. 10 treated sorghum seeds were put at one edge between the two filter papers. Then a third paper was put over them, all papers were well moisturized with sterilized deionized water, rolled and put into a plastic polythene bag. Each rolled filter paper was put into a flask and incubated in a growth chamber at 30/20°C (day/night) with a photoperiod of 12 hours. The rolled filter papers were moisturized daily with sterilized deionized water. After two weeks, sorghum root colonization by the bioagents was examined by aseptically taking small pieces from the root tip and from the middle of the root and cultured on PDA+PCNB agar with and without surface sterilization. Untreated seeds served as control. The experiment was conducted once.

3.1.6.2 Seed coating

For the first season field experiment, seed coating was achieved by mixing 100 g sorghum seeds with 10 ml Arabic gum (40%) and 10 g of fine (>1mm) “Pesta” granules containing the fungal isolates.

In the second season experiment sorghum seeds were coated by a private company SUET (Saat- und Erntetechnik GmbH, Eschwege, Germany).

To assist the company in the selection of the best material, 5 different adhesives (two types of cellulose, two organic polymers and a clay) provided by SUET were tested for their effects on the growth and sporulation of *Fusarium* isolates in solid and liquid medium. 10% (w/v) of the sticky materials were added to PDA, non-supplemented

PDA plates served as control. Each plate was inoculated with a 1 cm (dia.) agar plug from fungal cultures on SNA with five replicates per treatment. The plates were incubated at room temperature (21± 3°C) and the colony diameter was determined after 7 days.

To study the effects of the adhesives on sporulation, 5% (w/v) of each material (except for cellulose 2: 2.5% (w/v)) were added to Richard's solution. Each 100 ml medium in a 250-ml Erlenmeyer flask were inoculated with an agar plug (1cm dia.) of a fungal culture on SNA. The flasks were incubated on a rotary shaker at 150 rpm for five days, then the type and number of conidia were determined using a haemacytometer. Three replicates were used in this experiment.

For seed coating purposes, *Fusarium* isolates were fermented in aqueous sorghum straw (1.5 %(w/v)) medium for 48 hours using a bench-top fermenter as described above. The harvested biomass was centrifuged and filtrated and the filtrate left to air-dry over night. The dried biomass was ground to a fine powder using pestle and mortar or a laboratory mill (4000 rpm for 10 sec) and then passed through a 100 µm mesh screen. Cfu in the inoculum were determined by adding 0.1g of the powder to 10 ml water. The solution was vortexed from time to time for 30 min, serial dilutions were made and an aliquot of 0.1 ml was plated on half-strength PDA. Cfu/g were calculated after 3 days of incubation at room temperature. Seed coating was accomplished by SUET using this inoculum and Arabic gum and the one selected by the company as adhesive materials.

The number of cfu per coated seed was determined by taking four samples of 25 seeds each. The seeds were soaked in 25 ml sterilized deionized water for 30 min, an appropriate serial dilution was made and 0.1 ml were plated on half-strength PDA. Cfu per sorghum seed were determined after 3 days of incubation at room temperature.

Effects of the seed coating technique on sorghum seed germination was assayed by the blotter test and by sowing 20 coated seeds per treatment in the soil in petri-dishes (14 cm dia.).

3.1.7 Production of chlamydospores by *Fusarium* species

The effect of different media on chlamydospore production by the two fungal isolates was tested. These included the following:

1- SNB (Special Nutrient-poor Broth):

KH_2PO_4	1.0 g
KNO_3	1.0 g
$MgSO_4$. 7 H_2 O	0.5 g
KCl	0.5 g
Glucose	0.2 g
Sucrose	0.2 g
H_2O deion.	1000 ml

2- Celery medium:

Celery extract was prepared by autoclaving 20 g of celery in 100 ml distilled water. The filtrated extract (10 %, v/v) was added to deionized water.

3- SNB + celery:

Celery extract (10 %, v/v) was added to SNB.

4- SNB + yeast:

Yeast extract (0.2 %, w/v) was added to SNB

5- Sorghum straw medium

1.5 % (w/v) of very finely milled (> 500 µm) sorghum straw (from Sudan) was added to deionized water.

6- V8 + 0.03M Na_2SO_4

Vegetable juice	200.0 ml (albi®, Germany)
$CaCO_3$	2.0 g
Na_2SO_4	4.3 g
H_2O deion.	1000 ml

7- Czapex-Dox (CD) broth:

$NaNO_3$	2.0 g
KH_2PO_4	1.0 g
$MgSO_4$	0.5 g
KCl	0.5 g
$FeSO_4$	0.01g
Sucrose	30.0 g
H_2O deion.	1000 ml

8- Malt extract medium

Malt extract (Biomalt, Kirn, Germany, 2 % (v/v)) was added to deionized water.

9- SNB + Talc (ROTH®)

Talc (5 % (w/v)) was added to SNB.

10- SNB + Bentonite (ROTH®)

Bentonite (5 % (w/v)) was added to SNB.

200 ppm chloramphenicol was added to each medium. 100 ml of each medium in an Erlenmeyer flask were autoclaved at 121°C and 1.2 bars for 20 min, then inoculated with FN and FA agar plugs (1 cm dia.) taken from stock cultures of FN and FA on SNA. Then they were incubated in continuous darkness on a rotary shaker at 150 rpm. Chlamydospore formation was checked weekly for four weeks in two flasks from each medium. The content of the flasks was blended for a few seconds to detach chlamydospores from the mycelium and then filtered through a double layer of cheesecloth to remove mycelial fragments. The number of spores/ml was determined using a heamacytometer.

3.1.8 Identification of trichothecene mycotoxins

The ability of FA to produce trichothecene mycotoxins that could be a hazard to humans or animals was assessed from samples of the fungus growing on autoclaved wheat grains. After three weeks of incubation at 28-30°C, grains were air-

dried and ground in a laboratory mill. Non-inoculated wheat grains were produced in the same way as control. In addition the harvested seeds from the inoculated plots that contain 1.5 g of the mycoherbicides (“Pesta” and alginate granules) and seed teatment with both isolates using Arabic gum and from the untreated control were tested for the possibility of trichothecene mycotoxins translocation to seeds under field conditions. Random sample from each treatment and replicate were ground as described above. The identification of toxins using gas chromatography / mass spectrometry as described by Schollenberger and others (2005) was carried out by the Institute for Animal Nutrition, University of Hohenheim, Stuttgart, Germany.

3.2 Field experiments

3.2.1 Site description

The field experiments were conducted at the demonstration farm at Abuharaz Faculty of Agriculture and Natural Resources, University of Gezira, Wad Medani, Sudan, in 2003 and 2004. The location of the site was 33˚ 31' N and 14˚ 25' E with an altitude of 405 m. The climate is typical for central Sudan, which is tropical semi-arid characterized by a short summer rainy season (Walsh, 1991) with an annual precipitation of 200-300 mm generally falling between June and October reaching its peak during July/August. The mean maximum day temperature varied between 36.3-40.1°C and the mean minimum day temperature ranged from 20.1-24.9°C during the sorghum growing season (Meteorological station at Wad Medani, see appendix 1). The soil was a typical Gezira soil which is classified as Vertisol, characterized by deep dark color, a low organic matter content, low permeability, and deep cracks when dry. The clay content is 58%. The soil reaction is moderately alkaline (pH=8.1), non saline (EC<0.3dS/m) and slightly sodic (ESP=18%) (Agriculture Research and Biotechnology Corporation, 2005) The farm used to be planted with crops such as sorghum, maize, millet, sunflower, fodder sorghum, feed legumes and summer and winter vegetables. There is no fixed rotation adopted, but usually legume forage crops were sown after cereal crops.

3.2.2 First season experiments

3.2.2.1 Screening for the optimum dose of formulated bioagents and testing the seed coating technique for *Striga* control under field conditions

The area selected is known to be naturally infested with *Striga*, however artificial infestation with *Striga* seeds (0.1 g *Striga* seeds/ kg) (*Striga* seed viability > 80%) - applied by broadcasting followed by disc harrowing - was carried out to ensure a homogenous distribution of *Striga* seeds throughout the experimental plots. Sub-plots were 4.0m x 3.3m, arranged in a randomized complete block design with four replicates. The sub-plots were separated from each other by 2 meters and from the irrigation canal by 1.5 meters, so as to reduce the transmission of *Fusarium* spores from one plot to another. The experimental area was irrigated before sowing to close the soil cracks and to maintain adequate moisture for better fungal growth. Two application methods were tested: placement of formulated material in the planting holes and seed coating. In the placement method, four levels of “Pesta” granules were used (0.5, 1.0, 1.5 and 2.0 g from each fungal isolate and their combination), in addition to control 1 (untreated), control 2 (treated with the fungus-free granules) and chemical control using 2,4-D at a rate of 0.77 a.i. ha^{-1} in a post-emergence application (see table 1). Sorghum (cv. Wad Ahmed) seeds (treated with thiram 25% at 3.0 g/ kg sorghum seed against soil born disease) were sown by hand in ridges 80 cm apart with 15 cm distance between holes and thinned to two plants per hole 2 weeks after emergence. Weeds other than *Striga* were handpicked every 2 weeks. Urea at 95 kg ha^{-1} was applied by hand at the bases of sorghum plants 4 weeks after sowing. The crop was irrigated by furrow irrigation every 10-15 days depending on rainfall. Parameters evaluated include the following:

- *Striga* incidence (% of infested holes) and the total number of *Striga* plants/ plot (healthy, diseased, dead and flowering), counted in the middle rows in each plot every 10-15 days.
- Sorghum plant height measured 2 weeks after the emergence of *Striga,* then measured every 2 weeks.
- *Striga* biomass (dead *Striga* was collected throughout the season), measured at harvesting time.
- Sorghum straw yield (t/ha) and sorghum 100-seed weight determined also from the middle rows (because of the attack by sorghum head bugs (*Agonoscelis* sp.), sorghum 100 seed weight was determined instead of sorghum yield per unit area).

Table 1. Treatments applied in the first season experiment (2003/2004)

Treatment		No. of cfus g^{-1} formulated mycoherbicide or $seed^{-1}$
1.	Untreated control	-
2.	Just “Pesta” granules	-
3.	Chemical control	-
4.	0.5 g FN	$5.2x10^7$
5.	1g FN	"
6.	1.5 g FN	"
7.	2 g FN	"
8.	0.5 g FA	$5.7x10^7$
9.	1g FA	"
10.	1.5 g FA	"
11.	2 g FA	"
12.	0.5 g FN and FA	$5.5x10^7$
13.	1g FN and FA	"
14.	1.5g FN and FA	"
15.	2 g FN and FA	"
16.	Seeds coated with FN using fine “Pesta” granules	$10.4x10^2$
17.	Seeds coated with FA using fine “Pesta” granules	$8.5x10^2$

FN = *Fusarium nygamai*, FA = *Fusarium* Abuharaz. The formulated material was added to the planting hole. Chemical control of *Striga* was applied using 2,4-D at a rate of 0.77 kg a.i. ha^{-1} as post emergence application.

3.2.2.2 Testing the stability of the formulated bioagents in the soil

To assay the stability of both formulated biocontrol agents in the soil, soil samples were taken 0, 5, 15, 25, 45 and 75 days after sowing with an auger at 10cm depth from plots with heighest application dose of “Pesta” granules (2 g/hole) for both isolates and from the untreated control, samples were taken between holes. The samples within each replicate were mixed well together, then a representative sample was made from each replicate. Two sub-samples of 0.5 g were taken per sample. Each sample was put in a test tube and 10 ml water were added. The solution was vortexed for 30 sec and left for approximately 30 min. Afterwards, the

content was vortexed again for another 30 sec, then appropriate dilutions were made, from which two samples of 0.2 ml were pipetted and spread over plates containing PDA+PCNB agar. The agar plates were incubated at room temperature and cfu were determined after 3 days.

3.2.3 Second season experiments

3.2.3.1 Evaluation of "Pesta" and alginate formulations and seed coating technique to control *Striga*

The second season experiment was set to evaluate the efficacy of "Pesta" and alginate formulations and an advanced seed coating technique in controlling *Striga* under field conditions. The area selected was for the first time to be sown with sorghum, therefore artificial infestation with *Striga* seeds (1g *Striga* seed/ kg soil) (*Striga* seed viability > 80%) was carried out applied at a rate of 5 g/ sowing hole. During the first season, the *Striga* counting was erratic and this was attributed mainly to a high and uneven distribution of *S. hermonthica* seeds in the soil. This problem was tried to be avoided by increasing the number of replications and changing the method of artificial infestation with *Striga* seeds, which were then sown into the planting hole instead of broadcasting them followed by harrowing which had been applied during the first season. Sub-plots were 4.0m x 2.4m, arranged in a randomized complete block design with 6 replicates. The sub-plots were separated from each other by 2 m and from the irrigation canal by 1.5 m, so as to reduce the transmission of *Fusarium* spores from one plot to another. The experimental area was pre-irrigated. Two application methods for the biocontrol agents were tested: placement of formulated material in the planting holes and seed coating. In the placement method, 1.5 g of "Pesta" or alginate preparations for the two *Fusarium* species were used. Metalaxyl used against damping off with *Fusarium* seed treatment at a dose of 0.31 g kg^{-1} sorghum seed. The treatments applied are shown in Table 3. Sorghum (cv. Wad Ahmed) seeds were sown in ridges 80 cm apart and with 15cm distance between holes. The cultural practices and parameters evaluated were similar to the first season experiment. In addition, sorghum yield ha^{-1} was determined.

3.2.3.2 Testing the stability of the formulated biocontrol agents in the soil

The stability of the formulated biocontrol agents in the soil was assayed in the treatments where "Pesta" or alginate preparations had been applied in comparison to the untreated control as described above using 3 replicates out of 6 replicates. In addition data were corrected for percent soil moisture before analysis.

Table 2. Treatments applied in the second season experiment (2004/05)

Treatment	No. of cfus g^{-1} formulated mycoherbicide or $seed^{-1}$
1. Untreated control	-
2. Just "Pesta" granules	-
3. Just Alginate granules	-
4. Seed coated with SUET only	-
5. Seed coated with Arabic gum only	-
6. Chemical control	-
7. FN "Pesta" granules	$3.1x10^7$
8. FA "Pesta" granules	$3.4x10^7$
9. FN alginate granules	$7.2x10^7$
10. FA alginate granules	$11.5x10^7$
11. Seeds coated with FN using SUET	$31.7x10^3$
12. Seeds coated with FA using SUET	$7.9x10^3$
13. Seeds coated with FN using Arabic gum	$185x10^3$
14. Seeds coated with FA using Arabic gum	$42x10^3$
15. Seeds coated with FN using fine "Pesta" granules	$4.2x10^2$
16. Seed coated with FA using fine "Pesta" granules	$6.6x10^2$

FN = *Fusarium nygamai*, FA = *Fusarium* Abuharaz. The formulated material was added at a rate of 1.5 g per planting hole. Chemical control of *Striga* was applied using 2,4-D at a rate of 0.77 kg a.i. ha^{-1} as post emergence application.

3.2.4 Pot experiment to evaluate the efficacy of "Pesta" and alginate formulations and seed coating technique in *Striga* control.

An out-door pot experiment was conducted in the nursery of the Abuharaz Faculty of Agriculture and Natural Resources using plastic pots with 30cm diameter filled with 5kg of a mixture of non sterilized river alluvial soil and sand (1:1, v/v). Compromised eighteen different treatments as shown in table 2. Granular formulations were applied at 1.5 g per pot. All pots were artificially infested by adding 5 g *Striga*-infested soil (1g *Striga* seed / 1kg soil) (80 – 90% viability) to the planting holes except in the positive control. Each pot was sown with 5 sorghum seeds Wad Ahmed cultivar. Two weeks after germination, plants were thinned to two plants per pot. Pots were fertilized using urea at a rate of 90 kg ha^{-1} and irrigated every two days. Parameters evaluated include the total number of *Striga* and the proportion of diseased *Striga* shoots every two weeks after *Striga* emergence. At harvest, *Striga* biomass and sorghum plant height and shoot dry weight were determined.

3.3 Statistical analysis

Statistical analysis was performed using STATISTICA software version 5.0 (Stat Soft Inc., 1997) hence normal distribution and homogeneity of variance were tested before doing analysis of variance (ANOVA). Data, which were not normally distributed, were subjected to log-transformation. Percentage data were arcsine-transformed before analysis (Gomez and Gomez, 1984). Multiple mean comparisons were performed using Tukey's HSD-test or LSD test at $p \leq 0.05$. In addition multiple comparisons using a general linear model (GLM) was carried out to analyze the quantative treatments (mycoherbicide doses) using SAS® software version 8.2

Table 3. Treatments used in the pot experiment.

Treatment		No. of cfus g^{-1} formulated mycoherbicide or $seed^{-1}$
1-	Negative control (untreated)	-
2-	Positive Control (treated with *Striga* only)	-
3-	Just “Pesta” granules	-
4-	Just Alginate granules	-
5-	FN “Pesta” granules	$3.1x10^7$
6-	FA “Pesta” granules	$3.4x10^7$
7-	FN wheat (10% (w/v) alginate granules	$7.2x10^7$
8-	FA wheat (10% (w/v) alginate granules	$11.5x10^7$
9-	FN sorghum (6% (w/v) alginate granules	$57.7x10^7$
10-	FA sorghum (6% (w/v) alginate granules	$33.3x10^7$
11-	FN yeast (6% (w/v) alginate granules	$92x10^7$
12-	FA yeast (6% (w/v) alginate granules	$49.3x10^7$
13-	Seed coated with FN using SUET	$31.7x10^3$
14-	Seed coated with FA using SUET	$7.9x10^3$
15-	Seed coated with FN using Arabic gum	$185x10^3$
16-	Seed coated with FA using Arabic gum	$42x10^3$
17-	Seed coated with FN using fine “Pesta” granules	$4.2x10^2$
18-	Seed coated with FA using fine “Pesta” granules	$6.6x10^2$

* Granular materials were added at a rate of 1.5 g per planting hole.

4 Results

4.1 Laboratory experiments

4.1.1 Inoculum production

F. nygamai (FN) and *F.* sp. Abuharaz isolate (FA) were found to sporulate well in all liquid media: PDB, SNB + 0.2% yeast, Vegetable juice medium (V8) and Richard's solution (RS). V8 and RS media yielded a significantly higher number of conidia ml^{-1} after incubation for five days at room temperature compared to PDB (Table 4). Richard's solution gave the highest number of conidia (10^8 ml^{-1}) after five days of incubation. For this reason it was selected as growth medium for formulation purposes throughout this study.

Increasing the incubation period up to fourteen days didn't increase *Fusarium* sporulation markedly in all types of media tested. Spores produced by both *Fusarium* isolates in all tested media were micro- and macroconidia, however, few chlamydospores were observed in SNB + yeast after fourteen days.

Table 4. Sporulation of *Fusarium* species in different liquid culture

Medium	Conidia ml^{-1} x 10^7 *F. nygamai*	*F.* Abuharaz
PDB	2.3[a] (0.4)	0.9[a] (0.05)
SNB + Yeast	2.5[a] (0.2)	4.4[a] (0.5)
Vegetable juice	9.5[b] (1.3)	9.4[b] (1.0)
Richard's solution	11.0[b] (0.6)	14.6[b] (2.4)

Data are means of three replicates, numbers in parentheses indicate standard error of mean, means followed by the same letter(s) are not statistically different using Tukeys HSD – Test at $p \leq 0.05$.

4.1.2 Formulation of fungal isolates in “Pesta” granules

FN and FA were successfully formulated in “Pesta” granules. Cfus after encapsulation were found 3.1-5.2x10^7 and 3.4-5.7x10^7 per gram of formulated material for FN and FA, respectively (Table 5).

4.1.3 Formulation of fungal isolates in alginate granules

FN and FA were formulated in alginate granules amended either with 10% wheat flour or 6% sorghum flour or yeast extract. The size of the pellets was ≤ 3.15 mm and cfu were 7.2-11.5x10^7 (using wheat flour), 3.3-5.7x10^8 (using sorghum flour) and 4.9-9.2 x 10^8 (using yeast extract). Alginate preparations amended with 6% sorghum flour or yeast extract had significantly higher cfu per gram formulated material compared to the alginate formulation using 10% wheat flour for both isolates (Table 5). Yeast extract amendment significantly increased the number of cfu by 37.7 and 32.3% for FN and FA respectively compared to sorghum amendment. Generally FN alginate formulations amended with sorghum and yeast extract had significantly higher cfu than FA alginate formulation amended with sorghum. However, no statistical difference was found regarding the cfu from wheat alginate and “Pesta” formulations between the two isolates**.**

Table 5. Determination of cfu in “Pesta” and alginate formulations

Formulation type	cfux10^7 g^{-1} *F. nygamai*	*F.* Abuharaz
“Pesta”	3.1 [a] (0.4)	3.4 [a] (0.4)
Alginate granules amended with 10 % wheat flour	7.2 [c] (0.3)	11.5 [c] (1.9)
Alginate granules amended with 6 % sorghum flour)	57.7 [b] (2.2)	33.3 [d] (3.2)
Alginate granules amended with 6 % yeast extract	92.0 [a] (6.1)	49.3 [be] (1.5)

Data are means of three replicates, numbers in parentheses indicate the standard error of mean, means followed by the same letter(s) within one isolate and formulation are not statistically different using Tukeys HSD – Test at $p \leq 0.05$.

4.1.4 Comparison between the development of fungal populations after the application of different formulations in the soil

In all treatments, fungal propagules were detected in the soil until the end of the experiment, i.e. for 9 weeks, with approximately 10^5 cfu g^{-1} dry soil (Fig. 1). FN in alginate granules and FA formulated in "Pesta" granules showed an increase in propagule numbers in the third week by 122.7% and 88.7% respectively compared to the initial inoculum, respectively. In contrast FA in alginate granules and FN formulated in "Pesta" granules showed a decrease in propagule numbers in the third week by 60.6% and 64.8% compared to the initial inoculum, respectively. Thereafter, fungal populations in the soil decreased with time irrespective of the type of formulation. After 9 weeks, FN in alginate granules showed the lowest reduction in population by ca 51% followed by FA in "Pesta" granules (56%) compared to the initial inoculum.

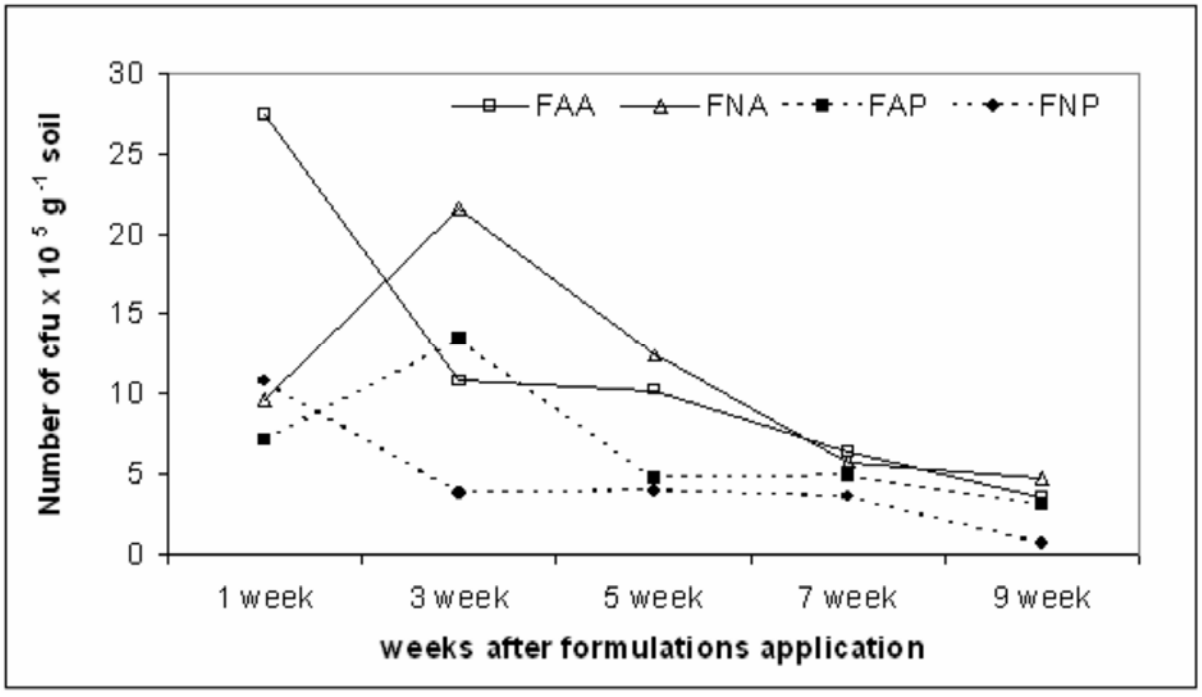

Fig. 1. Development of fungal populations in the soil after the application of different formulations. FAA= *F*. Abuharaz alginate formulation, FNA= *F. nygamai* alginate formulation, FAP= *F*. Abuharaz "Pesta" formulation, FNP= *F. nygamai* "Pesta" formulation. *Fusarium* population (cfu / g soil) was corrected to soil moisture and to *Fusarium* population background in the soil.

4.1.5 Seed treatment experiments

4.1.5.1 Seed soaking

Fusarium propagules were found to coat sorghum seeds when they had been soaked in fungal inoculum (10^9 cfu ml^{-1}) for 30 min. Cfu ranged from 3.3 to 30.0x10^4 per seed. Using a superabsorbent polymer or sodium alginate as additional amendment to the inoculum did not significantly increase the number of cfu on sorghum seeds compared to soaking in fungal inoculum only (Table 6).

Table 6. Determination of cfu on sorghum seeds soaked in fungal inoculum

Treatment	cfux10^4 $seed^{-1}$ *F. nygamai*	*F*. Abuharaz
Inoculum only	30.0 (9.8)	10.4 (0.4)
Inoculum+ superabsorbent polymer	25.7 (6.9)	3.3 (0.3)
Inoculum + alginate	29.7 (12.7)	16.3 (2.2)

Data are means of 3 replicates, numbers in parentheses indicate the standard error of mean. No statistically significant difference was found between the different treatments using Tukeys HSD – Test at $p \leq 0.05$.

Seed soaking in fungal inoculum had a negative effect on sorghum seed germination. *F. nygamai* amended with the polymer or alginate inhibited sorghum seed germination by 10-15% compared to the untreated control, while FA inoculum amended or not was found to inhibit germination by 5-20 % compared to the untreated control. Using the superabsorbent polymer and sodium alginate increased the adverse effect of seed soaking on sorghum seed germination compared to the untreated control and the treatment with non-amended fungal inoculum (Table 7).

Both fungi recovered easily from the soaked seeds and well colonized sorghum seeds. They also colonized the sorghum root externally up to 10 cm length. Both isolates could not be found on roots at 20 cm length or on root tips. They were neither reisolated from sorghum roots at any length after surface sterilization.

Table 7. Germination of sorghum seeds soaked in fungal inoculum

Medium	Germination (%)	
	F. nygamai	*F.* Abuharaz
Control	100	100
Inoculum only	100	95
Inoculum + polymer	85	75
Inoculum + alginate	90	80

4.1.5.2 Seed coating

A simple seed coating technique was adopted using fine "Pesta'' granules (>1 mm) and 40% Arabic gum as adhesive material. Using this technique, sorghum seeds were successfully coated with *Fusarium* spp. with 4.2-10.4x10^2 cfu per seed (FN) and 6.6-8.5x10^2 (FA) (Fig 2).

When testing the effect of adhesive materials on the growth of *Fusarium* species on solid media, cellulose 1 and organic polymer 1 significantly increased the colony diameter of FN and FA compared to non-amended PDA and other tested adhesive materials (Fig. 3). In contrast, organic polymer 2 significantly inhibited the growth of *F. nygamai* compared to PDA.

Fig. 2. Sorghum seeds coated with fine "Pesta" granules

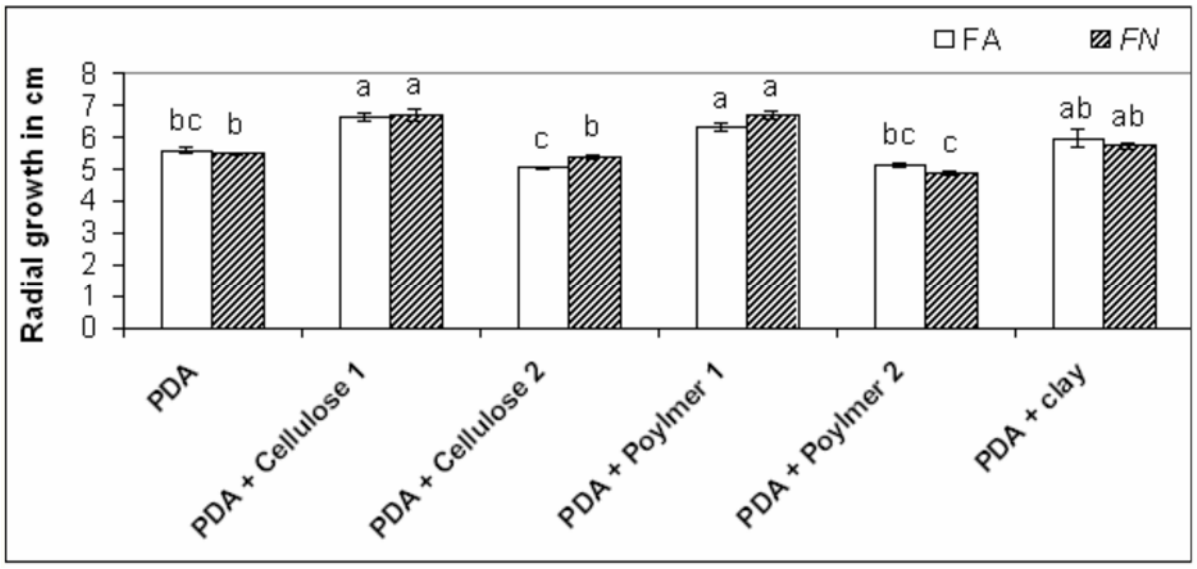

Fig. 3. Effect of adhesive materials on the colony diameter of *Fusarium* species on solid media Data are means of five replicates, adhesive materials were added to PDA at a rate of 10 % (w/v), ⌶ = standard error of means, bars with the same letter(s) are not statistically different using Tukeys HSD – Test at $p \leq 0.05$.

The adhesive materials were added to Richard's solution to study their effect on sporulation of *Fusarium* species. All tested materials significantly inhibited the production of micro- and macroconidia of both *Fusarium* species compared to the sporulation in Richard's solution without any adhesive materials (Fig 4a). Organic polymer 1 and clay gave 2-8 fold higher conidial numbers per ml compared to the other adhesive materials. Also they significantly enhanced the production of chlamydospores, especially by FA, compared to Richard's solution alone or amended with the other tested materials (Fig 4b).

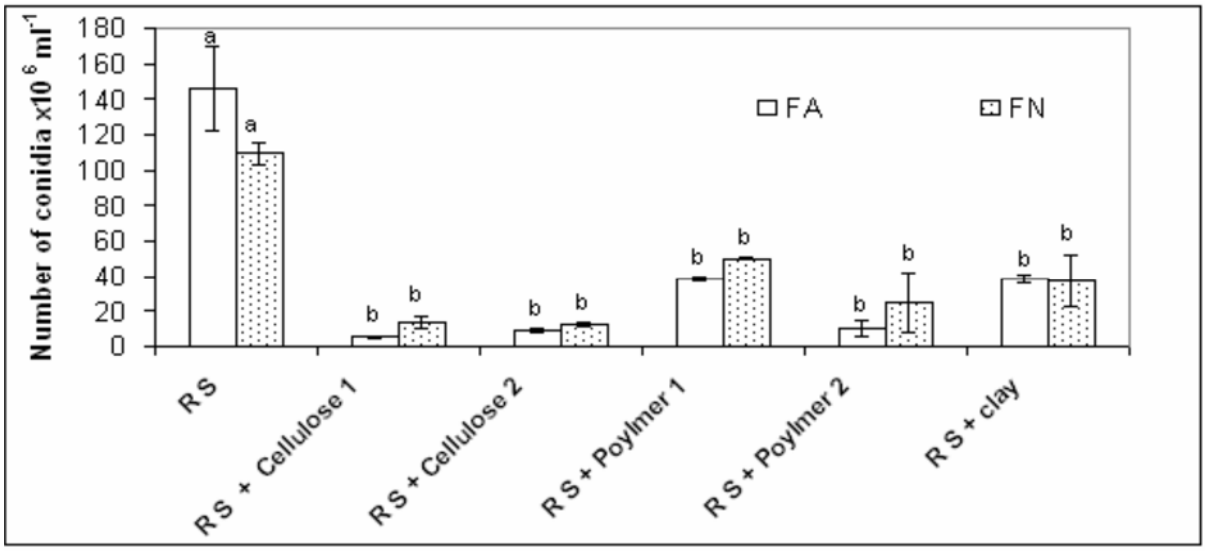

Fig. 4a. Effect of adhesive materials on conidia production by *Fusarium* species.

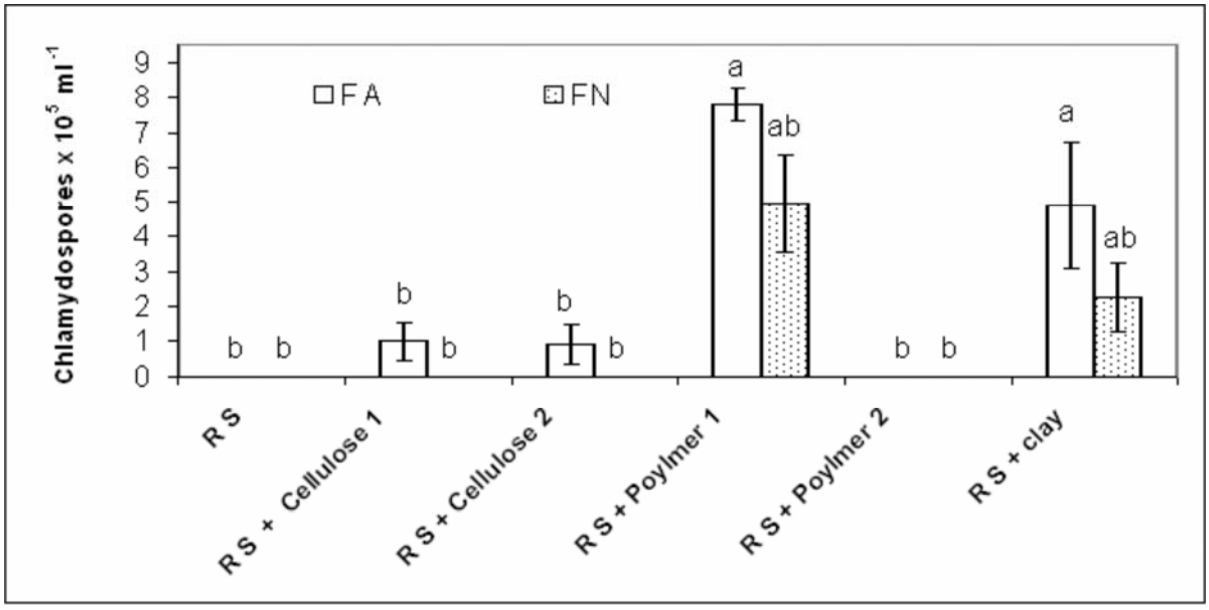

Fig. 4b. Effect of adhesive materials on chlamydospore production by *Fusarium* species. Data are means of three replicates, FA= *F*. Abuharaz, FN= *F. nygamai,* RS= Richard's solution medium, ⌶ = standard error of means, bars with the same letter(s) are not statistically different within the same isolate using Tukeys HSD – Test at $p \leq 0.05$.

Fungal biomass harvested from sorghum straw medium with $1.4x10^{8}$ cfu g^{-1} *(F. nygamai*) and $1.8x10^{8}$ g^{-1} (*F. Abuharaz*) was evenly adhered around sorghum seeds. Arabic gum was observed to give a better coverage of sorghum seeds compared to the adhesive materials provided by SUET, as shown in Fig. 5.

Fig. 5. Sorghum seeds coated with fungal inoculum fermented on sorghum straw

Cfu per sorghum seed was $7.9x10^3$ (FA) and $3.2x10^4$ (FN) using the adhesive material provided by SUET and $4.2x10^4$ g^{-1} (FA) and $1.9x10^5$ g^{-1} (FN) using Arabic gum. For FN, the number of cfu per seed obtained with Arabic gum was significantly higher compared to the preparation with the other adhesive material.

Seed coating with fungal biomass significantly affected sorghum seed germination compared to uncoated sorghum seeds and to those coated with adhesive materials only. Generally, seed coating with fungal biomass was observed to delay sorghum seed germination compared to the control. FA had a worse effect compared to FN, it reduced sorghum germination by 25-68.8 % in the blotter test (Table 8). However, when the germination test was performed in soil using Petri dishes, the germination of coated seeds was increased, irrespective to the fungal isolate and adhesive material used and became comparable to the control. Only the combination FA and Arabic gum resulted in a significantly lower germination than in the other treatments (Table 8).

Table 8. Effect of seed coating on germination of sorghum seeds

Treatments	cfu (x 10^3) per seed	Blotter test germination (%)	Soil test germination (%)
Control 1	0.0 [b] (0.0)	91.3 [a] (4.3)	42.5 [bc] (4.7)
Control 2	0.0 [b] (0.0)	86.3 [a] (2.4)	65.0 [ab] (8.7)
Control 3	0.0 [b] (0.0)	88.8 [a] (5.5)	82.5 [a] (2.5)
FA S	7.9 [b] (2.2)	36.3 [b] (15.3)	67.5 [ab] (2.5)
FN S	31.7 [b] (8.7)	48.8 [b] (4.2)	85.0 [a] (8.7)
FA G	42.0 [b] (8.1)	15.0 [b] (5.4)	27.5 [c] (2.5)
FN G	185.1 [a] (61.9)	48.8 [b] (10.9)	60.0 [ab] (9.1)

Control 1 = untreated control, Control 2 = seeds coated with adhesive material provided by SUET, Control 3 = seeds coated with Arabic gum, FAS = seeds coated with *F*. Abuharaz using adhesive material provided by SUET, FNS = seeds coated with *F. nygamai* using adhesive material provided by SUET, FAG = seeds coated with *F*. Abuharaz using Arabic gum, FNG = seeds coated with *F. nygamai* using Arabic gum. Numbers in parentheses indicate the standard error of mean, means followed by the same letter(s) are not statistically different using Tukeys HSD – Test at $p \leq 0.05$.

4.1.6 Media for chlamydospore production by *F. nygamai*

Different types of liquid media were tested for their ability to induce chlamydospore production in FN SNB + yeast was found to give the highest number of chlamydospores (10^5 ml^{-1}) throughout the incubation period among the tested media (Table 9a). On SNB and celery medium, the chlamydospore production started only in the second week of incubation and increased with increasing incubation period, giving chlamydospore numbers of $4.6x10^4$ and $3.3x10^4$ ml^{-1}, respectively. Adding celery extract to SNB at a rate of 10% (v/v) resulted in a 3 to 5 fold increased chlamydospore production ($5.5x10^4$ ml^{-1}) compared to SNB and celery medium alone, respectively, within the first week. Then the numbers of formed chlamydospores decreased with time. Czapek Dox medium stimulated the chlamydospore production within the first week and a slight increase was recorded with increasing incubation

time, giving a final rate of $5.5x10^4$ ml^{-1}. Vegetable juice + 0.03M Na_2So_4 had a similar stimulatory effect as SNB + celery extract. Malt extract enhanced the production of chlamydospores after three weeks of incubation, but after that no chlamydospores could be detected anymore. In sorghum straw medium, chlamydospores were observed in the third week and became countable in the fourth week ($2x10^4$).

Table 9a. Chlamydospore production by *F. nygamai* in different media

Medium type	I week	II week	III week	IV week
	Number of chlamydospores x 10^4 ml^{-1}			
SNA	0.0 [a] (0.0)	1.6 [b] (0.0)	4.5 [ab] (0.6)	4.6 [a] (2.6)
SNA +Yeast	4.0 [a] (4.0)	21.0 [a] (8.0)	21.5 [a] (8.5)	13.0 [a] (3.0)
Celery	0.0 [a] (0.0)	1.0 [b] (0.6)	2.8 [b] (0.8)	3.3 [b] (0.6)
SNA + Celery	0.0 [a] (0.0)	5.5 [ab] (0.0)	1.6 [b] (0.4)	1.0 [b] (0.2)
Czapek Dox	4.1 [a] (0.6)	3.8 [b] (1.0)	5.0 [ab] (1.4)	5.5 [ab] (2.0)
V8 + 0.03M Na_2So_4	0.0 [a] (0.0)	5.1 [a b] (0.4)	3.7 [b] (0.2)	2.4 [b] (0.4)
Malt extract	0.0 [a] (0.0)	0.0 [b] (0.0)	1.4 [b] (0.2	0.0 [b] (0.0)
Sorghum straw	0.0 [a] (0.0)	0.0 [b] (0.0)	0.0 [b] (0.0)	2.0 [b] (1.2)

Data are means of two replicates, SNB= Special nutrient-poor Broth, V8= Vegetable juice Broth + 0.03M Na_2So_4. Numbers in parentheses indicate the standard error of means, means followed by the same letter(s) are not statistically different using Tukeys HSD – Test at $p \leq 0.05$.

4.1.7 Media for chlamydospore production by *F. Abuharaz*

FA was also found to give the highest number of chlamydospores (10^5 ml^{-1}) in SNB + yeast (Table 9b). FA started to produce chlamydospores in the second week and did not reach a peak within the four weeks incubation time. Czapek Dox, V8 + 0.03M

Na_2So_4 and malt extract did not enhance chlamydospore production of FA. Sorghum straw medium had a similar stimulatory effect on chlamydospore production as observed with FN ($2x10^4$ ml^{-1}).

Table 9b. Chlamydospore production by *F*. Abuharaz in different media

Media type	I week	II week	III week	IV week
	Number of chlamydospores x 10^4 ml^{-1}			
SNA	0.0 [a] (0.0)	0.4 [b] (0.0)	1.2 [b] (0.4)	1.2 [bc] (0.8)
SNA + Yeast	0.0 [a] (0.0)	7.3 [a] (1.8)	7.7 [a] (0.1)	20.3 [a] (0.4)
Celery	0.0 (0.0)	0.8 (0.8)	1.4 [b] (0.2)	0.2 [c] (0.2)
SNB + Celery	0.6 [a] (0.6)	4.9 [b] (1.0)	5.1 [b] (3.1)	4.2 [b] (1.8)
Czapek –Dox	0.0 [a] (0.0)	0.0 [b] (0.0)	0.0 [b] (0.0)	0.0 [c] (0.0)
V8 + 0.03M Na_2So_4	0.0 [a] (0.0)	0.0 [b] (0.0)	0.0 [b] (0.0)	0.0 [c] (0.0)
Malt extract	0.0 [a] (0.0)	0.0 [b] (0.0)	0.0 [b] (0.0)	0.0 [c] (0.0)
Sorghum straw	0.0 [a] (0.0)	0.0 [b] (0.0)	0.0 [b] (0.0)	0.2 [c] (0.2)

Data are means of two replicates, SNB= Special nutrient-poor broth, V8= Vegetable juice broth + 0.03M Na_2So_4. Numbers in parentheses indicate the standard error of mean, means followed by the same letter(s) are not statistically different using Tukeys HSD – Test at $p \leq 0.05$.

4.1.8 Identification of trichothecene mycotoxins

FA propagated on wheat grain proved to produce none of the following trichothecene toxins: nivalenol, deoxynivalenol, 15-actetyldeoxynivalenol, fusarenon X, scirpentriol, monoacetoxyscirpenol, diacetoxyscirpenol, neosolaniol, T-2 triol, HT-2 toxin, and T-2

toxin. However, a faint spur of 3-acetyldeoxynivalenol had been observed indicating that FA could produce this toxin under certain conditions.

Similarly none of the above mentioned trichothecene toxins found to be translocated to harvested sorghum seeds after application of FN and FA mycoherbicides to the planting holes or the seeds.

4.2 Field experiment first season

4.2.1 Effect of *Fusarium* species on *Striga* incidence

Both *Fusarium* isolates were found to delay *Striga* emergence irrespective to the dose and method of application used. On the first count (30 days after sorghum sowing), 2g "Pesta" granules per planting hole containing FN delayed *Striga* emergence by 93% compared to the untreated control (Fig. 6a). At the end of the season the same dose of FN reduced *Striga* incidence only by 27% compared to the untreated control (Fig. 6a).

The results of the treatment with FN applied at a rate of 1.5g per planting hole were not presented, since most probably a technical problem occurred with this treatment.

Striga incidence was similarly reduced by 1.5 or 2.0g "Pesta" granules containing FA by 94.8% compared to the untreated control at the beginning of the season (Fig. 6b). By time, the reducing effect "Pesta" granules on *Striga* incidence decreased and the *Striga* incidence was reduced by only about 28% compared to the control using 1.5g of FA at the end of the season.

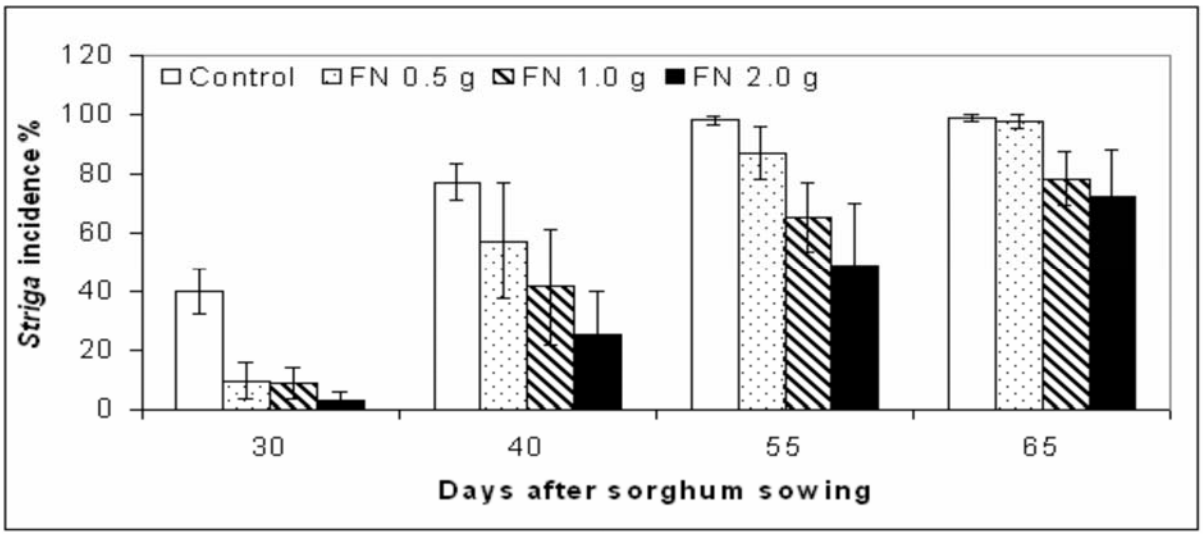

Fig. 6a. Effect of *Fusarium nygamai* applied in various doses on *Striga* incidence. FN= *Fusarium nygamai*, ⌶ = indicate ± standard error of means

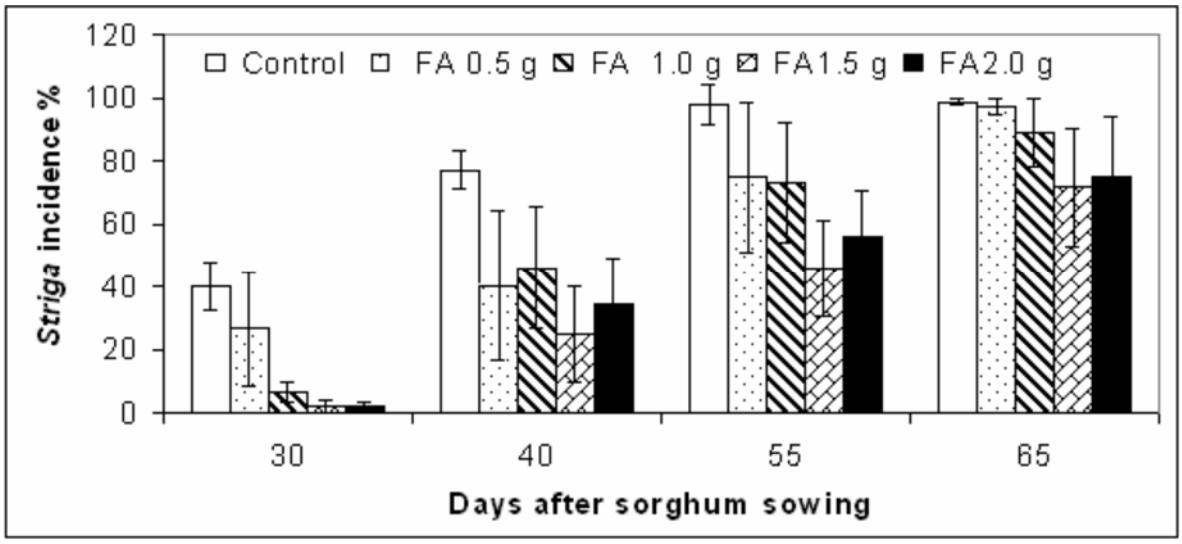

Fig. 6b. Effect of *Fusarium* Abuharaz applied in various doses on *Striga* incidence. FA= *Fusarium* Abuharaz isolate, ⌶ = indicate ± standard error of means.

The combination of the two fungal isolates at 1 or 1.5g also reduced *Striga* incidence early in the season by about 97% and 95% compared to the untreated control, respectively (Fig. 6c). Applying 1.5 g of the combined isolates still caused a reduction of 57% compared to the control on the third count (55 days after sorghum sowing).

Seed coating with both isolates similarly reduced *Striga* incidence by about 89% compared to the control early in the season (Fig. 6d). By the progress of the season the effect of seed coating treatments became insignificant compared to the control.

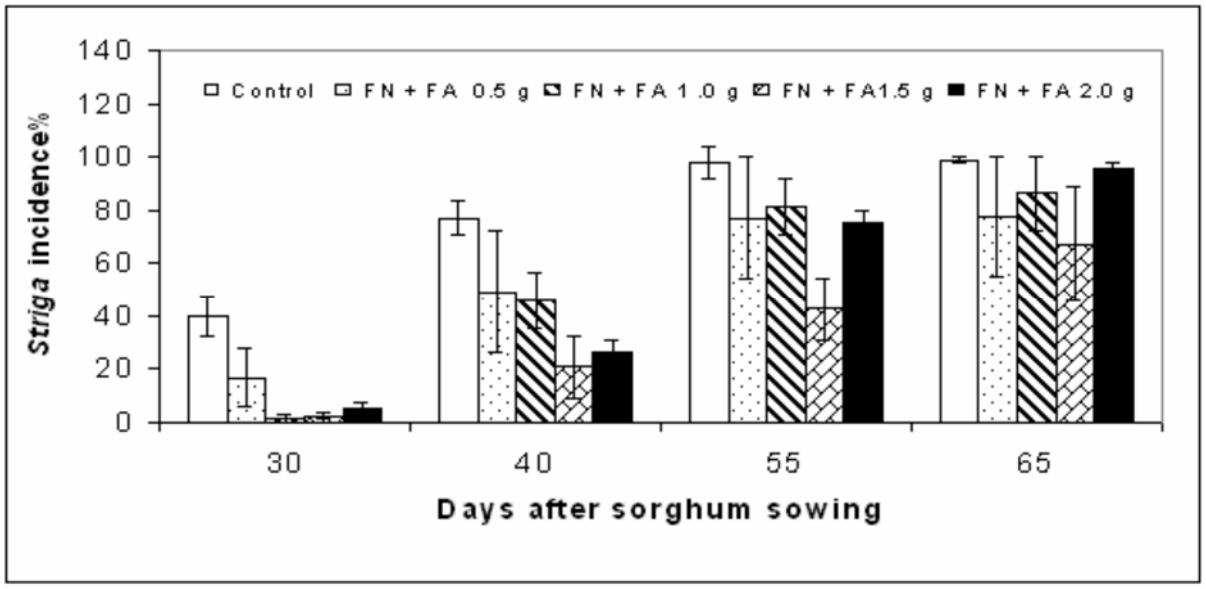

Fig. 6c. Effect of the combined fungal isolates on *Striga* incidence. FN= *Fusarium nygamai*, FA= *Fusarium* Abuharaz isolate, ⌶ = indicate ± standard error of means.

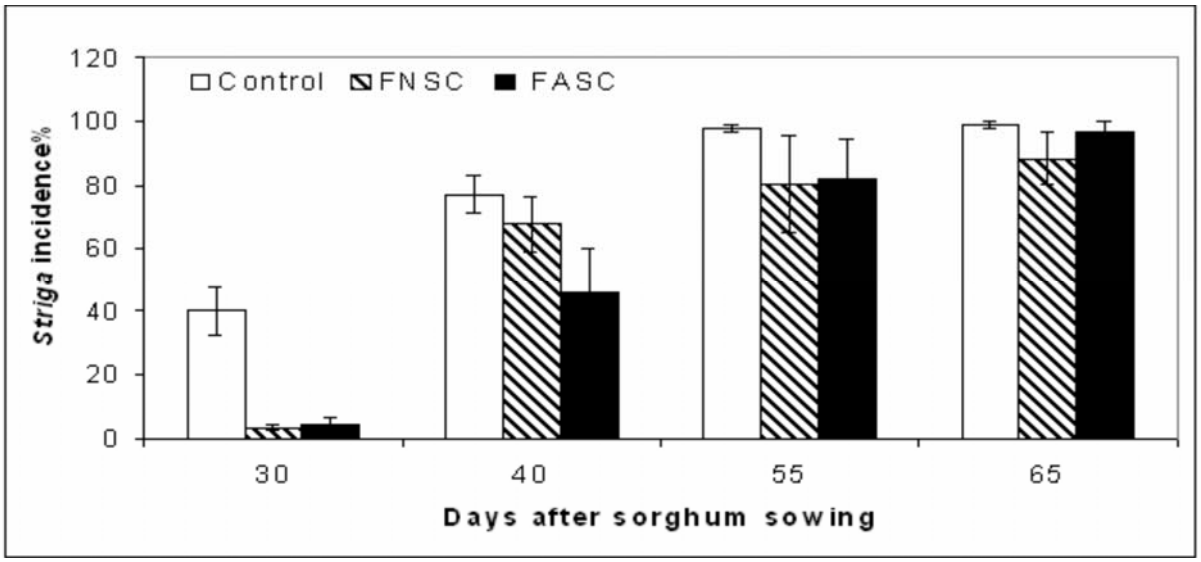

Fig. 6d. Effect of *Fusarium*-coated seeds on *Striga* incidence. FNSC= sorghum seeds coated with *F. nygamai* fine "Pesta" granules, FASC= sorghum seeds coated with *F.* Abuharaz fine "Pesta" granules. I = indicate ± Standard error of means.

Striga incidence was also reduced immediately after the application of the herbicide 2,4-D in the second count (40 days after sorghum sowing) by 90% compared to the control (Fig. 6e). However, by the end of the season the reduction was neither pronounced compared to the control nor to the fungal treatments.

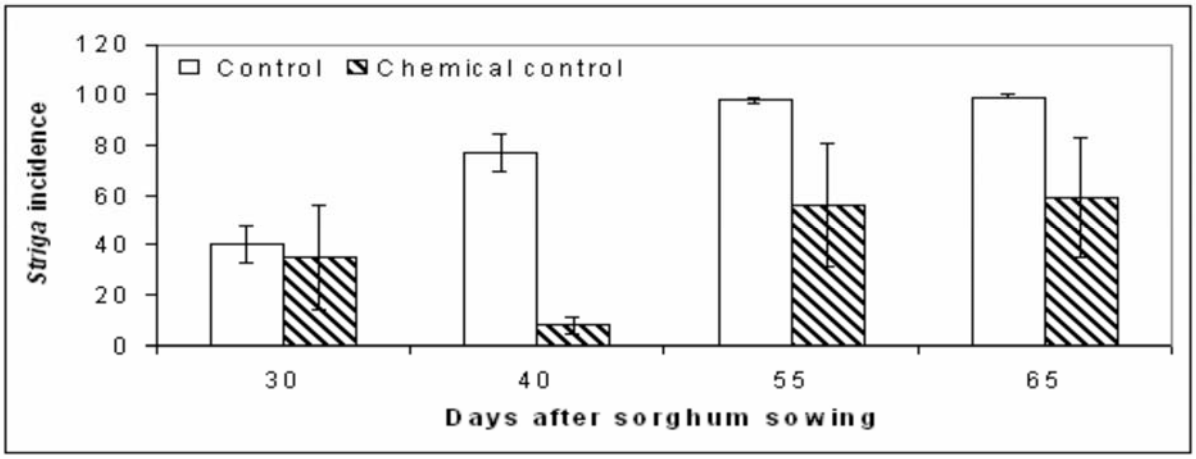

Fig. 6e. Effect of chemical control on *Striga* incidence

Using a general linear model (GLM) to analyze the quantitative treatments (different doses of granules applied), FA was the best in reducing *Striga* incidence significantly compared to the treatments without fungal doses until the third count as shown in table 10.

Table 10. Multiple comparisons using a general linear model (GLM) to show the effect of *Fusarium* species on *Striga* incidence

A: count I (30 DAS)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Block	3	480.5	160.2	0.55	0.7
Controls[a]	4	1178.5	294.3	1.00	0.4
FN[b]*Controls	1	6.1	6.1	0.02	0.9
FA[c]*Controls	1	2487.1	2487.1	8.47	0.01*
FN* FA[d]*Controls	1	84.7	84.7	0.29	0.6
Lack_of_fit	12	3834.8	319.6	1.09	0.4

B: count II (40 DAS)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Block	3	1342.4	447. 5	0.79	0.5
Controls[a]	4	3867.3	966.8	1.71	0.2
FN[b]*Controls	1	74.4	74.4	0.13	0.7
FA[c]*Controls	1	4081.3	4081.3	7.24	0.01*
FN* FA[d]*Controls	1	0.2	0.2	0.00	1.0
Lack_of_fit	12	4709.9	392.5	0.70	0.7

C: count III (50 DAS)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Block	3	1467.4	489.1	0.8	0.5
Controls[a]	4	1007.1	251.8	0.4	0.8
FN[b]*Controls	1	7.2	7.2	0.01	0.9
FA[c]*Controls	1	4821.5	4821.5	7.5	0.01*
FN* FA[d]*Controls	1	508.4	508.4	0.8	0.4
Lack_of_fit	12	6603.1	550.3	0.9	0.6

DAS= Days after sowing sorghum, [a] = treatments without fungal granular formulations. [b] =*F. nygamai* in 4 different doses, [c] =*F.* Abuharaz in 4 different doses, [d] =combination of the two isolates in 4 different doses, * = significant at P ≤ 0.05. Regression equation: *Striga* incidence (y)= Controls + xFN +xFA + xFN xFA.

4.2.2 Effect of *Fusarium* isolates on the total number of *Striga* shoots

The reduction of *Striga* incidence by the two fungal isolates was coupled with a reduction of the total number of *Striga* shoots. On the first count (30days after sorghum sowing), most doses of "Pesta" granules containing *Fusarium* isolates (FA 0.5 g and the combination 0.5 g) significantly reduced the total number of *Striga* shoots compared to the untreated control (Fig. 7a, b, c). By the end of the season, FN applied at 1.0 or 2.0 g per hole reduced the total number of *Striga* shoots by 78% and 72% compared to the control, respectively (Fig. 7a).

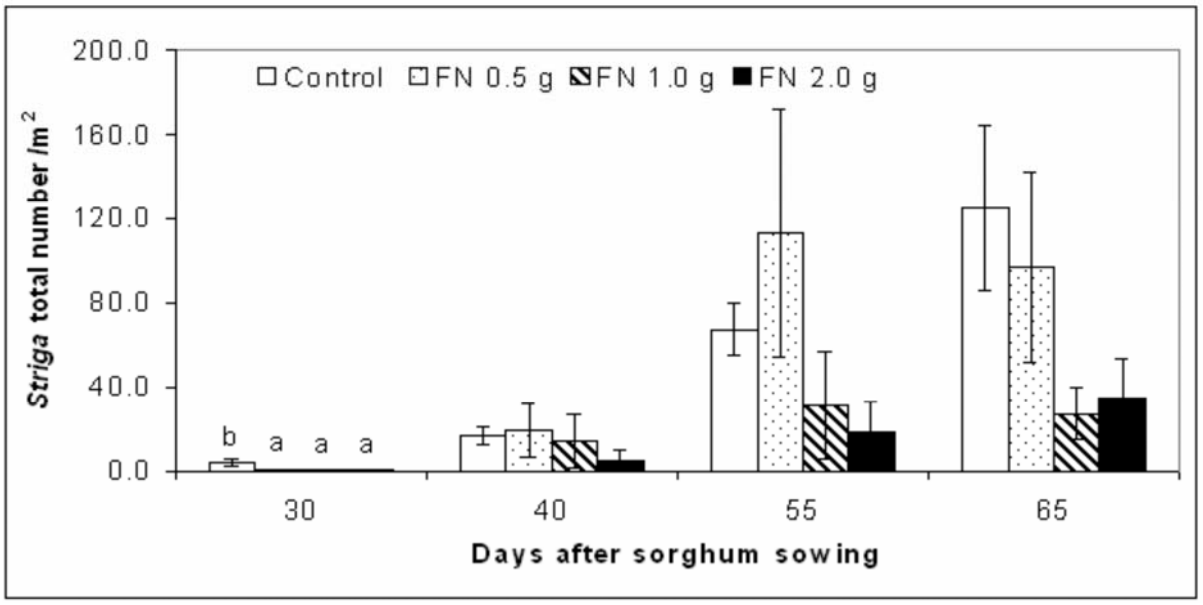

Fig. 7a. Effect of *Fusarium nygamai* on the total number of *Striga* shoots. N= *Fusarium nygamai*, ⌶ = indicate ± standard error of means, bars within the same count with same letter(s) are not statistically different after the protected LSD test at $p \leq 0.05$. No letters = data was not significant after analysis of variance.

FA reduced the total number of *Striga* shoots at the end of the season even more (by 82%) when applied as 1.5 g "Pesta" granules per planting hole. 2 g "Pesta" granules were less effective and reduced the total number of *Striga* shoots by 67% compared to the control (Fig. 7b).

The combination of the two isolates at 1 g was more effective in reducing the total number of *Striga* shoots compared to the single applications of FA and FN at 0.5 g (by 27% and 52% respectively). Using higher dosages, combining the two fungi did not have a more pronounced effect on the total number of *Striga* shoots compared to the single applications. The combination of the isolates applied as 1.5 g "Pesta"

granules per hole was able to reduce the number of the parasite shoots at the end of the season by 76% compared to the control (Fig. 7c), which is a comparable effect of FA applied alone at 1.5g.

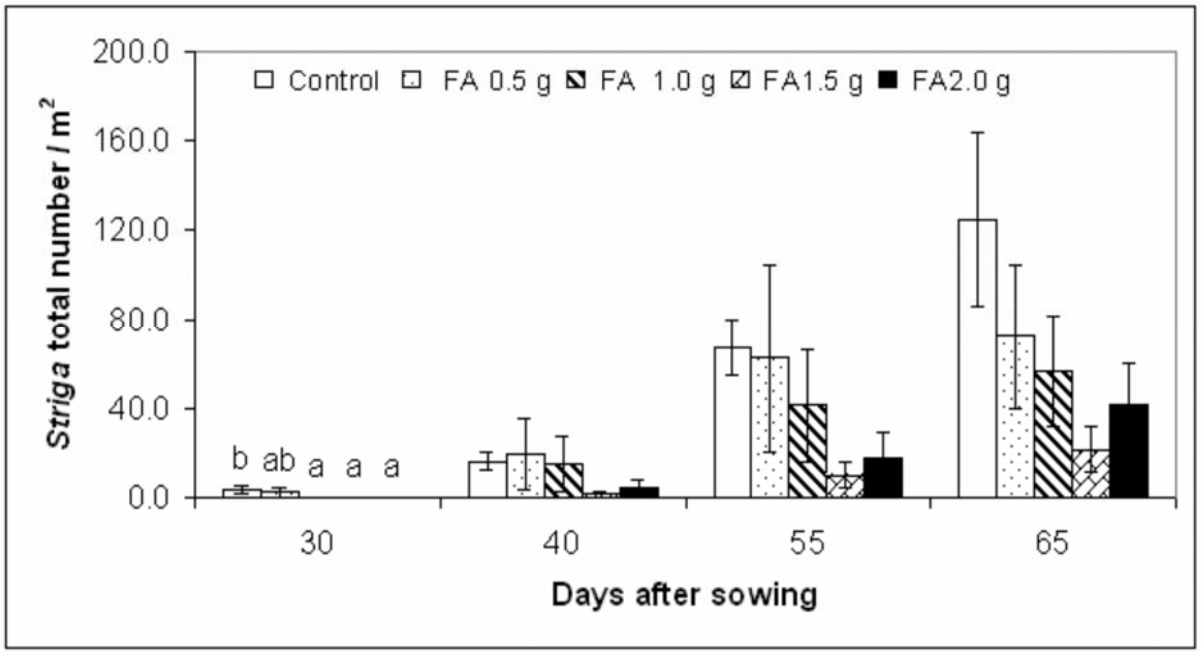

Fig. 7b. Effect of *Fusarium* Abuharaz on the total number of *Striga* shoots. FA= *Fusarium* Abuharaz isolate, ⌶ = indicate ± standard error of means, bars within the same days with same letter(s) are not statistically different after the protected LSD test at $p \leq 0.05$. No letters = data was not significant after analysis of variance.

Seed coating with both isolates significantly reduced the total number of *Striga* shoots compared to the control early in the season. The significant effect no longer existed, however, at the end of the season (Fig 7d).

The chemical herbicide had a pronounced effect on causing a quick knockdown of the *Striga* population, which was reduced by 97% on the second count immediately after application (40 days after sorghum sowing, Fig. 7e). At the end of the season the total number of *Striga* shoots was reduced by 68.4% compared to the control, which is less than the reduction achieved by some of the fungal treatments applied at 1g or more *(*Fig. 7b).

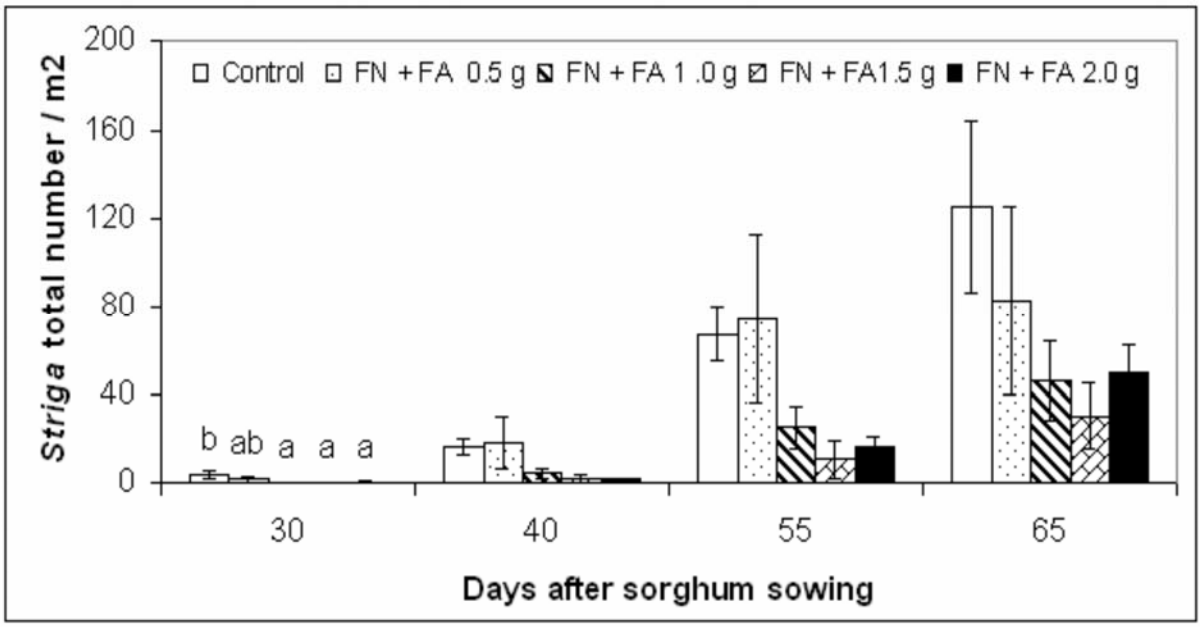

Fig. 7c. Effect of the combined fungal isolates on the total number of *Striga* shoots. FN= *Fusarium nygamai*, FA= *Fusarium* Abuharaz isolate, ⌶ = indicate ± standard error of means, bars within the same count with same letter(s) are not statistically different after the protected LSD test at $p \leq 0.05$. No letters = data was not significant after analysis of variance.

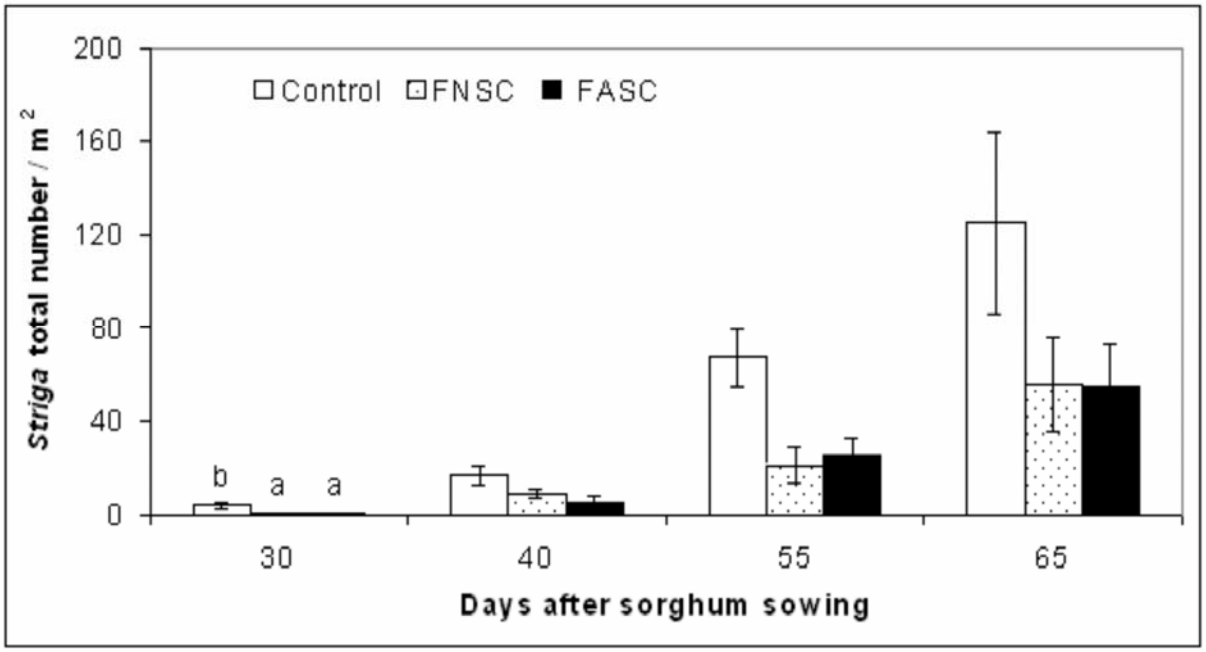

Fig. 7d. Effect of *Fusarium*-coated seeds on the total number of *Striga* shoots. FNSC= sorghum seeds coated with *F. nygamai* fine "Pesta" granules, FASC= sorghum seeds coated with *F.* Abuharaz fine "Pesta" granules. ⌶ = indicate ± standard error of means, bars within the same count with same letter(s) are not statistically different after the protected LSD test at $p \leq 0.05$. No letters = data was not significant after analysis of variance.

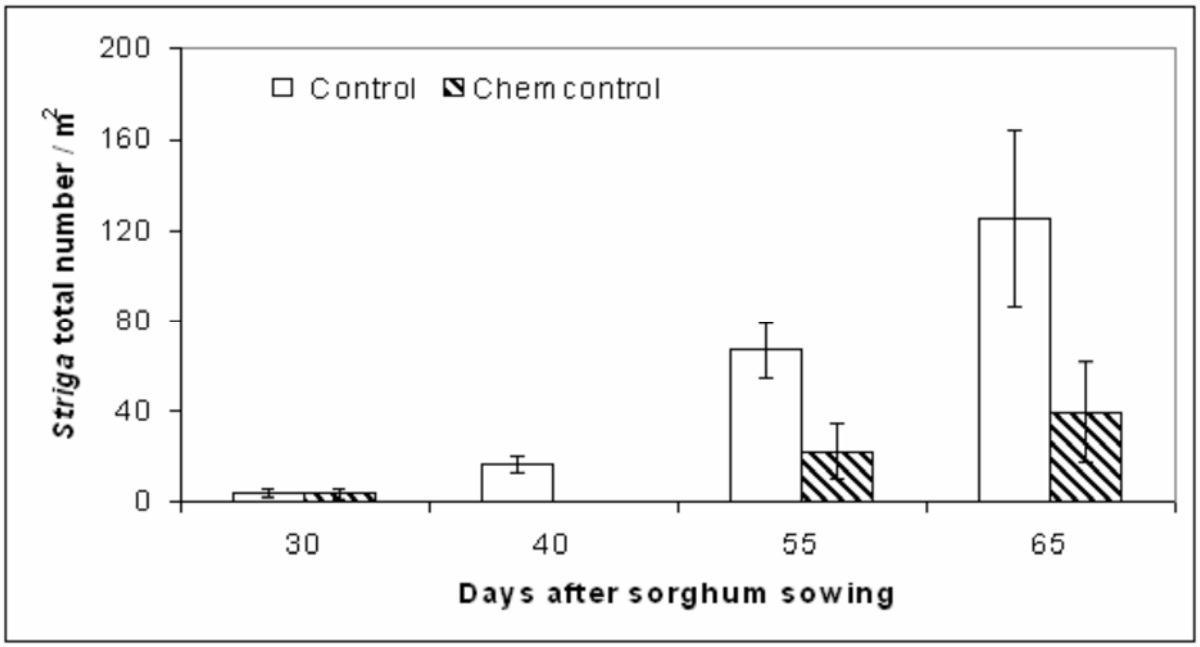

Fig. 7e. Effect of chemical control on the total number of *Striga* shoots. ⌶ = indicate ± Standard error of means.

Similar to the reduction of *Striga* incidence, FA showed consistency in reducing the total number of *Striga* shoots significantly compared to the treatments without fungal doses throughout the season using a GLM (Table 11 A, B, C, D).

Table 11. Multiple comparisons using a general linear model (GLM) to show the effect of *Fusarium* species on the total number of *Striga* shoots

A: count I (30 DAS)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Block	3	0.2	0.07	0.76	0.5
Controls[a]	4	0.5	0.1	1.37	0.3
FN[b]*Controls	1	0.01	0.00	0.01	0.9
FA[c]*Controls	1	0.7	0.7	8.1	0.01*
FN* FA[d]*Controls	1	0.02	0.02	0.2	0.6
Lack_of_fit	12	1.5	0.1	1.4	0.2

DAS= Days after sowing sorghum, [a] = treatments without fungal granular formulations. [b] =*F. nygamai* in 4 different doses, [c] =*F.* Abuharaz in 4 different doses, [d] =combination of the two isolates in 4 different doses, * = significant at P ≤ 0.05. Regression equation: *Striga* total number (y)= Controls + xFN +xFA + xFN xFA.

Table 11 continued

B: count II (40 DAS)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Block	3	0.4	0.1	0.4	0.7
Controls[a]	4	1.5	0.4	1.4	0.3
FN[b]*Controls	1	0.1	0.1	0.5	0.5
FA[c]*Controls	1	1.8	1.8	6.4	0.01
FN* FA[d]*Controls	1	0.00	0.0	0.0	1.0
Lack_of_fit	12	0.00	0.2	0.8	0.6

C: count III (55 DAS)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Block	3	0.5	0.6	0.4	0.7
Controls[a]	4	0.5	0.1	0.3	0.9
FN[b]*Controls	1	0.0	0.0	0.01	0.9
FA[c]*Controls	1	3.4	3.4	8.9	0.004*
FN* FA[d]*Controls	1	0.2	0.2	0.5	0.5
Lack_of_fit	12	4.0	0.3	0.9	0.6

D: count IV (65 DAS)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Block	3	1.2	0.4	1.3	0.3
Controls[a]	4	0.7	0.2	0.6	0.7
FN[b]*Controls	1	0.1	0.1	0.3	0.6
FA[c]*Controls	1	1.7	1.7	5.3	0.02
FN* FA[d]*Controls	1	0.3	0.3	1.1	0.3
Lack_of_fit	12	2.9	0.2	0.7	0.7

DAS= Days after sowing sorghum, [a] = treatments without fungal granular formulations. [b] =*F. nygamai* in 4 different doses, [c] =*F.* Abuharaz in 4 different doses, [d] =combination of the two isolates in 4 different doses, * = significant at P ≤ 0.05. Regression equation: *Striga* total number (y)= Controls + xFN +xFA + xFN xFA.

4.2.3 Effect of *Fusarium* spp. on *Striga* growth

In addition to the reduction of *Striga* incidence and emergence, *Fusarium* spp. induced disease symptoms on emerged *Striga* shoots. The symptoms started to appear 2-3 weeks after the emergence of *Striga.* Diseased *Striga* showed symptoms of blackening on the apex of the oldest leaves. Infected stems turned dark brown and at the end the diseased plants showed symptoms of wilting followed by the death of the whole plants (Fig. 8).

FN applied at 2 g and FA at 1 g gave a significantly higher proportion of diseased *Striga* plants compared to the untreated control and the chemical control (Table 12a, b). The overall efficacy of the treatments was calculated as the reduction of healthy *Striga* shoots compared to the untreated control (Bedi, 1994). The highest efficacy (88%) was achieved by applying FA at 1.5 g, followed by FN at 1 g (84%). The combination of the two isolates at 1.5 g gave an efficacy of 77%. Again, the combined isolates at 1 g reduced healthy *Striga* shoots to a greater extent compared to the control than the respective single applications of FA and FN at 0.5 g (by 23.2 and 27.0% respectively**).** This effect was not observed when comparing 2 g of the combined treatment with 1 g of both isolates applied singly.

Fig.8. Typical symptoms caused by *Fusarium* sp. on diseased *Striga* shoots parasitizing sorghum plants

Table 12a. Effect of *F. nygamai* on *Striga* and sorghum plant growth

Treatment	Diseased *Striga* shoots (%)	Efficacy	*Striga* dry biomass (t/ha)	Sorghum height (cm/plant)	Sorghum straw yield (t/ha)	Sorghum 100 seed weight (g)
Control	16.3 [bc] (1.9)		0.7 (0.1)	80.6 (9.1)	2.4 (0.3)	1.0 [b] (0.2)
Chemical control	13.3 [c] (5.1)	67.1	0.3 (0.2)	92.2 (15.4)	3.5 (0.9	1.5 [ab] (0.2)
0.5 g	43.6 [a] (11.6)	46.1	0.5 (0.1)	93.7 (8.0)	3.3 (0.7)	1.9 [a] (0.3)
1.0 g	38.7 [ab] (5.2)	84.0	0.2 (0.1)	99.2 (12.5)	5.0 (1.2)	2.0 [a] (0.3)
2.0 g	46.6 [a] (9.6)	82.4	0.2 (0.1)	100.9 (9.3)	4.0 (1.2)	1.7 [a] (0.1)
Seed coating	42.7 [a] (9.1)	67.4	0.4 (0.1)	106.4[a] (10.2)	3.6 (1.0)	1.7 [a] (0.2)

Data are means of four replicates, numbers in parentheses indicate standard error of means, means with the same letter(s) are not statistically different using the protected LSD at $p \leq 0.05$. Efficacy calculated as the reduction of healthy *Striga* shoots compared to the untreated control

The seed coating treatments and the chemical control reduced the number of healthy *Striga* shoots by about 67-63% (Table 12a, b, c).

FA at 1 g or more reduced *Striga* biomass by 71 - 86% compared to the untreated control, the highest reduction was achieved by applying 1.5 g (Table 12b). FN at the levels of 1 g or 2 g also reduced *Striga* biomass by 71% (Table 12a). Similarly 1.5 g of the combined isolates reduced *Striga* biomass by 71% compared to the control (Table 12c). All fungal treatments using a single isolate at a rate of 1 g were more effective in reducing *Striga* biomass than the tested chemical control, which gave a similar efficacy as obtained by the combined fungal isolates (57%) (Table 12c). Again, after multiple comparisons using GLM, FA had the most pronounced effect on reducing *Striga* biomass comparing to the blocked treatments (treatments without mycoherbicides doses) as shown in table 13.

4.2.4 Effect of *Fusarium* species on sorghum growth

As a result of the above mentioned adverse effects of *Fusarium* species on *S. hermonthica*, the performance of the sorghum plants was positively improved by increasing sorghum growth and yield. However, all effects were not statistically significant. Only the 100-seed weight increased significantly in all “Pesta”-treated plots, which was confirmed by regression analysis (Table 14). The combination of the two isolates at a rate of 1.5 g gave the most pronounced improvement of sorghum growth. It increased the sorghum straw yield by 142% and the sorghum 100-seed weight by 140% compared to the untreated control and by 90% compared to the chemical control (Table 12a, b, c).

Table 12b. Effect of *F.* Abuharaz isolate on *Striga* and sorghum plant growth

Treatment	Diseased *Striga* shoots (%)	Efficacy	*Striga* dry biomass (t/ha)	Sorghum height (cm/plant)	Sorghum straw yield (t/ha)	Sorghum 100 seed weight (g)
Control	16.3 ^{bc} (1.9)		0.7 (0.1)	80.6 (9.1)	2.4 (0.3)	1.0 ^{b} (0.2)
Chemical control	13.3 ^{c} (5.1)	67.1	0.3 (0.2)	92.2 (15.4)	3.5 (0.9)	1.5 ^{ab} (0.2)
0.5 g	27.7 ^{abc} (4.7)	49.9	0.4 (0.2)	93.0 (13.2)	4.1 (1.3)	2.2 ^{a} (0.5)
1.0 g	48.0 a (11.8)	71.6	0.2 (0.1)	93.6 (8.6)	2.9 (0.7)	1.7 a (0.3)
1.5 g	41.7 ^{ab} (3.9)	87.8	0.1 (0.1)	92.9 (13.3)	4.5 (0.7)	2.1 ^{a} (0.2)
2.0 g	31.6 ^{abc} (10.6)	72.7	0.2 (0.1)	102.3 (9.6)	4.9 (1.2)	2.1 ^{a} (0.2)
Seed coating	29.5 ^{abc} (5.5)	63.0	0.4 (0.1)	105.4 (5.1)	3.7 (0.6)	1.7 ^{a} (0.2)

Data are means of four replicates, numbers in parentheses indicate standard error of means, means with the same letter(s) are not statistically different using the protected LSD test at $p \leq 0.05$. Efficacy calculated as the reduction of healthy *Striga* shoots compared to the untreated control

Table 12c. Effect of combined fungal isolates on *Striga* and sorghum plant growth

Treatment	Diseased *Striga* shoots (%)	Efficacy	*Striga* dry biomass (t/ha)	Sorghum height (cm/plant)	Sorghum straw yield (t/ha)	Sorghum 100 seed weight (g)
Control	16.3 [ab] (1.9)		0.7 (0.1)	80.6 (9.1)	2.4 (0.3)	1.0 [bc] (0.2)
Chemical control	13.3 [c] (5.1)	67.1	0.3 (0.2)	92.2 (15.4)	3.5 (0.9)	1.5[c] (0.2)
0.5 g	45.7 [a] (8.0)	57.3	0.3 (0.2)	98.4 (12.0)	3.9 (0.9)	1.8 [ac] (0.1)
1.0 g	39.6[ab] (5.2)	73.1	0.3 (0.1)	93.2 (9.0)	3.8 (1.5)	2.1 [ac] (0.3)
1.5g	21.2 [ab] (9.6)	77.2	0.2 (0.1)	95.7 (12.6)	5.8 (1.9)	2.4 [a] (0.2)
2.0 g	30.4 [ab] (5.1)	66.7	0.3 (0.1)	86.0 (14.6)	3.6 (0.9)	1.8 [ac] (0.3)

Data are means of four replicates, numbers in parentheses indicate standard error of means, means with the same letter(s) are not statistically after the protected LSD test at $p \leq 0.05$. Efficacy calculated as the reduction of healthy *Striga* shoots compared to the untreated control

Table 13. Multiple comparisons using a general linear model (GLM) to show the effect of *Fusarium* isolates on *Striga* dry weight

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Block	3	0.0	0.0	0.2	0.9
Controls[a]	4	0.0	0.0	0.3	0.8
FN[b]*Controls	1	0.0	0.0	0.01	0.9
FA[c]*Controls	1	0.1	0.1	9.7	0.003[*]
FN* FA[d]*Controls	1	0.0	0.0	0.1	0.8
Lack_of_fit	12	0.1	0.1	1.3	0.3

[a] = treatments without fungal granular formulations. [b] =*F. nygamai* in 4 different doses, [c] =*F.* Abuharaz in 4 different doses, [d] =combination of the two isolates in 4 different doses, * = significant at $P \leq 0.05$. Regression equation: *Striga* dry weight (y)= Controls + xFN +xFA + xFN xFA.

Table 14. Multiple comparisons using a general linear model (GLM) to show the effect of *Fusarium* isolates on sorghum 100-seed weight.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Block	3	0.7	0.2	0.8	0.5
Controls[a]	4	0.7	0.2	0.6	0.7
FN[b]*Controls	1	1.3	1.3	4.4	0.04*
FA[c]*Controls	1	1.5	1.5	5.2	0.02*
FN* FA[d]*Controls	1	0.3	0.3	1.1	0.29
Lack_of_fit	12	9.5	0.8	2.68	0.008

[a] = treatments without fungal granular formulations. [b] =*F. nygamai* in 4 different doses, [c] =*F.* Abuharaz in 4 different doses, [d] =combination of the two isolates in 4 different doses, * = significant at P ≤ 0.05. Regression equation: sorghum 100-seed weight (y)= Controls + xFN +xFA + xFN xFA.

4.3 Field experiment second season

4.3.1 Effect of "Pesta" and alginate formulations and the *Fusarium*-coated sorghum seeds on *Striga* incidence

In contrast to the first field experiment, none of the *Fusarium* isolates formulated as "Pesta" granules had a significant effect on *Striga* incidence (Fig. 9a and Fig. 6a, b).

Both isolates encapsulated in alginate granules were able to delay *Striga* incidence significantly compared to the untreated control in the first count (30 days after sorghum sowing). By the progress of the season, no statistical difference in *Striga* incidence was found between treatments (Fig. 9b).

FA was able to reduce *Striga* emergence significantly compared to the control in the second count (40 days after sorghum sowing) when it was applied as a seed coating using Arabic gum as adhesive material. The effect of seed coating on reducing *Striga* incidence became irrelevant by the end of the season (Fig. 9c).

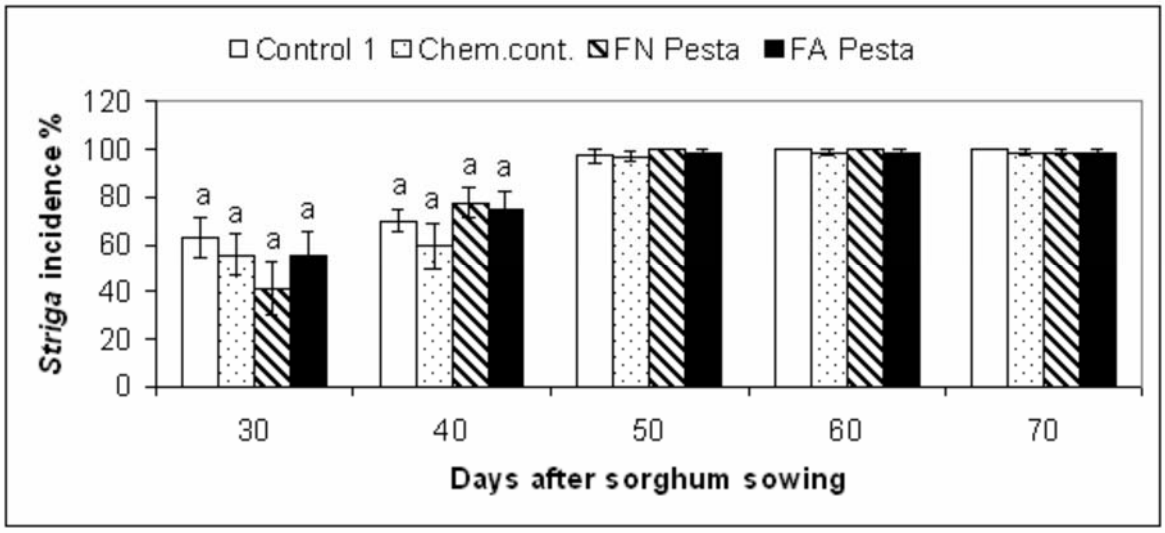

Fig. 9a. Effect of *Fusarium* spp. formulated as "Pesta" granules on *Striga* incidence. Data are means of 6 replicates, Control 1 = untreated control, Chem.cont. = Chemical control using 2,4D; FN = *Fusarium nygamai*, FA = *Fusarium* Abuharaz isolate. ⌶ = indicate ± standard error of mean, bars with the same letter(s) within the same count are not statistically different after the protected LSD test at $p \leq 0.05$.

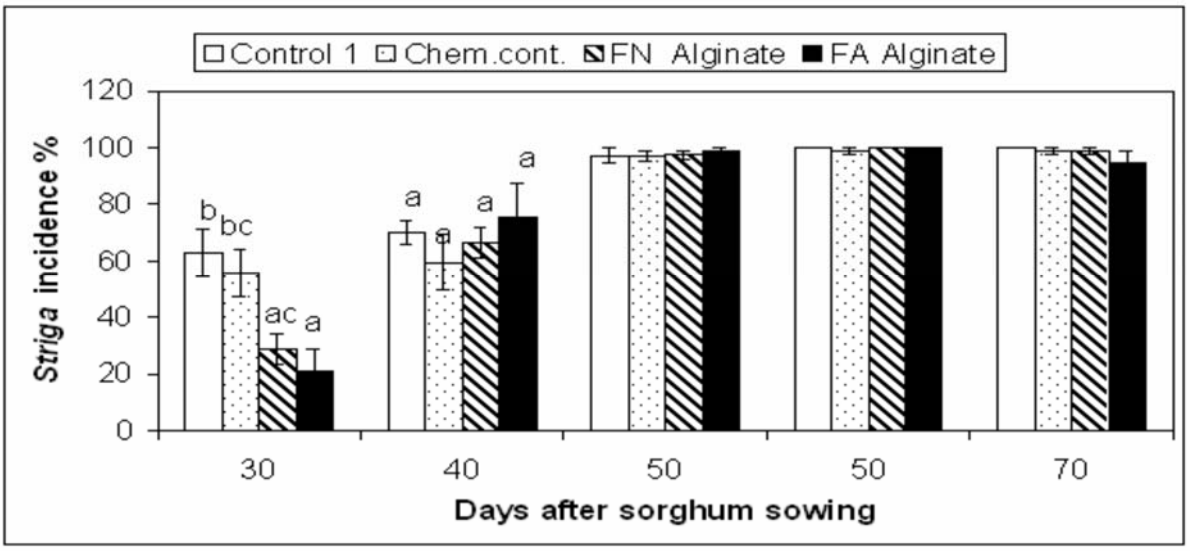

Fig. 9b. Effect of *Fusarium* spp. formulated as alginate pellets on *Striga* incidence. Data are means of 6 replicates, Control 1 = untreated control; Chem. cont. = chemical control using 2,4D; FN = *Fusarium nygamai*, FA = *Fusarium* Abuharaz isolate. ⌶ = indicate ± standard error of mean, bars with the same letter(s) within the same count are not statistically different after the protected LSD test at $p \leq 0.05$.

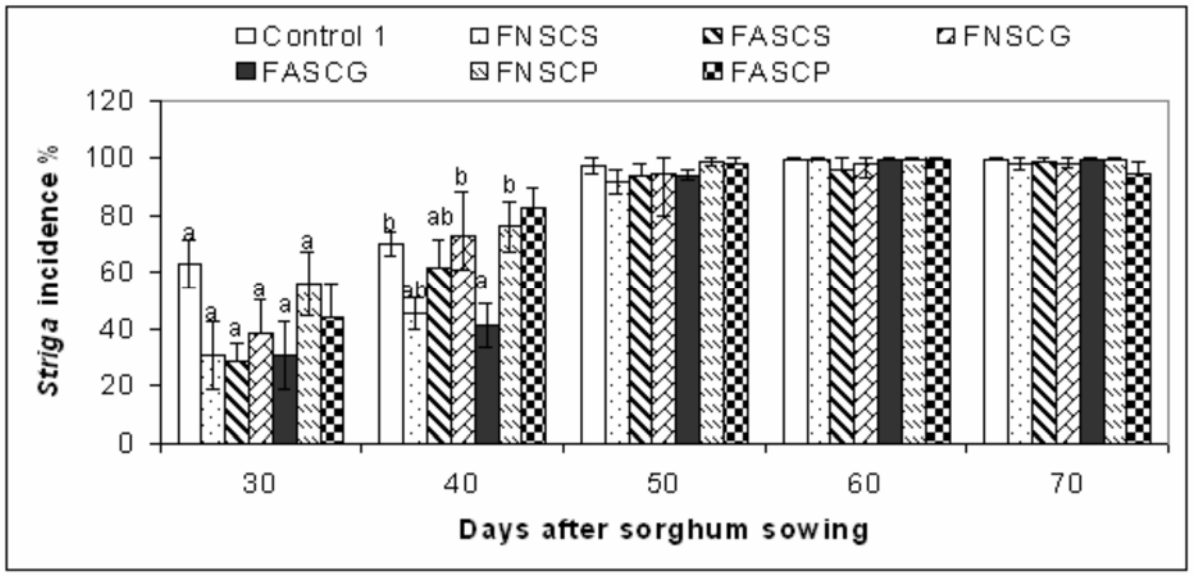

Fig. 9c. Effect of *Fusarium*-coated seeds on *Striga* incidence, control 1 = untreated control, FNSCS = *Fusarium nygamai* seed coating using sticky material selected by SUET, FASCS = *Fusarium* Abuharaz isolate seed coating using sticky material selected by SUET, FNSCG = *Fusarium nygamai* seed coating using Arabic gum, FASCG= *Fusarium* Abuharaz isolate seed coating using Arabic gum, FNSCP = *Fusarium nygamai* seed coating using fine "Pesta" formulate, FASCP = *Fusarium* Abuharaz isolate seed coating using fine "Pesta" formulate. ⌶ = indicate ± standard error of mean, bars with the same letter(s) within the same count are not statistically different after the protected LSD test at $p \leq 0.05$.

4.3.2 Effect of "Pesta" and alginate formulations and the *Fusarium*-coated sorghum seeds on the total number of *Striga* shoots

Similar to the effect of both "Pesta"-granulated *Fusarium* species on *Striga* incidence none of them had a significant effect on the total number of *Striga* shoots. However, they reduced the total number of shoots by 42.2-54.5 % compared to the control early in the season. By the end of the season, only FA showed a slightly lower number of emerged *Striga* shoots compared to the control (Fig.10a).

Comparable to the effect on *Striga* incidence, FA formulated as alginate pellets significantly reduced the total number of *Striga* shoots by 77.8 % compared to the untreated control at the beginning of the season. This effect became insignificant by the progress of the season (Fig.10b).

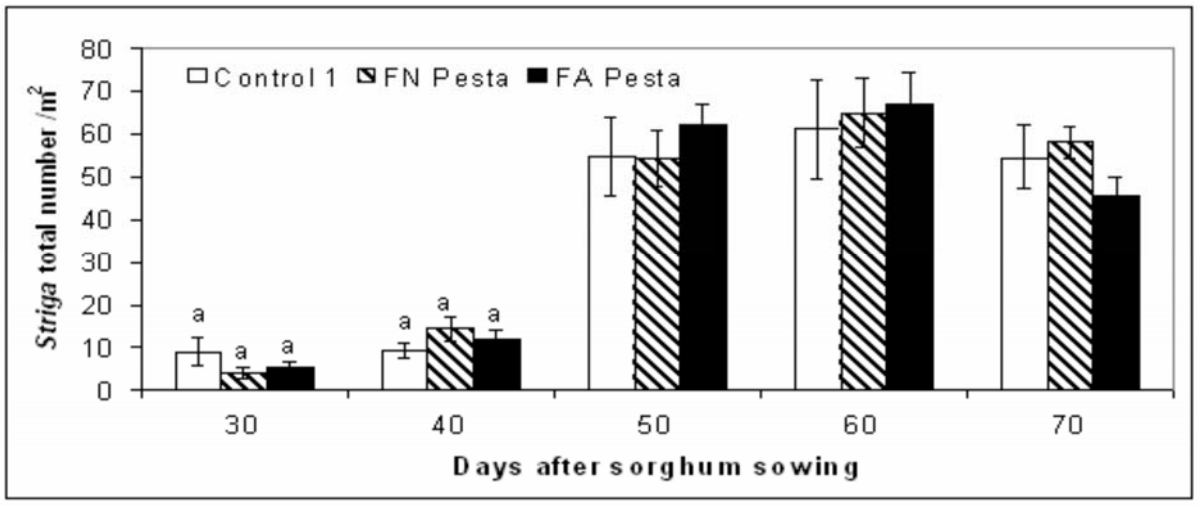

Fig. 10a. Effect of *Fusarium* spp. formulated as "Pesta" granules on the total number of *Striga* shoots. Control 1 = untreated control; FN = *Fusarium nygamai*, FA = *Fusarium* Abuharaz isolate, ⌶ = indicate ± standard error of mean, bars with the same letter(s) within the same count are not statistically different after the protected LSD test at $p \leq 0.05$.

Coating sorghum seeds with both isolates using fine "Pesta" granules or FN applied with the adhesive material provided by SUET significantly reduced the number of *Striga* shoots compared to the control early in the season. In the second count (40 days after sorghum sowing), FN applied with the adhesive material provided by SUET reduced the total number of *Striga* shoots significantly compared to the seed coating treatments using fine "Pesta" granules with $p \leq 0.004$-0.03. Also FA with Arabic gum reduced the total number of *Striga* shoots significantly compared to seed coating treatments with fine "Pesta" granules with $p \leq 0.001$-0.01 (Fig.10c).

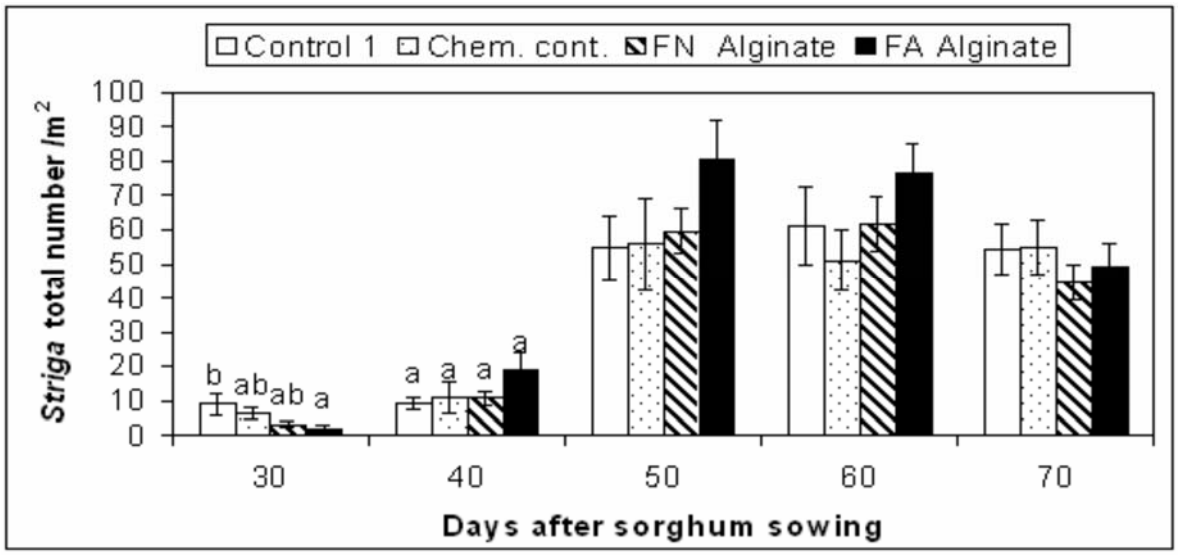

Fig. 10b. Effect of *Fusarium* spp. formulated as alginate pellets on the total number of *Striga* shoots. Control 1 = untreated control, Chem. cont. = chemical control using 2,4D**,** FN = *Fusarium nygamai*, FA = *Fusarium* Abuharaz isolate, ⌶ = indicate ± standard error of mean, bars with the same letter(s) within the same count are not statistically different after the protected LSD test at $p \leq 0.05$.

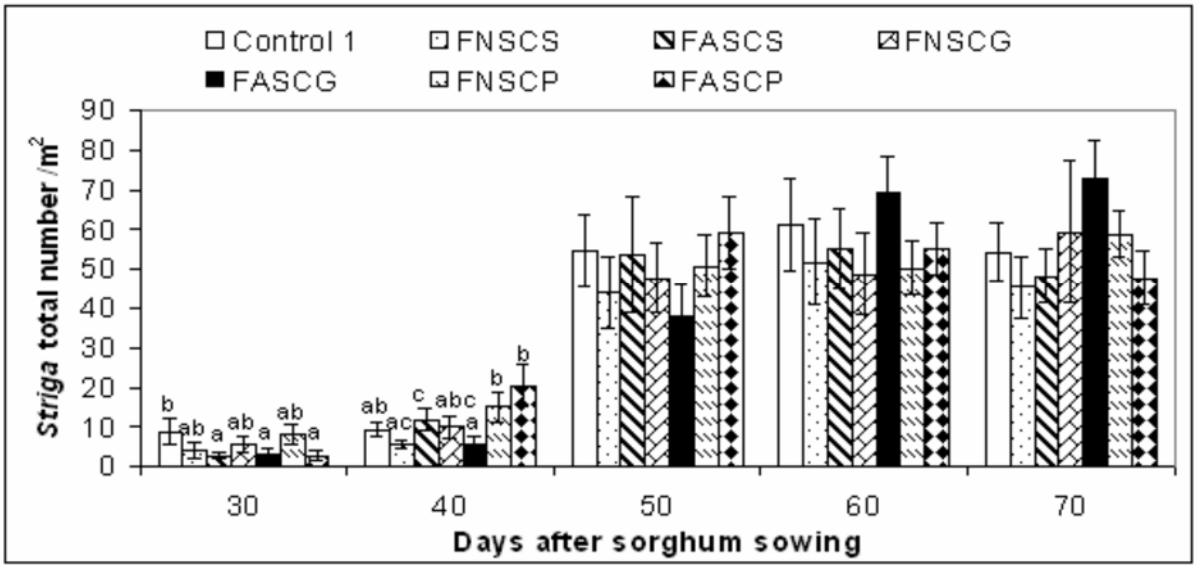

Fig. 10c. Effect of *Fusarium*-coated sorghum seeds on the total number of *Striga* shoots. Control 1 = untreated control, FNSCS = *Fusarium nygamai* seed coating using sticky material selected by SUET, FASCS = *Fusarium* Abuharaz isolate seed coating using sticky material selected by SUET, FNSCG = *Fusarium nygamai* seed coating using Arabic gum, FASCG= *Fusarium* Abuharaz isolate seed coating using Arabic gum, FNSCP = *Fusarium nygamai* seed coating using fine "Pesta" formulate, FASCP = *Fusarium* Abuharaz isolate seed coating using fine "Pesta" formulate. ⌶ = indicate ± standard error of mean, bars with the same letter(s) within the same count are not statistically different after the protected LSD test at $p \leq 0.05$.

4.3.3 Effect of *Fusarium spp.* on *Striga* growth

Fusarium isolates exerted no significant effect on *Striga* dry weight per unit area compared to the control, irrespective of their formulation and application method (Table 15a, b, c). However, FA applied as “Pesta” granules was found to be the most effective treatment in reducing *Striga* biomass by 57.7% compared to the control, followed by FN formulated in alginate pellets (42.3%).

Fusarium species encapsulated in “Pesta” and alginate granules or applied as seed coating induced disease symptoms on *Striga* shoots early in the season and the disease development progressed with time. “Pesta” containing FN and FA was found to increase the proportion of diseased *Striga* significantly in the fourth count (67% and 51%, respectively), and the significant effect of FA lasted until the final count (76%) The overall efficacy of this treatment calculated as the reduction in the total number of healthy *Striga* shoots compared to the untreated control was 55 % (Fig. 11a).

Similarly, both isolates formulated in alginate granules significantly increased the disease incidence on *Striga* shoots compared to the untreated control in the last two counts. FA was found to cause the highest disease incidence in the final count (80%), with an overall efficacy calculated as 60% (Fig.11b).

Also some of the seed-coating treatments were very efficient in inducing disease symptoms on *Striga* shoots. Only FA formulated as “Pesta”-granules and applied as seed coating significantly increased the proportion of diseased *Striga* compared to the control, but both isolates significantly caused an increased disease incidence compared to the other seed coating treatments in the first count (Fig.11c).

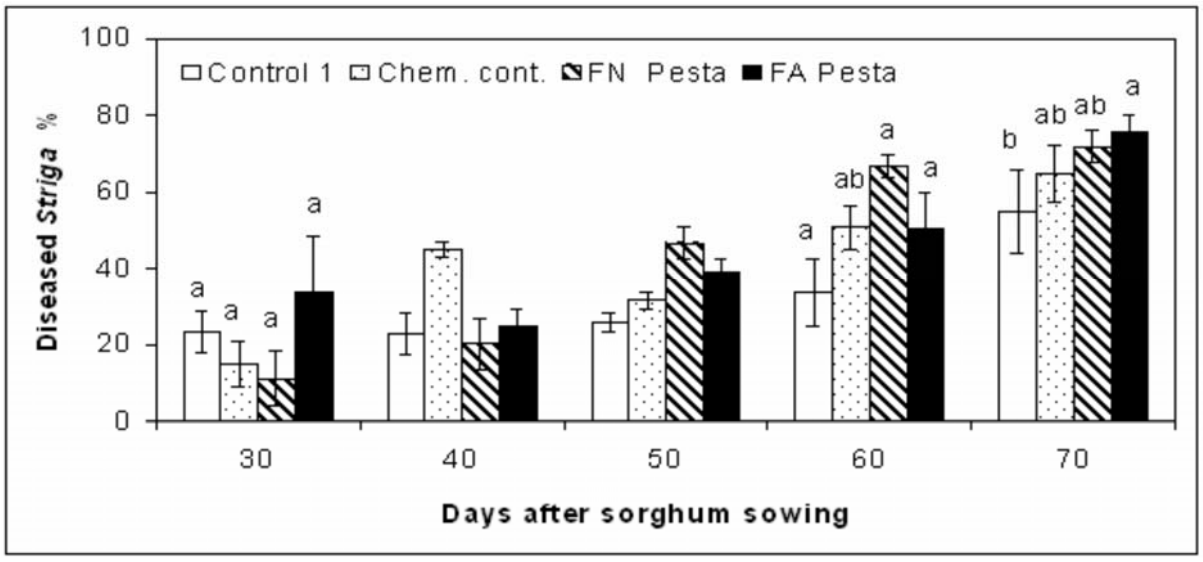

Fig. 11a. Effect of *Fusarium* spp. formulated as "Pesta" granules on the proportion of diseased *Striga* shoots

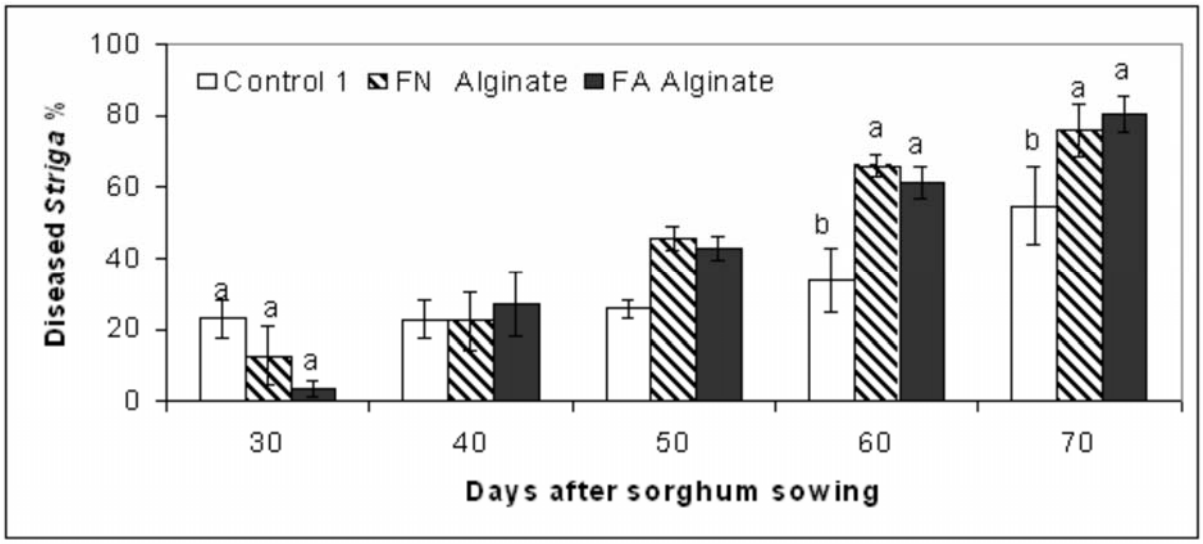

Fig. 11b. Effect of *Fusarium* spp. formulated as alginate pellets on the proportion of diseased *Striga* shoots. Control 1 = untreated control, Chem. cont. = Chemical control using 2,4D, FN = *Fusarium nygamai*, FA = *Fusarium* Abuharaz isolate. ⌶ = indicate ± standard error of mean, bars with the letter(s) within the same count are not statistically different after the protected LSD test at $p \leq 0.05$.

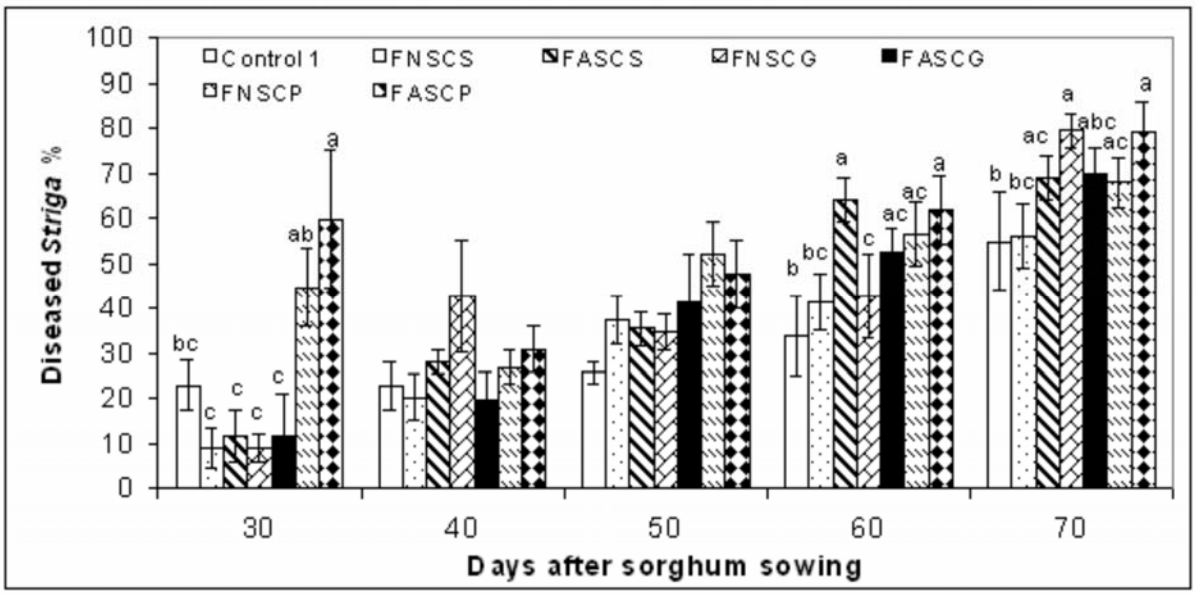

Fig. 11c. Effect of *Fusarium*-coated sorghum seeds on the proportion of diseased *Striga* shoots. Control 1 = untreated control, FNSCS = *Fusarium nygamai* seed coating using sticky material selected by SUET, FASCS = *Fusarium* Abuharaz isolate seed coating using sticky material selected by SUET, FNSCG = *Fusarium nygamai* seed coating using Arabic gum, FASCG= *Fusarium* Abuharaz isolate seed coating using Arabic gum, FNSCP = *Fusarium nygamai* seed coating using fine "Pesta" formulate, FASCP = *Fusarium* Abuharaz isolate seed coating using fine "Pesta" formulate. I = indicate ± standard error of mean, bars with the same letter(s) within the same count are not statistically different after the protected LSD test at $p \leq 0.05$.

In the fourth count, seed coating using "Pesta" granules with both fungal isolates and seed coating with FA using the adhesive material selected by SUET or Arabic gum increased the proportion of diseased *Striga* (57-62%, 64% and 53%, respectively) significantly compared to the untreated control. In the fifth count most of the seed coating treatments increased the proportion of diseased *Striga* shoots significantly compared to the control, except the treatment containing FN and adhesive material selected by SUET and FA plus Arabic gum. Overall, seed coating using Arabic gum and fine "Pesta" granules containing FA caused the highest increase in the proportion of diseased *Striga* shoots (79.2%). Thus the calculated efficacy (60%) was comparable to that induced by FA formulated in alginate granules (Fig.11b, c).

4.3.4 Effect of *Fusarium* spp. on sorghum growth

Fusarium spp. encapsulated in “Pesta” or alginate granules or applied directly to the sorghum seeds had no pronounced effect on increasing sorghum plant height compared to the control (Table 15a, b and c).

Fusarium spp. in form of “Pesta” or alginate granules showed positive effects on increasing sorghum head number, sorghum grain yield and sorghum straw yield per hectare compared to the control, however, the effect was statistically insignificant (Table 15a, b and c). The high *Striga* control efficacy of FA formulated in “Pesta” granules was positively reflected in improving sorghum performance, by increasing sorghum grain yield (62.5%) and sorghum straw yield (72.5%) compared to the control. FA applied directly to sorghum seeds using SUET adhesive materials increased sorghum grain yield similar to FA applied as “Pesta” granules.

The chemical control had no effect on *Striga* biomass compared to the control, but it was found to insignificantly increase sorghum grain yield by 75% and sorghum straw yield by 64.4%, which was less than that achieved by FA applied as “Pesta” granules (Table 15a).

Table 15a. Effect of *Fusarium* spp. formulated as “Pesta” granules on *Striga* and sorghum plant growth

Treatments	*Striga* dry biomass (t/ha)	Sorghum height (cm/plant)	Sorghum head no. (1000/ha)	Sorghum grain yield (t/ha)	Sorghum straw yield (t/ha)
Control 1	0.3 (0.1)	112.7 (7.6)	92.4 (11.3)	2.4 (0.5)	7.9 (1.4)
Chem.cont	0.3 (0.1)	118.7 (3.3)	125.0 (8.4)	4.2 (1.2)	13.3 (1.7)
FN	0.2 (0.0)	117.1 (6.7)	106.2 (31.2)	3.9 (1.6)	10.5 (3.4)
FA	0.1 (0.0)	116.2 (3.1)	129.9 (8.0)	3.9 (0.9)	13.6 (2.7)

Control 1 = untreated control, Chem.cont. = Chemical control using 2,4D, FN = *Fusarium nygamai*, FA = *Fusarium* Abuharaz isolate. Numbers in parentheses indicate standard error of mean. None of the parameters showed significant differences after performing ANOVA.

Table 15b. Effect of *Fusarium* spp. formulated as alginate pellets on *Striga* and sorghum plant growth

Treatments	*Striga* dry biomass (t/ha)	Sorghum height (cm/plant)	Sorghum head no. (1000/ha)	Sorghum grain yield (t/ha)	Sorghum straw yield (t/ha)
Control 1	0.3 (0.1)	112.7 (7.6)	92.4 (11.3)	2.4 (0.5)	7.9 (1.4)
FN	0.2 (0.1)	120.7 (7.6)	135.4 (26.8)	3.8 (1.1)	11.2 (2.0)
FA	0.2 (0.1)	118.4 (6.0)	110.4 (17.0)	3.6 (0.8)	11.3 (1.4)

Table 15c. Effect of *Fusarium*-coated sorghum seeds on *Striga* and sorghum plant growth

Treatments	*Striga* dry biomass (t/ha)	Sorghum height (cm/plant)	Sorghum head no. (1000/ha)	Sorghum grain yield (t/ha)	Sorghum straw yield (t/ha)
Control 1	0.3 (0.1)	112.7 (7.6)	92.4 (11.3)	2.4 (0.5)	7.9 (1.4)
FNSCS	0.3 (0.1)	103.1 (9.6)	85.4 (18.8)	2.7 (1.1)	9.5 (2.4)
FASCS	0.2 (0.1)	114.6 (9.3)	112.5 (28.0)	3.9 (2.1)	9.7 (3.0)
FNSCG	0.4 (0.1)	107.1 (7.6)	111.8 (19.6)	2.9 (1.0)	11.1 (3.0)
FASCG	0.4 (0.1)	94.2 (7.8)	95.8 (16.6)	1.9 (0.9)	9.1 (1.9)
FNSCP	0.2 (0.1)	118.4 (3.4)	119.4 (8.7)	3.3 (0.6)	9.6 (2.1)
FASCP	0.3 (0.1)	112.0 (6.7)	93.1 (21.6)	2.8 (0.9)	9.9 (1.5)

Control 1 = untreated control, FN = *Fusarium nygamai*, FA = *Fusarium* Abuharaz isolate FNSCS = *Fusarium nygamai* seed coating using adhesive material selected by SUET, FASCS = *Fusarium* Abuharaz isolate seed coating using adhesive material selected by SUET, FNSCG = *Fusarium nygamai* seed coating using Arabic gum, FASCG= *Fusarium* Abuharaz isolate seed coating using Arabic gum, FNSCP = *Fusarium nygamai* seed coating using fine “Pesta” granules, FASCP = *Fusarium* Abuharaz isolate seed coating using fine “Pesta” granules. The number between parentheses indicates standard error of mean. None of the parameters showed significant differences after performing ANOVA.

4.3.5 Development of the fungal populations after the application of "Pesta" and alginate granules to the soil

In the first season, the populations of both FA and FN rapidly declined in the soil when "Pesta" granules had been applied pre-planting. Although FA readily proliferated from the granules and was detected in the soil at approximately 1 x 10^4 cfu per g soil (5 days after application), the population almost completely disappeared after two more weeks. The population of FN grew very slowly and it was detected in the soil only 2 weeks after application. After 45 days, the populations of FA and FN were reduced to very low levels in the soil (Fig. 12). However, the number of cfu was not corrected by the moisture content of the soil, which might differ among sampling dates.

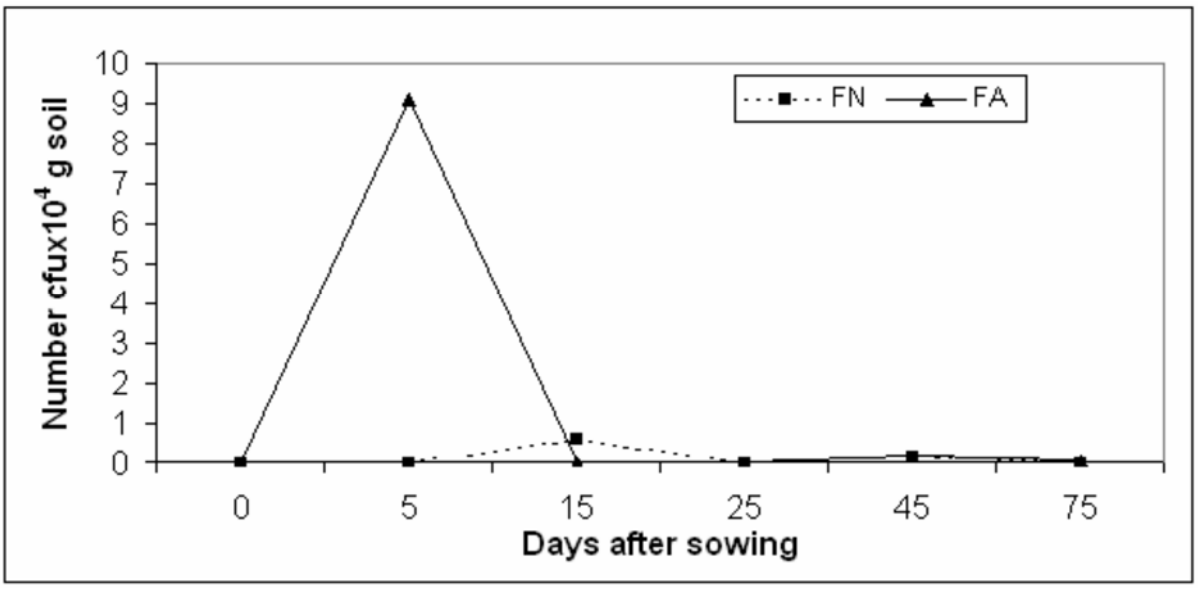

Fig. 12. Development of *Fusarium* populations in the soil after pre-planting application. FA = *F*. Abuharaz "Pesta" formulation, FN= *F. nygamai* "Pesta" formulation. Background soil population densities of *Fusarium* sp. recorded in the untreated control have been subtracted from the treatments before analysis.

In the second season, both FA and FN proliferated well out of "Pesta" and alginate granules and were detected in the soil 7 days after application at levels of 10^3 cfu per g dry soil. In contrast to the first season, fungal population counts fluctuated a lot**,** but generally increased with time. Only the population of FA formulated as alginate pellets showed a stable increase of cfu per g soil from five weeks until nine weeks after application. The highest population counts were detected after seven weeks,

irrespective of the formulation applied. After 9 weeks, the populations of FA and FN applied in “Pesta” granules showed an increase compared to the initial inoculum (after 7 days from application) by 80.0 and 242%, respectively. The populations originating from the alginate formulation were increased by 410% (FA) and 1100% (FN, Fig. 13).

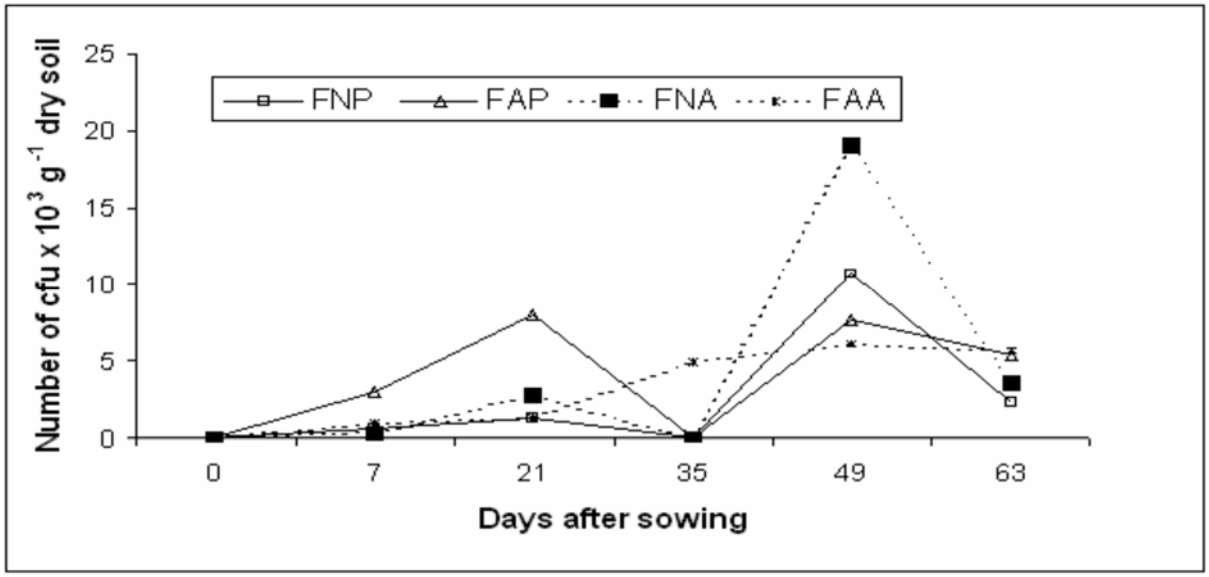

Fig. 13. Development of *Fusarium* populations in the soil after pre-planting application of “Pesta“ and alginate granules (season 2004). FAA= *F*. Abuharaz alginate formulation, FNA= *F. nygamai* alginate formulation, FAP= *F*. Abuharaz “Pesta” formulation, FNP= *F. nygamai* “Pesta” formulation. Background soil population densities of *Fusarium* sp. recorded in the untreated control have been subtracted from the treatments before analysis

4.4 Pot experiment

4.4.1 Effect of "Pesta" and alginate formulations and seed coating on the total number of *Striga* shoots

The fungal isolates formulated as "Pesta" granules were able to delay *Striga* emergence and to reduce the total number of *Striga* shoots significantly until 50 days after sorghum sowing by 80 to 100% compared to the control. *F.* Abuharaz isolate highly significantly inhibited *Striga* emergence until the end of the season (Fig. 14a).

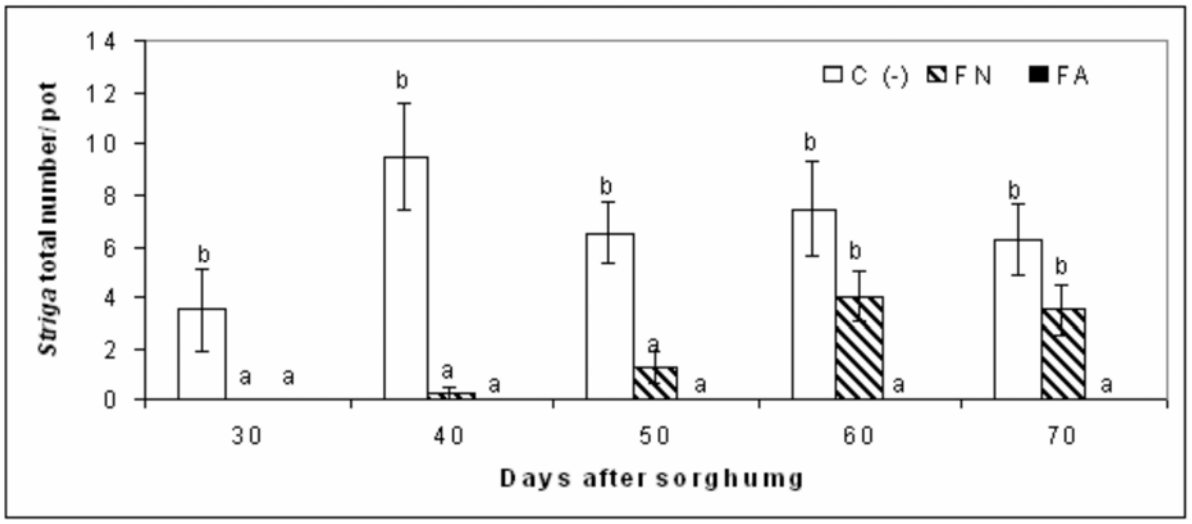

Fig. 14a. Effect of *Fusarium* spp. formulated as "Pesta" granules on the total number of *Striga* shoots. C (-) = Negative control treated with *Striga* only, FN = *Fusarium nygamai*, FA = *Fusarium* Abuharaz isolate, ⌶ = indicate ± standard error of mean, bars sharing same letter(s) within the same count are not statistically different after the LSD test at $p \leq 0.05$.

Fusarium species in alginate pellets also delayed *Striga* emergence and reduced the total number of *Striga* throughout the growing season. *F.* Abuharaz isolate encapsulated in wheat, sorghum or yeast alginate granules significantly inhibited *Striga* emergence compared to the control until the end of the experiment. In the last three counts, *F.* Abuharaz isolate encapsulated in alginate granules with sorghum (FAAS) or yeast extract (FAAY) significantly reduced the total number of *Striga* shoots compared to *F. nygamai* in alginate granules amended with 10% wheat flour. By the end of the season alginate granules containing FA reduced the total number of *Striga* shoots significantly by 84% (using 6% sorghum flour or yeast extract) compared to the negative control (Fig. 14b).

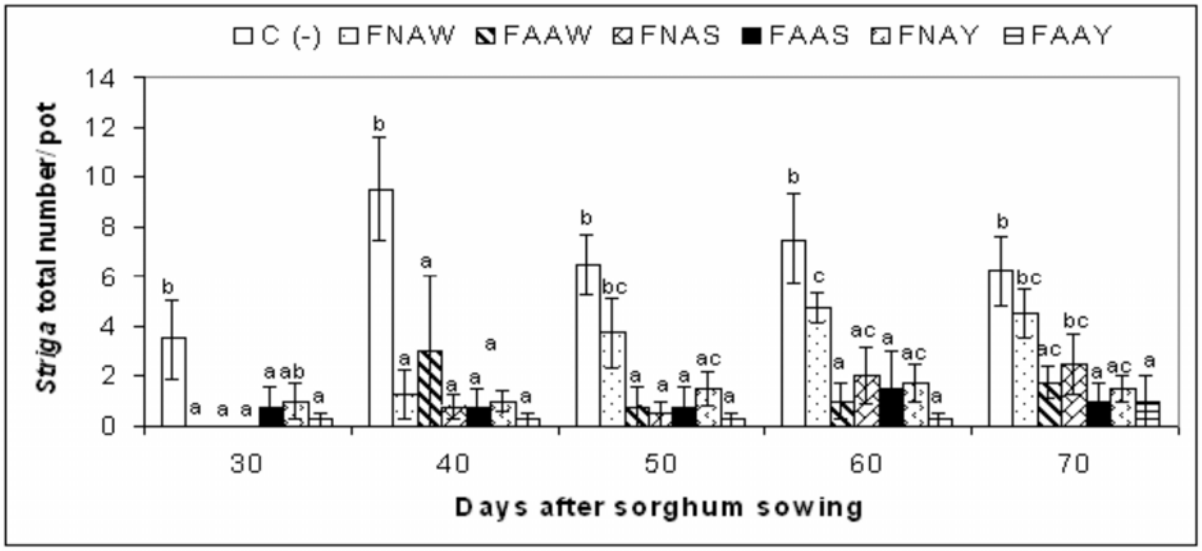

Fig. 14b. Effect of *Fusarium* spp. formulated as alginate pellets on the total number of *Striga* shoots. FNAW = *Fusarium nygamai* alginate wheat pellets, FAAW = *Fusarium* Abuharaz isolate alginate wheat pellets, FNAS = *Fusarium nygamai* alginate sorghum pellets, FAAS = *Fusarium* Abuharaz isolate Alginate sorghum pellets, FNAY = *Fusarium nygamai* alginate yeast pellets, FAAY = *Fusarium* Abuharaz isolate alginate yeast pellets, I = indicate ± standard error of mean, bars sharing same letter(s) within the same count are not statistically different after the LSD test at $p \leq 0.05$.

Generally, The fungal isolates applied as seed coating did not result in a significant reduction in *Striga* shoots. Furthermore, none of the seed treatments differed significantly from others regarding its efficacy. Overall, FA coated to sorghum with materials provided by SUET adhesive materials gave the best results, reduced the total number of *Striga* shoots by 52% compared to the negative control at the end of the season (Fig. 14c).

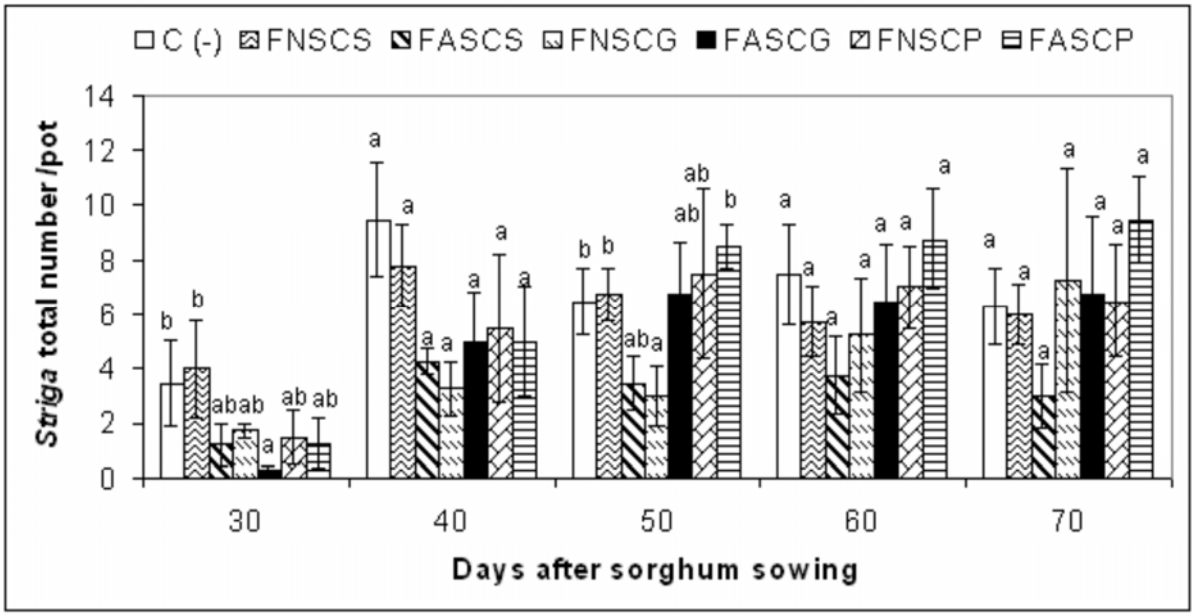

Fig. 14c. Effect of *Fusarium*-coated sorghum seeds on the total number of *Striga*. C (-) = negative control treated with *Striga* only, FNSCS = sorghum seeds coated with *F. nygamai* using adhesive material SUET, FASCS = sorghum seeds coated with F. Abuharaz isolate using adhesive material SUET, FNSCG = sorghum seeds coated with *F. nygamai* using Arabic gum, FASCG = sorghum seeds coated with F. Abuharaz isolate using Arabic gum, FNSCP = sorghum seeds coated with F. *nygamai* using fine "Pesta" granules and Arabic gum, FASCP = sorghum seeds coated with F. Abuharaz isolate using fine "Pesta" granules and Arabic gum. ⌶ = indicate ± standard error of mean, bars with the same letter(s) within the same count are not statistically different after the LSD test at $p \leq 0.05$.

4.4.2 Effect of *Fusarium* spp. on *Striga* growth

Fusarium spp. encapsulated in "Pesta" and alginate granules or applied directly as seed coating induced disease symptoms on *Striga* shoots within 2 - 4 weeks after the emergence of *Striga* and the disease development progressed with the season. After applying FN encapsulated into "Pesta" granules, 30 % of the *Striga* shoots were diseased at the end of the season, which was not statistically different from the control. FN in alginate granules amended with yeast significantly increased the proportion of diseased *Striga* compared to the control and to most of the other alginate preparations in count II and IV (40 and 60 day after sorghum sowing, Figure 15a). The overall efficacy for the alginate preparations calculated as the reduction in the number of healthy *Striga* shoots at the end of the season was about 84% (granules amended with yeast), 65 - 88% (granules amended with sorghum flour) and 49-71% (granules amended with wheat flour).

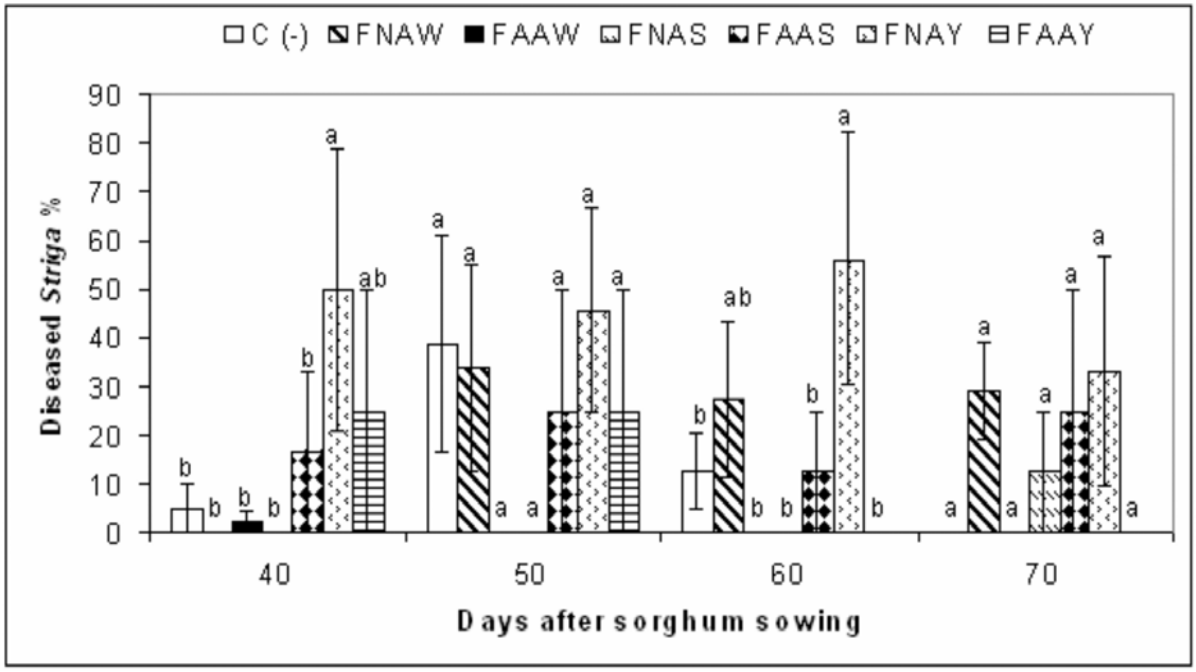

Fig. 15a. Effect of *Fusarium* spp. formulated as alginate pellets on the proportion of diseased *Striga* shoots. C (-) = Negative control treated with *Striga* only, FN = *Fusarium nygamai*, FA = *Fusarium* Abuharaz isolate FNAW = *Fusarium nygamai* alginate wheat pellets, FAAW = *Fusarium* Abuharaz isolate alginate wheat pellets, FNAS = *Fusarium nygamai* alginate sorghum pellets, FAAS = *Fusarium* Abuharaz isolate Alginate sorghum pellets, FNAY = *Fusarium nygamai* alginate yeast pellets, FAAY = *Fusarium* Abuharaz isolate alginate yeast pellets, ⌶ = indicate ± standard error of mean, bars sharing same letter(s) within the same count are not statistically different after the LSD test at $p \leq 0.05$.

Fungi directly applied to sorghum seeds using different adhesive materials successfully induced disease symptoms on emerged *Striga* shoots within two weeks after its emergence. Especially *F. nygamai* together with the sticky material selected by the seed company SUET significantly increased the proportion of diseased *Striga* by 77% at the end of the season (Fig. 15b). It was therefore the treatment with the highest efficacy (78%) (Calculated as the reduction in the total number of healthy *Striga* shoots compared to the control) among all seed coating treatments.

The impact of *Fusarium* species on *Striga* growth using different formulations and application methods was reflected negatively in the development of *Striga* biomass dry weight. FA formulated as "Pesta" granules highly significantly reduced *Striga* biomass, which was found to be reduced by 99% as shown in Table 16a.

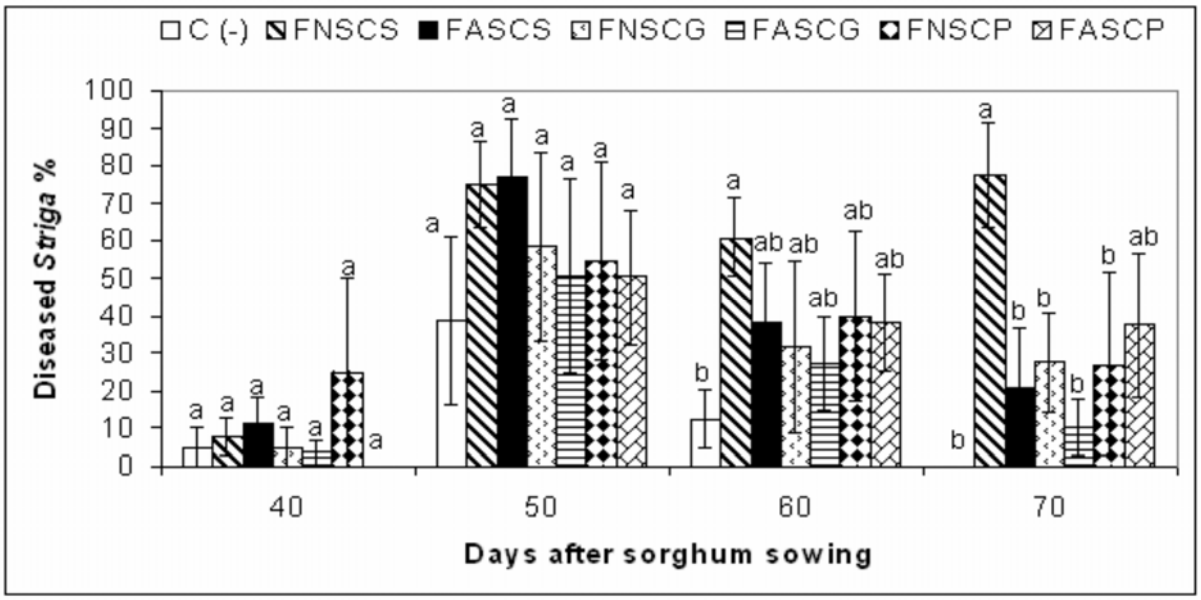

Fig. 15b. Effect of *Fusarium*-coated sorghum seeds on the proportion of diseased *Striga* shoots. C (-) = Negative control treated with *Striga* only, FNSCS = sorghum seeds coated with *F. nygamai* using adhesive material SUET, FASCS = sorghum seeds coated with F. Abuharaz isolate using adhesive material SUET, FNSCG = sorghum seeds coated with *F. nygamai* using Arabic gum, FASCG = sorghum seeds coated with F. Abuharaz isolate using Arabic gum, FNSCP = sorghum seeds coated with F. *nygamai* using fine "Pesta" granules and Arabic gum, FASCP = sorghum seeds coated with F. Abuharaz isolate using fine "Pesta" granules and Arabic gum. ⌶ = indicate ± standard error of mean, bars with the same letter(s) within the same count are not statistically different after the LSD test at $p \leq 0.05$.

All alginate granules except those containing FN amended with 10% wheat flour reduced *Striga* biomass significantly compared to the control. Among the alginate preparations, *F*. Abuharaz formulated with sorghum flour or yeast extract caused the highest reduction on *Striga* biomass (81 and 89%, respectively) compared to the control (Table 16b).

Seed coating treatments reduced *Striga* biomass consistently to a lower extend than the application of granules, and none of the reductions caused a significant difference from the control (Table 16c).

Table 16a. Effect of *Fusarium* spp. formulated as "Pesta" granules on *Striga* biomass dry weight and sorghum plant growth

Treatments	*Striga* dry biomass (g/pot)	Reduction (%)	Sorghum height (cm/plant)	Increase (%)	Sorghum dry weight (g/plant)	Increase (%)
C (-)	13.93 [b] 2.2		29.8 [b] (4.7)		6.9 [b] (2.1)	
FN	7.33 [b] (3.9)	48	46.5 [a] (4.1)	56	17.7 [c] (2.6)	157
FA	0.03 [a*] (0.0)	99	53.8 [a] (2.1)	80	33.9 [a] (2.5)	391

C (-) = Negative control, FN = *Fusarium nygamai* "Pesta" formulation, FA = *Fusarium* Abuharaz isolate "Pesta" formulation, numbers in parentheses indicate standard error of mean, means followed by the same letter(s) are not statistically different after using the LSD test at $P \leq 0.05$. * = Highly significant (p=0.000003)

4.4.3 Effect of *Fusarium* spp. on sorghum growth

Both isolates formulated as "Pesta" granules had a significant positive effect in increasing sorghum plant height and sorghum dry weight. FA significantly increased sorghum plant height by 80 % compared to the negative control and sorghum shoot dry weight by 391% compared to the negative control and to FN (Table 16a).

Alginate formulations with different additives also significantly increased sorghum plant height by 67-79% and sorghum shoot dry weight by 209-425% compared to the negative control (Table 16b).

The impact of the seed coating on *Striga* emergence and development was also positively reflected in the performance of the host plant. Coating the sorghum seeds with both isolates using the adhesive material selected by SUET or with FN using Arabic gum significantly increased sorghum shoot dry weight compared to the control (Table 16c). Again the increase on sorghum plant height and dry matter was generally lower than that caused by the granular formulations.

Table 16b. Effect of *Fusarium* spp. formulated as alginate pellets on *Striga* biomass dry weight and sorghum plant growth

Treatments	*Striga* dry biomass (g/pot)	Reduction (%)	Sorghum height (cm/plant)	Increase (%)	Sorghum dry weight (g/plant)	Increase (%)
C (-)	13.93 [b] (2.2)		29.8 [b] (4.7)		6.9 [b] (2.1)	
FNAW	5.45 [abc] (3.6)	60	44.0 [a] (3.0)	48	21.3 [a] (2.5)	209
FAAW	6.85 [cb] (2.4)	50	53.4 [a] (3.2)	79	34.4 [a] (9.1)	399
FNAS	6.73 [cb] (1.9)	52	53.4 [a] (3.5)	79	36.0 [a] (2.0)	422
FAAS	2.60 [ac] (0.6)	81	51.4 [a] (3.9)	72	33.5 [a] (4.4)	386
FNAY	5.65 [cb] (1.4)	59	49.8 [a] (3.5)	67	33.9 [a] (2.9)	391
FAAY	1.48 [a] (1.2)	89	52.3 [a] (3.0)	75	36.2 [a] (3.0)	425

C (-) = Negative control, FNAW = *Fusarium nygamai* alginate wheat pellets, FAAW = *Fusarium* Abuharaz isolate alginate wheat pellets, FNAS = *Fusarium nygamai* alginate Sorghum pellets, FAAS = *Fusarium* Abuharaz isolate alginate sorghum pellets, FNAY = *Fusarium nygamai* alginate yeast pellets, FAAY = *Fusarium* Abuharaz isolate Alginate yeast pellets, number in parentheses indicate standard error of mean, means followed by same letter(s) are not statistically different after the LSD test at $P \leq 0.05$.

Table 16c. Effect of *Fusarium*-coated sorghum seeds on *Striga* biomass dry weight and sorghum plant growth

Treatments	*Striga* dry biomass (g/pot)	Reduction (%)	Sorghum height (cm/plant)	Increase (%)	Sorghum dry weight (g/plant)	Increase (%)
C (-)	13.93 [a] (2.2)		29.8 [b] (4.7)		6.9 [bcd] (2.1)	
FNSCS	9.43 [a] (2.5)	32.4	41.4 [a] (1.0)	38.93	14.0 [ac] (1.6)	102.9
FASCS	10.04 [a] (3.3)	28.1	40.5 [a] (1.9)	35.91	18.0 [ac] (8.9)	160.9
FNSCG	7.63 [a] (1.8)	45.3	47.4 [a] (7.8)	59.06	25.0 [a] (7.0)	262.3
FASCG	13.16 [a] (7.0)	5.0	42.1 [a] (3.5)	41.28	7.9 [cd] (1.4)	2.9
FNSCP	7.60 [a] (2.8)	45.3	45.0 [a] (6.2)	56.7	10.8 [bc] (2.6)	56.5
FASCP	8.78 [a] (2.2)	36.7	37.3 [ab] (6.0)	25.17	5.2 [d] (0.8)	

C (-) = Negative control, FNSCS = *Fusarium nygamai* seed coating using sticky material selected by SUET, FASCS = *Fusarium* Abuharaz isolate seed coating using sticky material selected by SUET, FNSCG = *Fusarium nygamai* seed coating using Arabic gum, FASCG= *Fusarium* Abuharaz isolate seed coating using Arabic gum, FNSCP = *Fusarium nygamai* seed coating using fine “Pesta” formulate, FASCP = *Fusarium* Abuharaz isolate seed coating using fine “Pesta” formulate. Numbers in parentheses indicate standard error of mean. Means followed by the same letter(s) are not statistically different after the LSD test at $P \leq 0.05$.

5 Discussion

5.1 Inoculum Production

For commercialization of bioherbicides, the method of inoculum production should be inexpensive and yield sufficient biomass containing viable, highly virulent propagules, which ideally can be long-term stored within dry formulations. Submersed fermentation was found to be the more economical method for the production of fungal biocontrol agents compared to solid fermentation (Jackson *et al.,* 1996). The selection of a suitable medium that enables good growth and propagule formation is the first step in optimizing inoculum production.

Chlamydospores, the resting stages of *Fusarium* species, play an important role in long-term survival of the fungi in the soil due to their ability to withstand adverse environmental conditions and to easily germinate under favorable conditions (Nash *et al.,* 1961; Schippers and Van Eck, 1981). This characteristic makes them a suitable propagule type to be used within a mycoherbicide formulation. For example, chlamydospores formulated in “Pesta” granules retained a higher viability (up to 100%) compared to mycelia and/or microconidia, irrespective of the inoculum concentration and storage temperature (Elzein and Kroschel, 2004a). However**,** no differences in efficacy were observed between formulated microconidia and chlamydospores of *F. oxysporum* f.sp. *orthoceras* in controlling *Orobanche cumana* under greenhouse conditions (Müller-Stöver *et al*., 2002).

One of the objectives of this study was to find a medium suitable for chlamydospore production by FA and FN, the potential bioagents for *Striga* control. A variety of media were tested for their capability to enhance chlamydospore production in both isolates. Among these, Special Nutrient-poor Broth (SNB) + yeast was found to give the highest number of chlamydospores (10^5 ml^{-1}) in both isolates throughout the incubation period (Table 9a, b). However, the number is still low compared to what can be achieved with other *Fusarium* strains (Ciotola *et al*., 2000; Müller-Stöver *et al*., 2002; Elzein and Kroschel, 2004a). The initial inoculum concentration suitable for bioherbicide formulation in terms of cfu per ml depends on many factors such as type of inoculum and formulation and the loss of viable propagules occurring during the formulation process. Generally, “Pesta” granules containing a higher amount of viable inoculum (approximately 10^7 cfu g^{-1}) was associated with a higher efficacy in

controlling *Striga* or *Orobanche* using *F. oxysporum* (Müller-Stöver, 2001; Elzein, 2003; Elzein and Kroschel, 2004a).

Chlamydospore production was found to be affected by many factors such as nutritional components of the medium (carbon, nitrogen and minerals), nutrient stress, light, temperature and hydrogen ion concentration (Huang *et al.,* 1983; Nagao and Hattori, 1983; Mondal *et al.,* 1996; Sood, 1996; Diarra *et al.,* 1996; Hebbar *et al.,* 1996). Various plant extracts have been tested for their ability to induce chlamydospore formation by *Fusarium* spp. For *F. oxysporum* f. sp. *niveum,* celery (*Apium graveolens* L.) extract had the highest stimulatory activity among the tested plant extracts (Huang *et al.,* 1983), an effect that was confirmed by Müller-Stöver *et al*. (2002) with *F. oxysporum* f. sp. o*rthoceras,* a potential biocontrol agent for *Orobanche cumana*. Sood (1996) reported that onion (*Allium cepa* L.) bulb extract enhanced the production of chlamydospores in seven different species of *Fusarium* after 18 hours of incubation and reached a peak after 7 days. Huang *et al*. (1983), also recorded that the addition of Na_2SO_4 to the celery extract greatly increased the number of chlamydospores produced and that light had an inhibitory effect on chlamydospore production in this medium. The enhancing effect of Na_2SO_4 on chlamydospore production by *F. oxysporum* f. sp. *orthoceras* was confirmed by Bedi and Sauerborn (1999). However, in our study 0.03M Na_2SO_4 in V-8 broth stimulated the production of chlamydospores in FN only, while 2% celery extract increased chlamydospore formation by both *Fusarium* isolates and also had an enhancing effect when added to Special Nutrient-poor Broth. Bedi and Sauerborn (1999) also reported that the effect of Na_2SO_4 was increased when the bioagent was subjected to nutrient stress by transferring the produced inoculum into sterilized water. This is in accordance to Nagao and Hattori (1983) who argued that chlamydospore production occurs after a limited period under starvation conditions.

Hebbar *et al.* (1996) also proved that chlamydospores of *F. oxysporum* strain EN4 were readily produced in an abundant amount in substrates with a low content of utilizable carbon (extract of soybean (*Glycine max* (L.) Merr.) hull and corncobs), whereas chlamydospore formation was inhibited in substrates with a high content of utilizable carbon (e.g. cotton (*Gossypium* spp.) seed embryo). They also found a significant interaction between fungal variants, the substrate used and the substrate concentration, indicating that the optimization of the inoculum production is complex. Diarra *et al*. (1996) reported that sorghum straw is a suitable medium for

chlamydospore production by *F. oxysporum* M12-4A. High numbers of chlamydospores of this strain were produced in a two-stage fermentation system using 5% sorghum straw under black light (UV) at 30 ^{o}C for 14 days, and the produced chlamydospores caused a complete inhibition of *Striga* emergence when they were applied as dry powder to the soil at seeding or applied directly to the sorghum seed (Ciotola *et al.,* 2000). Müller-Stöver *et al.* (2002) optimized chlamydospore production by *F. oxysporum* f.sp. *orthoceras* in a one step liquid fermentation using sorghum stover and wheat-based stillage (the spent fermentation broth of ethanol production) for 16 days. Similarly, Elzein and Kroschel (2004b) obtained 4x10^{7} chlamydospores ml^{-1} by *F. oxysporum* (Foxy 2) within only 12 days in a one step liquid fermentation system using the combination of maize stover and wheat-based stillage. In contrast, during this study FN and FA in sorghum straw yield only 2x10^{4} chlamydospores ml^{-1} within four weeks.

Thus, chlamydospore production for both FA and FN which could help to prolong the shelf life of the prepared formulations, still needs to be optimized taking into consideration the environmental and nutritional factors that affect chlamydospore production.

5.2 Efficacy of “Pesta” granules in controlling *Striga*

So far, most of the research on the efficacy of “Pesta” preparations for biological control of parasitic weeds was performed under controlled conditions (i.e. greenhouses), thus the main task of this study was to investigate the efficacy of the granules to control *Striga* under natural conditions. During the first season (2003) the main objective was to determine the effective dose of the formulated fungal isolates, applied singly and in combination, to control *Striga.* These treatments have been compared with a simple seed coating treatment as an alternative delivery system of the bioagents and a standard herbicide treatment (2,4D).

The “Pesta” technology showed a potential to be used as a delivery system to control *S. hermonthica* under field conditions. Both “Pesta” -formulated *Fusarium* species were able to delay *Striga* emergence, reduce the total number of *Striga* shoots and induce disease symptoms on all growth stages of *Striga* plants, irrespective of the dose and method of application used. FN applied at 1.0 or 2.0 g per hole reduced the total number of *Striga* shoots compared to the control by 72%. FA reduced the total number of *Striga* shoots at the end of the season even more (by 82%) when applied

as 1.5 g “Pesta” granules per planting hole (Fig. 7 b). The efficacy of the whole treatments was calculated as the reduction of healthy *Striga* shoots compared to the control (Bedi, 1994). The highest efficacy (88%) was achieved by applying FA at 1.5 g, followed by FN at 1 g (84%). The combination of the two isolates at 1.5 g gave an efficacy of 77% (Table 5a, b, c). In this study, 1.5 g (FA) or 2 g (FN) of the formulated material per planting hole gave better results in controlling *Striga* in sorghum than the use of *F. nygamai*-infected sorghum grains (50g/planting hole) to control *Striga* in maize under field conditions of Cote d’ Ivoire in 1993/1994 (Sauerborn *et al*., 1996b). This clearly shows the advantage of a suitable formulation in reducing the amount of the inoculum needed and increasing the efficacy of the bioagent. Under Burkina Faso field conditions (season 1997/1998) the efficacy of *F. oxysporum* isolate 4-3-B to control *Striga* was evaluated using different growth media (millet straw and compost) and application methods (spot application and incorporation in the upper 10 cm of the whole plot at rate of 30 or 1500 g, respectively; Yonli *et al*., 2004). Irrespective to growth media and application method used, this *Fusarium* isolate delayed *Striga* emergence by 9 to 13 days and reduced *Striga* emergence by 27 to 33% compared to the untreated control and consequently sorghum performance improved significantly (Yonli *et al*., 2004). The authors observed that millet straw inoculum increased the infestation of sorghum with termites especially under dry conditions, adding another advantage to the use of granular formulations. Under Nigeria field conditions *F. oxysporum* (PSM-197) incorporated in the soil using spot application at rate of 10 g of fermented sorghum grains reduced the number of emerged *Striga* up to 89% (Marley and Shebayan, 2005). This is comparable to the effect achieved by FA used in this study at 1.5 g/hole, which again shows that granular formulations can markedly reduce the amount of the inoculum needed. Very recently the efficacy of two isolates of *F. oxysporum* (Foxy 2 and PM-197) entrapped in “Pesta” granules (applied at 2 g/planting hole) to control *Striga* was evaluated in combination with *Striga*-resistant cultivars of sorghum and maize under Nigeria field conditions (Schaub *et al*., 2006). The authors reported that the two isolates reduced the total number of *Striga* shoots by 75% and *Striga* biomass by about 74% and consequently sorghum and maize performance was increased compared to the untreated control. These results are generally in accordance to those achieved in the current study, although both investigations were carried out under different *Striga* infestation levels. In the present study, about 125 *Striga* plants/m^2 emerged in the untreated control

plots, compared to a maximum *Striga* population of about 0.6 and 2 plants/m^2 reported by Schaub *et al*. (2006) in untreated sorghum and maize, respectively. Also they observed that the combination of “Pesta”granules and resistant cultivars had the strongest suppressive effect on *Striga* which proves the suitability of the formulated fungi to be integrated with other control measures to control *Striga*.

From the results obtained in this study, 1 or 1.5 g of the formulated material applied per planting hole is suitable for field application, since increasing the amount did not result in an increased control effect when comparing the doses among each other. FN at 1 g or 2 g had the same effect on reducing the total number of *Striga* shoots or reducing *Striga* biomass and FA at 1.5 g had the best efficacy in controlling *Striga* throughout the season. Nevertheless, the selection of the suitable dose to be used under field conditions still needs to be optimized taking into consideration the type of inoculum to be used (such as chlamydospores), environmental conditions, the method of application and the level of *Striga* infestation.

Synergism between plant pathogens has been found beneficial for the biological control of weeds. In nature, often more than one fungal pathogen infects a host whereas only one may be primarily responsible for the disease symptoms. Secondary infection can further weaken the host, an effect that could be utilized for the development of a mycoherbicide (Cother, 1992). The efficacy of *Fusarium lateritium* Nees ex Fr. to control spurred anoda (*Anoda cristata* (L.) Schlecht) was increased when the plants were infected by *Alternaria macrospora* Zimm before they were inoculated with *F. lateritium* (Crawley and Walker, 1983). Guske *et al*. (2004) effectively used a mixture of four pathogens (*Phoma hedericola, Ph. destractiva, Ph. nebulosa* and Mycelia sterilia) against *Cirsium arvense*. Chandramohan *et al*. (2002), evaluated a mixture of three fungi (*Drechslera gigantea, Exerobilum longirostratum and E. rostratum)* to control seven weedy grasses in citrus under field conditions. Although the efficacy of the pathogen mixture was equal to that of the individual pathogens in controlling the target weeds, the authors preferred to use two or three combined pathogens to achieve broad spectrum control for the following reasons: a) synergism is only a possibility, whereas a combination of at least three pathogens is mainly an insurance against any possible failure of one or even two of the pathogens in any given combination under field conditions, b) more pathogens with different modes of action and sites of action in a combination have a much greater chance of reducing the potential risk of development of resistance to a single pathogen used

repeatedly and c) more pathogens allow for custom mixing based on the weed present (Chandramohan *et al*., 2002). However, when thinking about the benefit that could be gained from the synergistic effect of this “multi-pathogen strategy”, the high costs for the development and registration procedure needed for each isolate should be taken into consideration. Although the development and registration costs for bioherbicides are much less than for chemical herbicides (Auld and Morin, 1995), still the production costs are considered the major constraint to commercialization.

During the course of this study the effect of combining the two *Fusarium* isolates on controlling *Striga* has been studied using various dose levels. The results proved that the combination of the two fungal isolates had the most pronounced effect on reducing *Striga* incidence. Early in the season, these treatments significantly delayed *Striga* emergence compared to the control when applied as 1.0, 1.5 or 2.0 g per planting hole, respectively (Chapter 4, Fig. 6c). Applying 1.5 g of the combined isolates caused the highest reduction by 57% compared to the control until mid of the season. The combination of the isolates applied as 1.5 g “Pesta”granules per hole was able to reduce the number of the parasite shoots at the end of the season by 76% compared to the control (Fig. 7c), which is a similar effect to that of FA applied alone at 1.5 g. The combination of the two isolates at a rate of 1.5 g gave the most pronounced improvement of sorghum growth. It increased the sorghum straw yield by 142% and the sorghum 100-seed weight by 140% compared to the untreated control and by 70% compared to the chemical control. Thus it was possible to manage *Striga* under field conditions using the formulation with the individual fungal strain as well as with the combination of the two isolates. Increasing the amount of the combined inoculum up to 3 g per planting hole might result in improving the efficacy of the mycoherbicides in controlling *Striga* but on the other hand would mean to double the amount of the inoculum needed and consequently increase the total production cost. It would be better to increase the observed effect by improving other factors that would lead to a better interaction between the two fungi (Bailey, 1990), rather than increasing the amount of the dose, such as nutritional amendment that enhance the rapid growth and sporulation of both fungi as well as optimization of inoculum type and inoculum concentration.

The adverse effects of *Fusarium* spp. on *Striga* growth were consequently followed by an improvement of the performance of the host plant sorghum. Due to the attack with sorghum head bug (*Agonoscelis pubescens*) at sorghum milky stage, we were

unable to determine the sorghum yield per unit area during the first season (2003). Instead, the sorghum 100-seed weight was measured as parameter for sorghum yield. The 100-seed weight was significantly increased by all “Pesta” treatments (except FN at 1.5 g) by 70-140% (chapter 4, table 9a, b, c). The improvement of sorghum growth has been recorded by all researchers who used *Fusarium* species for the biological control of *Striga* under controlled or under field conditions. *F. oxysporum* (isolate M12-4A) in combination with ammonium nitrate fertilizer increased sorghum yield by 100% in inoculated plots (Citola *et al.,* 1996). Similar results were obtained by Mohamed (2002) who recorded that FA grown on sorghum grains alone or in combination with urea significantly increased the sorghum yield by 100-130% compared to the control. Some observations suggest that the effect of *Fusarium* species on the host plant cannot be attributed to the control of the parasite alone but also to interactions between the bioagent and the host plant. Numerous *Fusarium* species were reported to produce plant growth regulators. *F*. *nygamai* and *F*. *semitectum* var. *majus* produce different kinds and amounts of cytokinins such as zeatin or zeatinriboside and other plant hormones such as indoleacetic acid, absisic acid and gibberellins (Abbasher, 1994). Since *Striga* parasitism causes a drastic shortage of growth hormones such as cytokinins and gibberellins in the host plant (Drennan and El Hiweris, 1979), it may benefit even more from growth-promoting substances provided by the biocontrol fungi.

The efficacy of “Pesta” granules in controlling *Striga* shoots was much lower in the second season: e.g. FA reduced the number of parasites only by 16% compared to the control, which means a reduction in efficacy by 66% using the same dose (1.5g/ hole), although the total number of shoots per m^2 was higher in the first season compared to the second season. Reflected in lower *Fusarium* soil populations (see chapter 4, Fig 12 and 13), the reduced efficacy of the “Pesta” formulation compared to the first experiment can probably be attributed to climatic conditions, which differed from the first season in higher temperatures coupled with lower rainfall and low relative humidity (see appendix 1). Similarly, when *F. lateritium* was formulated in alginate granules and applied for pre-emergence control of *A. theophrasti* and *S. spinosa,* relatively low levels of weed control (46 and 59% mortality, respectively) were achieved under field conditions which were attributed partially to high summer temperatures and drought during the trials (Green *et al.*, 1998). The importance of moisture on disease development by plant pathogens has been well documented,

and most studies demonstrated a positive correlation between the length of the dew period and the extent of disease (TeBeest *et al.*, 1992). The successful use of the mycoherbicide Collego® can be attributed to the high relative humidity in rice and soybean fields in which it is used (Templeton *et al.*, 1979). Generally, temperature has not been considered to be as critical as moisture for mycoherbicide development, since most pathogens studied (especially soil borne pathogens) were infectious over a wide range of temperatures. However, it has been found that the development of a disease during the season is a function of accumulative degree days (ADD); these ADDs vary between regions and years, resulting in different degrees of control (TeBeest *et al.*, 1992). In field experiments conducted in Côte d' Ivoire during 1993/1994 to control *Striga hermonthica* in maize using *F. nygamai*, it was reported that the plots that were planted and treated with the bioherbicide in the morning gave different results compared to those treated in the afternoon (Sauerborn *et al*., 1996b). This variation was attributed to the high mid-day temperature, affecting the viability of the treatments applied in the morning. Propagule numbers of *F. oxysporum* f.sp. *erythroxyli* formulated in alginate granules used for the control of *Erythroxylum coca* var. *coca* under controlled and under field conditions were mostly influenced by factors such as soil type, soil moisture and soil conduciveness (soil favorable conditions; with physical and chemical properties suitable for mycoherbicide growth) to the fungus (Bailey *et al.*, 1997).

In this study, the reduced efficacy of the formulated bioagents in the second season could further be attributed to the alkaline soil in the fields of the demonstration farm in Abuharaz in which sodicity (sodium rich soil) is known to occur in patches with ESP (exchangeable sodium percent) = 18%. A soil is considered to be sodic when the ESP > 15% (Bohn *et al.*, 2001; Sparks, 2003). Sodic soil produces a poor soil - water - air relationship, resulting in a poor plant growth, also due to toxicity of excess sodium, carbonates and bicarbonates and deficiency of iron or other micronutrients caused by the resultant high pH (Gill and Abrol, 1991; Costa and Godz, 1999, Sparks, 2003). Sodicity might have affected the proliferation of the fungi in the soil by the high soil pH (8.5-10) and poor physical soil properties (Sparks, 2003) resulting in a reduced *Striga* control.

Another reason for the reduced effect of the bioagents may be an inhibitory effect of the applied insecticide Sevin (active ingredient Carbaryl (1-naphthyl N-methylcarbamate) (Laveglia and Dahm, 1977) at a rate of 1.9 kg/ha in two

applications (the first one month after sowing and the second a month later) to control stem borers, the larvae of *Sesamia cretic*a and *Chilo partellus*, that cause major yield losses in subsistence cereal production throughout sub-Saharan Africa (Khan *et al*., 2000). Sevin belongs to the carbamate insecticide group with a half-life of carbaryl in the soil of approximately 8 days at all concentrations. The insecticide is degraded within 40 days when applied to the soil (and less than 2 weeks after foliar application) by soil microorganisms including *F. solani* utilizing it as a carbon source and producing water soluble metabolites (Laveglia and Dahm, 1977). These metabolites might affect the virulence of soil fungi. Sevin significantly inhibited the germination of primary conidia of *Entomophthora muscae,* a well known bioagent for adult diptera (e.g. *Musca domestica*, Mullens and Rodriguez, 1986). Similarly carbofuran (Furdan), another carbamate insecticide, significantly reduced the mycelial growth of some soil fungi such as *Trichoderma viride*, *T. reesei*, *Rhizobium stolinfere* and *Penicillium vermiculatum* at a rate of 100 ppm (Beatty and Sohn, 1986). The reduction of *Fusarium* populations (after 35 and 63 days) coincides with the application of the insecticide (Fig. 13). Therefore it is essential to study the compatibility of the potential bioagents with the used pesticides in sorghum production.

The lower efficacy in the second season could also be attributed to a different soil microflora that could colonize the granules and exert antagonistic effects on the *Fusarium* isolates (Rhodes, 1990). Therefore, it could be possible to co-incorporate other biological or chemical agents (e.g. antibiotics) into the granules that inhibit the growth of other microorganisms and enhance the growth and the release of the bioagents (Weidemann, 1988; Connick *et al*, 1990).

5.3 Efficacy of seed treatment in controlling *Striga*

To control root parasitic weeds biologically, it is important to put the bioagent at the infection site along the host root where the parasite attack takes place. A seed treatment with the fungal propagules could establish the biocontrol agent in the root zone of the host plant, hence preventing the attachment of the parasite to the host plants, reducing the amount of the inoculum needed, and offering a simple and easy delivery system (Elzein, 2003; Elzein *et al*., 2006).

Some seed treatments with biocontrol agents are already commercially available. For example, Quantum- 4000^{TM} (Gustafson inc., Dallas, TX) is a biofungicide containing

Bacillus subtilis, which is applied to the seeds of sugar beet. This bacterium colonizes the developing root system and protects the seedling from soilborne fungal diseases throughout the season, even more effective than chemical fungicides that provide protection for only few weeks early in the season (Connick *et al.*, 1990; Suslow and Schroth, 1982). The technique is also used with biocontrol fungi, e.g. powdered biomass of *Pythium oligandrum* containing an abundant number of oospores was mixed with a clay carrier and coated to seeds of cress, carrot and sugarbeet to control damping–off (Lutchmeah and Cooke, 1985).

In this study, two different approaches to treat the seeds were tested: soaking the seeds in a conidial suspension and coating the seeds with fungal propagules using adhesive materials. The effectiveness of seed soaking as simple and economic delivery system had been proved under controlled and under field conditions to control sclerotinia stem rot (*Sclerotinia minor*) in sunflower using *Gliocladium virens* as bioagent. Soaking sunflower seeds in a conidial suspension of *Gliocladium virens* for 2 hours reduced disease incidence from about 59% to about 18%, which is statistically equivalent to that resulting from direct application to pasteurized soil (Burgess and Heppowerth, 1996). Also Georgakopoulos *et al*. (2002) applied a seed soaking treatment for the control of cucumber and sugar beet damping-off caused by *Pythium ultimum* using bacterial antagonists. The authors observed that the survival of the bacteria on germinating seeds was often poor after application in a liquid suspension, especially during the period immediately after planting.

Seed germinability was slightly affected when sorghum seeds were soaked in the microconidial suspension of FA and FN alone or amended with an organic polymer or sodium alginate for 30 min. Using the superabsorbent polymer and sodium alginate increased the negative effect of seed soaking on sorghum seed germination compared to the untreated control and the treatment with non-amended fungal inoculum. However, when increasing time of soaking up to 2 hours, sorghum seed germination was adversely affected. The fungi were able to recover from the soaked seeds and well colonized sorghum seeds and the sorghum root up to 10 cm after 2 weeks. The efficacy of the seed soaking technique in controlling *Striga* could not be assessed in root chambers because *Striga* seed failed to germinate in the untreated control. Nevertheless, disease symptoms were observed on *Striga* shoots in root chambers sown with soaked sorghum seeds (Fig. 14).

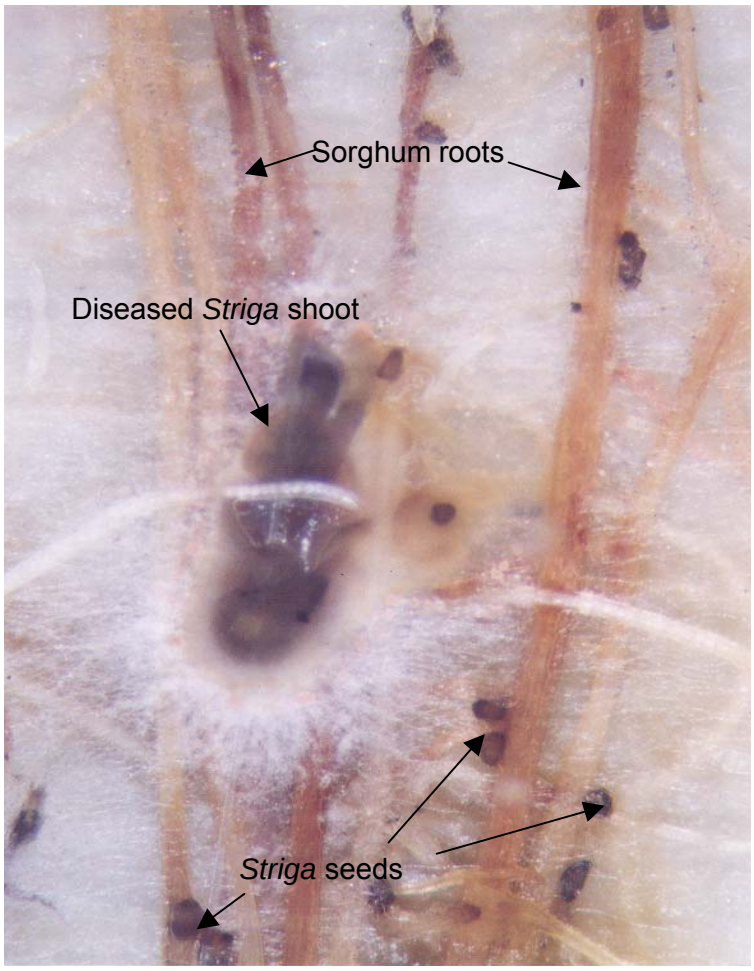

Fig. 14. *Fusarium nygamai* colonizing sorghum roots and causing disease in *Striga* shoot, applied as sorghum seed treatment.

The seed coating technique proved to be a promising alternative delivery system for a potential mycoherbicide to control *Striga.* Ciotola *et al*. (2000), proved complete *Striga* inhibition under field conditions of Mali when sorghum seeds were coated with chlamydospores of *Fusarium oxysporum* M12-4A using Arabic gum. Dried chlamydospores ($1x10^4$ g^{-1}) of *F. oxysporum* (Foxy 2) applied directly to sorghum seeds using Arabic gum were found to cause disease in 77% of the germinated *Striga* seeds and to all attached tubercles after 25 days in root chamber trials, and significantly reduced the number of emerged *Striga* shoots in a pot experiment (Elzein, 2003; Elzein *et al*., 2006).

In this study, coating sorghum seeds with both *Fusarium* species using fine “Pesta” granules and Arabic gum as adhesive material showed a potential to control *Striga* under field conditions, despite the low number of cfu per seed achieved with this

technique. The results obtained in the first field experiment encouraged us to test in the second season experiment the technology was improved by selecting suitable adhesive materials that do not reduce or even enhance the growth and sporulation of the fungi and by using professional seed coating machinery. The effect of five types of adhesive materials on growth and sporulation of FA and FN were tested in solid and liquid media. An organic polymer and a clay significantly enhanced the production of chlamydospores, especially by FA, compared to Richard's solution alone or amended with the other tested materials. One of them was therefore used besides Arabic gum for the production of coated seeds to be used in the field experiment. Arabic gum was observed to give a better coverage of sorghum seeds and hence higher cfus per seed compared to the other adhesive material.

Arabic gum was also chosen by Ciotola *et al*. (2000) for sticking the chlamydospores of *Fusarium oxysporum* M12-4A to sorghum seeds, because Arabic gum has the highest water solubility index among all gums and provided a carbon source that stimulated germ tube elongation and production of a new generation of chlamydospores in the absence of the host. In addition, Arabic gum is available at local markets of the Sahelian and Subsahara countries including Sudan.

It is vital that the application of the bioagent does not affect germinability and vigor of growth, which are important quality parameters of seeds (McQuilken *et al*. 1998). During this study sorghum seed germination assessed with the Blotter test was significantly affected by coating with fungal biomass compared to uncoated sorghum seeds and to those coated with adhesive materials only. However, when the germination test was repeated in soil, the germination of coated seeds was increased irrespective to the fungal isolate and adhesive material used and became comparable to the control. Generally, seed coating with fungal biomass was observed to delay sorghum seed germination, which could possibly be attributed to fungal metabolites that affect the germinability. Or maybe the high inoculum concentration (mycelium growing on the seed surface) and the adhesive material form a solid layer around the seed that slow down the imbibition rate of water by sorghum seeds resulting in delayed germination. The delaying effect was not observed under field conditions where sufficient water was applied through irrigation.

During the second season, seed coating with FA and Arabic gum (10^4 cfu $seed^{-1}$) was able to reduce *Striga* emergence as well as the total number of *Striga* shoots significantly compared to the control for approximately one month from sorghum

emergence, indicating that the bioagent was able to colonize the sorghum root and prevented *Striga* attachment. With the progress of the season the effect became insignificant. Seed coating using fine formulated “Pesta” and Arabic gum (10^2 cfu $seed^{-1}$) as adhesive material did not show a significant effect on *Striga* emergence and total number compared to the control. Nevertheless, it was able to increase the proportion of diseased *Striga* significantly compared to the untreated control and other seed coating treatments. Despite the lower concentration of the inoculum around the seed compared to the granular formulation dose, the seed treatment containing FA and SUET adhesive material had a comparable effect in controlling *Striga* and improving sorghum yield to the granular preparations placed in the planting hole at the rate of 1.5 g, this could be attributed to high disease incidence on *Striga* shoots about 70% (induced by FA and SUET adhesive material) comparable to that caused by “Pesta” granules 74%. Thus, seed coating as a simple delivery system showed a high potential to control *Striga.* However, its efficacy needs to be improved through optimizing the type of inoculum. Investigations should concentrate on using chlamydospores because they can withstand extreme environmental conditions (Nash *et al*., 1981) and readily germinate when conditions are favorable (Schippers and Van Eck, 1981). Also the amount of inoculum and the selection of appropriate adhesive materials need to be optimized.

5.4 Efficacy of alginate pellets in controlling *Striga*

To increase the efficacy of the potential mycoherbicides used to control *Striga* under field conditions, an additional type of formulation (alginate granules) has been prepared and used in the second season of this study. The formulation of propagules in alginate pellets is a type of solid or granular formulation that is suitable for pathogens infecting their host below the soil surface, thus it seems to be a convenient delivery system for pre-emergence mycoherbicides (Daigle and Cotty, 1992). An alginate formulation was firstly developed by Walker and Connick (1983) by adding propagules of bioherbicidal fungi to a sodium alginate solution, which is then dripped into a calcium chloride, or calcium gluconate solution. Alginate pellets were formed due to ionotrophic gelation (Connick, 1988). Alginate formulation proved to be effective for a number of potential bioherbicides and has many advantages over other formulations since the granules are uniform in size, low weight, convenient to

store, and non toxic. Furthermore, they can be produced on a large scale and applied using the existing agricultural machinery such as convential granular application equipment (Fravel *et al.*, 1985; Lumsden and Locke, 1989; Daigle *et al.,* 1997).

In the conducted experiment, alginate preparations using different substrates (wheat flour, sorghum flour and yeast extract) were prepared using the method described by Walker and Connick (1983) and modified by Müller-Stöver (2001). Alginate formulations gave a higher concentration of cfu per g formulated material (about 10^8 cfu g^{-1}) compared to "Pesta" granules, which gave about 10^7 cfu g^{-1}. This could be due to higher losses of viable propagules during the "Pesta" formulation process, which was also observed by Hebbar *et al*. (1998). The tested additives also had an influence on the number of viable cfu per g formulation: alginate preparations of both fungal isolates amended with sorghum flour or yeast extract had significantly higher cfu compared to those with wheat flour, and yeast extract amendment significantly increased the number of cfu compared to sorghum amendment. This coincides with results of Müller- Stöver (2001) who reported that, on the surface of alginate granules of *F. oxysporum* f.sp. *orthoceras* amended with brewer's yeast a higher number of conidia were produced than on a preparation containing wheat bran, which was also reflected in the effectiveness of the two preparations. Corresponding relations were also reported by Weidemann (1988) for alginate pellets of *F. solani* f.sp*. cucurbitae* amended with ground corn meal, oatmeal or soy flour. The amendments increased the conidial production on the surface of the granules within 14 days of soil application.

Only the efficacy of one alginate formulation amended with wheat flour was tested under field conditions in comparison to the "Pesta" formulation at a rate of 1.5g per hole. The other alginate preparations were tested in an outdoor pot experiment. Under field conditions, the *Fusarium* isolates formulated in alginate granules were able to significantly delay *Striga* emergence and reduce the total number of *Striga* shoots compared to the control indicating that the bioagents were able to proliferate well on the granules and attacked *Striga* early in the season. In contrast, the "Pesta" preparations did not delay *Striga* emergence (as they did in the first season). Though "Pesta"-granulated *Fusarium* species were able to reduce the total number of *Striga* shoots early in the season, the reduction was less than that caused by alginate pellets. *Fusarium* species encapsulated in "Pesta" and alginate granules were found to induce disease symptoms on *Striga* shoots early in the season and the disease

development progressed with time. At the end of the season, about 70 to 80% of the shoots were diseased, irrespective of the formulation applied. The overall efficacy calculated as the reduction of healthy *Striga* shoots compared to the control (Bedi, 1994) ranged between 34 - 56% for “Pesta” preparations and 56-60% for alginate pellets.

The preparations tested in the field gave excellent results in controlling *Striga* when tested in an outdoor pot experiment. Especially FA incorporated in “Pesta” granules was very efficient causing a complete inhibition of *Striga* emergence throughout the season. The main differences between the pot and field experiment are good water management and the type of soil used (pot experiment: river alluvial soil mixed with sand 1:1 (v/v)). This fact strengthens the assumption that soil factors play an important role in affecting the efficacy of the formulations under field conditions, which needs to be investigated in more detail. The better efficacy of “Pesta” compared to alginate formulation in the pots could be attributed to the different type and amount of food base in the formulation that allowed a rapid growth of the fungus over the surface of the wheat flour-granule. A positive correlation was reported between the amount of conidia of *F. oxysporum* applied in pots and its effect on *Orobanche cumana* by Thomas *et al*. (1998). In contrast, alginate formulation showed a better efficacy in controlling *Striga* under field conditions compared to “Pesta” especially early in the season. This difference could be due to the ability of the alginate matrix to provide a better protection to the bioagents to withstand adverse environmental conditions (Connick *et al*., 1990)

5.5 Persistence of *F. nygamai* and *F*. Abuharaz in the soil

The application of mycoherbicides to the soil in a granular formulation can be advantageous over a liquid application, not only because of greater stability but also because the bioagent is able to produce secondary spores on the granule surface (Weidemann *et al*., 1995).

In the present study, FA was able to recover from “Pesta” granules and abundant propagules (about 10^5 cfu per g soil) could be detected in the soil a few days after its application. Population counts declined rapidly within two weeks, but the fungal propagules were still detected in the soil for the sampling period (about 10 weeks, 10^4 cfu per g soil) during the first season. The establishment of FN from “Pesta” granules in the soil was slower, fungal propagules (at a rate of 10^4 cfu per g soil)

could be detected after 2 weeks only, compared to 5 days for FA. Abbasher *et al.* (1996) assumed that *F. nygamai* does not have the ability to penetrate the soil effectively, as only limited movement from the treated area was observed.

In the second season, the bioagents could develop out of both types of formulations ("Pesta" and alginate granules) in the soil under controlled as well as under field conditions. In all treatments, fungal propagules were detected in the soil until the end of the sampling period (9 weeks after application), with 10^6 cfu g^{-1} dry soil (under controlled conditions) and 10^3 cfu g^{-1} soil (under field conditions). A positive correlation between a high population of *Fusarium* early in the season and a high control efficacy is found. Furthermore, the highest fungal populations originating from the alginate formulations of both isolates and from FN formulated in "Pesta" granules were associated with a high proportion of diseased *Striga* shoots. Comparing the establishment of fungal populations out of "Pesta" granules during the two field seasons, the detected cfus were higher during the first season for both isolates. This may be attributed to the high inoculum dose used in the first season (2 g/hole) and / or could be due to more favorable environmental circumstances in the first season that allowed a better proliferation of the fungi.

The persistence of a fungal biocontrol agent in the soil can be influenced not only by the type of formulation used, but also by the type and concentration of propagules incorporated, amendments added to the formulation, and the initial dosage applied to the soil. It was found that the amendment of soy flour and oatmeal increased the soil persistence of *F. solani* f.sp. *cucurbita*, a biocontrol agent for Texas gourd, incorporated in an alginate formulation (Weidemann, 1988). In addition, formulated macroconidia were more persistent in the soil than microconidia. Hebbar *et al.* (1998) working with *F. oxysporum* EN-4, a pathogen of *Erythroxylum coca* var. *coca*, concluded that the substrates amended to an alginate formulation not only determined the number of microconidia produced but also enhanced their conversion to chlamydospores which is critical for long term persistence in the soil. For *Talaromyces flavus,* a biocontrol agent for plant pathogens formulated in alginate pellets, it was found that the propagule type, the initial inoculum level in the pellets, the type of filler material used to make the pellets and the initial concentration added to the soil each significantly affected fungal survival in and proliferation into soil from the pellets. Preparations incorporating conidia or ascospores using bran or Pyrex as bulky material resulted in a greater number of cfu in the soil compared to

formulations prepared using fungal biomass (Papavizas *et al.*, 1987). These factors need to be optimized for FA and FN also to improve their persistence in the soil and hence their efficacy in controlling *Striga* under field conditions.

5.6 Chemical control vs. bioherbicides

Numerous chemicals, such as fumigants, germination stimulants, antitranspirants, or organic acids to harden host-crop seeds have been investigated to destroy either the dormant *Striga* seeds in the soil, the germinating seeds, or the emerged weed (Lagoke *et al.*, 1991). However, few methods have been developed to make a practical use of those natural or synthetic substances under field conditions for the control of *Striga* (Parker and Riches, 1993). This is mainly because most of the subsistence farmers are generally not able to take the advantage of chemical herbicides due to their costs, the skills required for their safe application, and because they carry a greater risk of crop damage that prevent their use in most mixed cropping systems (Parker and Riches, 1993). However, *Striga* infestation may be one single situation where it is feasible to introduce herbicides, in particular as an aid to prevent seeding and as an alternative to hand pulling for destruction of the emerged parasite.

The ideal herbicide is one that controls *Striga* before or immediately after attachment so that damage to the host plant is prevented (Parker and Riches, 1993). 2,4-dichlorophenoxacetic acid (2,4-D) has a high activity at the germination stage of *Striga* and can destroy also the emerged weed, thus reducing damage to the crop. However, its success depends greatly on irrigation or rainfall washing the chemical into the soil at the critical time of *Striga* germination, 2-3 weeks after planting of sorghum (Parker and Riches, 1993). In Sudan, 2,4-D was found to be effective in controlling *Striga* when applied 2-3 times after sowing of sorghum (Last, 1960). The herbicide can also be applied as a post emergence knockdown herbicide as an alternative to hand weeding and is considered as important component of the routine *Striga* eradicating program in the USA. In an experiment using 2, 4-D to prevent further *Striga* seed production, it was observed that some of the plants are completely destroyed but generally the meristematic apical zone is most sensitive. Therefore, the herbicide needs to be sprayed several times directly on the parasites during the growing season, because *Striga* seedlings that are still in their

underground stage are unaffected (Paré *et al.,* 1997). However, repeated herbicide application seems to be uneconomical for the subsistence farmer.

During this study 2,4-D had a pronounced effect, reducing *Striga* incidence and causing a quick knockdown of the total number of *Striga* shoots, which was significantly reduced by 97% at the second count. However, by the end of season the chemical herbicide reduced the total number of *Striga* insignificantly by 68 %, an effect which was similar to that caused by FA applied at 2.0 g per hole. *Striga* biomass was also insignificantly reduced by 2,4-D, comparable to or less than the reduction caused by the *Fusarium* isolates. Sorghum performance improved after the chemical *Striga* control and sorghum 100-seed weight was increased significantly, as also achieved by most of the biocontrol treatments. This indicates that formulated FA and FN have a similar efficacy as the chemical herbicide. Similar to the biocontrol agents, the efficacy of the herbicide was also affected by the adverse environmental factors in the second season. It was found to induce only wilting symptoms on the treated *Striga* shoots instead of causing a knockdown as in the first season.

Combination of low or sublethal doses of herbicides and biocontrol agents could result in an increased weed control since an affected plant could be predisposed to infection by a pathogenic fungus. Additionally, several herbicides had a stimulatory effect on hyphal growth and spore production of pathogens, as it was the case with trifluralin and glyphosate. (Altman *et al.,* 1990). Furthermore, sublethal doses of glyphosate can inhibit the plant's ability to produce phytoalexins and hence increase fungal virulence (Levesque and Rahe, 1992.)

There are several examples of an increased efficacy of mycoherbicides when they had been combined with chemical herbicides. *Fusarium solani* f.sp. *cucurbita* (FSC), a potential mycoherbicide for Texas gourd (*Cucurbita texana*) in soybean, was successfully integrated with trifluralin in tank-mixture treatments. This combination reduced weed seedling emergence more than either FSC or trifluralin alone (Weidemann, 1988; Roy and Smith, 1991). The addition of a sublethal rate of the herbicides linuron, imazaquin and lactofen to *Alternaria cassiae* spores in an invert emulsion resulted in a significantly increased control of sicklepod (Boyette *et al*., 1991). The control of velvetleaf (*Abutilon theophrasti*) significantly improved by the sequential application of 2,4-DB (2,4-dichlorophenoxybutaric acid) and spores of *Fusarium lateritium* (Boyette and Walker*,* 1985)*.*

Recently herbicide seed treatment using imazapyr or 2,4-DB appears to be a promising approach for the control of *Striga* in maize or sorghum (Kanampiu *et al*. 2001; Dembélé *et al*., 2005). Therefore this simple and cost effective technique can be integrated with mycoherbicides for more sound *Striga* control using herbicide such as imazapyr or 2,4-DB, which are proved to be suitable for seed treatment and effective in controlling *Striga*. Nevertheless, the compatibility of these herbicides with the *Fusarium* isolates should be tested.

5.7 Mycotoxins

For environmental safety the mycoherbicide candidate should not produce toxins that are hazardous to humans and animals. Trichothecenes, zearalenones (ZEN) and fumonisins are the major *Fusarium* mycotoxins occurring on a worldwide basis in cereal grains, animal feeds and forages. Other important *Fusarium* mycotoxins include moniliformin and fusaric acid. Spontaneous outbreaks of *Fusarium* mycotoxicoses have been recorded in Europe, Asia, New Zealand and South America and, in addition, chronic exposure occurs on a regular and more widespread scale (D'Mello and Macdonald 1997; D'Mello *et al.*, 1999).

The trichothecene skeleton is chemically stable and the 12,13-epoxide ring is stable to nucleophilic attack. Furthermore, trichothecenes are heat stable and are not degraded during normal food processing or autoclaving (Wannemacher *et al*. 2000), cited in Rocha *et al*., 2005). The common effects of trichothecenes on both animal and plant cells include inhibition of protein, DNA and RNA synthesis, inhibition of mitochondria functions, inhibition of cell division and membrane effects. In animal cells, trichothecenes induce apoptosis, a programmed cell death (PCD) response (Rocha *et al*., 2005). Recently it is concluded that trichothecenes are not likely to cause any harm in ruminants unless fed visibly damaged feed and no limit is probably needed. Poultry are more sensitive to trichothecenes than ruminants. Levels from 9 mg DON/kg feed have lead to negative effects in chickens, while no effect was found in chickens fed 5mg DON/kg feed, and a limit of 2.5 mg DON/kg feed is proposed. The available information about nivalenol (NIV) does not allow a limit to be set, but the finding of minor pathological changes in chicken fed 1mg NIV/kg feed indicates that NIV may be more toxic to poultry than DON. Oral lesions are observed in chickens and hens fed 1mg T-2 toxin/kg feed. Other effects, including a reduction in feed intake, are found with increasing levels of T-2 toxin. Pigs

are more sensitive to trichothecenes than other farm animals. The effects occurring at the lowest levels of trichothecenes are reduced feed intake and weight gain, normally occurring at levels from 0.6 mg DON/kg feed in naturally contaminated feed. Pigs fed 0.5 mg T-2 toxin/kg feed reduced their feed intake. Impairment of the immune system has also been observed in pigs at this level of T-2 toxin in the feed. Guidelines of 0.3 mg DON/kg feed and 0.2 mg T-2 toxin/kg feed is proposed in pig feed (Eriksen and Petterson, 2004). Recently it was proved that trichothecenes are rapidly excreted from animals and residues of trichothecenes in animal-derived food products are not considered to pose any threat to consumers (Eriksen and Petterson, 2004).

There is now undisputed evidence that ZEN and its metabolites possess estrogenic activity in pigs, cattle and sheep. T-2 toxin has also been identified to cause reproductive disorders in farm livestock. Fumonisins are positively linked with pulmonary edema in pigs, leukoencephalomalacia in equines and with deranged sphingolipid metabolism in these animals. *Fusarium* mycotoxins have also been provisionally implicated in ovine ill-thrift, acute mortality of poultry and in duodenitis/proximal jejunitis of horses (D'Mello and Macdonald 1997, D'Mello *et al.,* 1999).

Toxins produced by FA on inoculated wheat grains have been screened and none of the following trichothecene toxins could be detected: nivalenol, deoxynivalenol, 15-actetyldeoxynivalenol, neosolaniol, fusarenon-x, scripentriol, monoactoxyscripenol, diacetoxyscripenol, T-2 triol, HT-2 toxin and T-2 toxin. Also FN proved to produce none of the above mentioned trichothecene toxins (Abbasher, 1994). Harvested sorghum seeds from the plots treated with both biocontrol agents have been free of trichothecene toxins as well.

The crude filtrate of *Fusarium* Abuharaz reduced *Striga* germination by 50% after three days following conditioning *Striga* seeds with the filtrate but the germination rate increased with time (Mohamed, 2002). The same author proved that *Striga* seed germination is strongly inhibited by *F*. Abuharaz conidial suspension, which indicates that the effect of *Fusarium* Abuharaz and its possible phytotoxins on *Striga* seeds needs further investigations.*F. nygamai* was found to produce phytotoxins such as fusaric acid, dehydrofusaric acid and their corresponding methyl esters in a higher amount (Capasso *et al.*, 1996). These substances proved to be very phytotoxic against *Striga* seeds even at lower concentrations. Zonno and Vurro (1996)

concluded that phytotoxins produced by *Striga* pathogens could be used in the field as natural and safe herbicides for the suppression of weed seed germination or could be combined with other cultural and biological methods for an integrated management of the noxious weed. *F. nygamai* is also known to produce fumonisin B_1 (Capasso *et al.*, 1996) that showed cancer promoting activity in rats at high doses over a long period of time (Gelderblom *et al.*, 1988). Recently, Kroschel and Elzein (2004) proved that fumonisin B_1 could be a potential natural herbicide for controlling *Striga*, however, before its commercialization as a bioherbicide investigations in the field will be necessary to assess the natural degradation and the contamination of cereals after application.

5.8 Conclusions and prospective

The “Pesta” formulation proved to be an easy and cost-effective delivery system for the tested fungal isolates. FA and FN singly encapsulated in “Pesta” granules applied at 1.5g in the planting hole showed a high efficacy to control *Striga* effectively and consequently improved sorghum performance under Sudan field conditions. However, the procedure could be further optimized through:

- Finding a cost-effective medium for inoculum mass production, preferablely for chlamydospore production by the two fungi.
- Optimizing the formulation itself to improve the persistence in the soil and hence the efficacy in controlling *Striga* by finding the optimum type and amount of inoculum to be incorporated and by finding additives that allow rapid colonization of the granules by the biocontrol agents and maintain their viability.
- Studying and optimizing storage conditions and shelf-life of the “Pesta” formulation for both FA and FN.
- Studying the compatibility of mycoherbicides used in this study with the chemicals and pesticides used in sorghum production so as to achieve an appropriate integrated pest management strategy.

The alginate formulation also seems to be an appropriate technique to control *Striga*, when incorporated in the soil at 1.5 g per planting hole and could be preferable to “Pesta” under field conditions. Since alginate granules amended with sorghum flour or yeast extract showed an increased efficacy compared to pellets amended with wheat

flour, the efficacy of alginate pellets with different amendments needs to be studied under field conditions.

Seed treatment with biological agents is an attractive delivery system since it combines specific effects with a limited environmental impact and delivers the bioagents at the right amount and the right time to the right place. Seed treatment could possibly be used effectively under field conditions to control *Striga*. However, its efficacy needs to be increased by:

- Selection of an appropriate seed coating material and carriers suitable for rapid growth and sporulation.
- Optimizing the inoculum type and concentration around the seed.
- Studying the effect of storage conditions (such as temperature and relative humidity) and shelf life on the treated seeds.
- Combining the biocontrol agents with other compatible pesticides such as fungicides used already as a regular seed treatment in sorghum production.

Since disease severity of several plant pathogens is known to increase in response to various chemical herbicides, the efficacy of FA and FN could possibly be increased if they would be integrated with compatible chemical herbicides.

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7 Summary

Striga hermonthica is a parasitic flowering plant belonging to the family Orobanchaceae. It is a root parasite that attacks sorghum, maize, millet and several grass weeds in the semi-arid Tropics. In Sudan, *Striga* is widespread in irrigated and rainfed areas and considered the main biotic constraint in production of sorghum, the main staple food for the majority of Sudanese people. More than 500,000 hectares under rainfed cultivation are heavily infested with *Striga*, which commonly results in significant yield losses of 70 – 100%. It has become obvious that there is no simple, fast and inexpensive solution to the *Striga* problem in Africa. Biological control is considered a potential cost-effective and environmentally safe means for reducing weed populations in crops, forests, or rangelands where low profit margins prevent large herbicide expenditure.

Biological control using microorganisms (especially phytopathogenic fungi) showed a high efficacy in controlling *S. hermonthica* under controlled and field conditions. However, so far it did not come to practical field application. This could be attributed to environmental obstacles or due to the lack of appropriate delivery systems. The pathogenicity of two fungal isolates indigenous to Sudan (*Fusarium nygamai* [FN] and *F*. "Abuharaz" [FA] isolate) against *Striga* has been studied using infected sorghum grains or a spore suspension as inoculum. These formulations were very effective in controlling *Striga* under controlled and natural conditions; however, a high level of fungal inoculum (approximately 800 kg ha^{-1} for the grain inoculum) would be required for effective control, which arises a lot of problems e.g. concerning sterilization and transportation. Such problems can be overcome by adopting an appropriate formulation technology. Granular formulations such as "Pesta" and alginate pellets were found to be suitable delivery systems for controlling weeds. "Pesta" granules are made by encapsulating bioagents in a gluten matrix. Alginate formulations are prepared by incorporating the biocontrol agent's propagules in a sodium alginate solution, which is dripped to a calcium chloride or calcium gluconate solution. Alginate pellets are then formed by ionotrophic gelation.

The main objectives of this study were: (a) to study the efficacy of the two *Fusarium* species in controlling *Striga* under field conditions using "Pesta" and alginate formulations, (b) evaluate the effect on sorghum yield, (c) determine the optimum dose of the formulated material, (d) investigate the persistence of the formulated

fungal isolates in the soil, and (e) study the efficacy of seed treatments as an alternative delivery system. Furthermore, for environmental safety reasons the newly isolated *F.* "Abuharaz" isolate was tested for its ability to produce some of the most important mycotoxins. Harvested sorghum seeds out of the fungus-treated plots were also investigated for their mycotoxins content.

A prerequisite to be able to formulate biocontrol fungi is the development of an inexpensive method of inoculum production that yields sufficient biomass containing viable, highly virulent propagules. Chlamydospores are the soil-persisting propagules of many *Fusarium* species and considered as ideal propagules to be used in granular formulations. For this reason, finding a medium suitable for the production of chlamydospores by the two *Fusarium* isolates was one of the specific objectives of this study. Different media were tested among them Special Nutrient-poor Broth (SNB) + yeast gave the highest number of chlamydospores (10^5 ml^{-1}) in both isolates throughout the incubation period. However, both isolates generally did not form sufficient chlamydospores to be used within a bioherbicide formulation. Richard's solution gave the highest number of microconidia (10^8 ml^{-1}) after five days of incubation and hence it was selected as growth medium for formulation purposes throughout this study.

FN and FA were successfully formulated in "Pesta" and alginate granules amended either with 10% wheat flour or 6% sorghum flour or yeast extract. Alginate granules generally gave higher numbers of colony forming units (cfu) per g of formulated material compared to "Pesta". Alginate preparations amended with 6% sorghum flour or yeast extract had significantly higher cfu compared to the alginate formulation using 10% wheat flour. Yeast extract amendment further increased the number of cfu by about 38 and 32% for FN and FA, respectively, compared to sorghum amendment.

In the first field experiment (2003/2004), a screening for the suitable dose of "Pesta" granules per planting hole to control *Striga* was conducted together with the investigation of a seed coating treatment as an alternative delivery system of the biocontrol agents. The "Pesta" technology showed a potential to be used as a delivery system to control *S. hermonthica* under field conditions. Both "Pesta"-formulated *Fusarium* isolates were able to delay *Striga* emergence, reduce the total number of *Striga* shoots and induce disease symptoms on all growth stages of *Striga* plants, irrespective to the dose and method of application used. The highest control

efficacy was achieved by applying FA at 1.5g, which reduced the total number of parasite shoots by 82 % and the number of healthy *Striga* shoots by 88% compared to the untreated control. As a consequence, sorghum biomass and sorghum 100-seed weight were increased by 86 and 110%, respectively. FN and the combination of the fungal isolates were slightly less efficient in controlling the parasites. 1.5 g "Pesta" granules per planting hole was found to be the optimum dosage for *Striga* management since increasing the dosage did not result in a significant improvement of control.

In the second season (2004/05), the efficacy of alginate formulations amended with 10% wheat flour applied at 1.5g/planting hole was evaluated in addition to the "Pesta" formulation for controlling *Striga* under field conditions. Alginate granules were able to delay *Striga* incidence significantly and reduce the total number of *Striga* shoots by 64 – 78 % compared to the control early in the season. In contrast to the first season, fungal isolates formulated in "Pesta" granules had no pronounced effect on delaying *Striga* emergence, however, "Pesta"-granulated *Fusarium* species were able to reduce the total number of *Striga* shoots by 42 – 55 % compared to the control early in the season. By the end of the season, the effect of both formulations on the total number of *Striga* shoots became negligible, but they significantly increased disease incidence on *Striga* shoots compared to the untreated control. FA formulated in "Pesta" or alginate pellets was especially effective in this regard, causing disease in 74 and 80% of the *Striga* plants and reducing the total number of healthy *Striga* shoots by 55 and 60% compared to the control, respectively. FA applied as "Pesta" granules was the most effective treatment in reducing *Striga* biomass by 58 % compared to the control which was positively reflected in an increased sorghum grain yield (63%) and sorghum straw yield (73%) compared to the control.

The reduction of the efficacy of the "Pesta" formulation in controlling *Striga* in the second season compared to the first season can probably be attributed to three reasons. These include a) climatic conditions, which differed from the first season in higher temperatures coupled with lower rainfall and low relative humidity, b) sodicity problems in the fields which might have affected the proliferation of the fungi in the soil and c) an inhibitory effect of the metabolites of the applied insecticide Sevin (active ingredient Carbaryl (1-naphthyl N-methylcarbamate)) on the virulence of soil fungi.

Furthermore, an outdoor pot experiment was conducted to study the efficacy of alginate formulations with different amendments (wheat flour, sorghum flour and yeast extract) in comparison to the “Pesta” formulation and seed treatments on controlling *Striga*. In this experiment, FA formulated as “Pesta” granules was the most effective treatment and successfully inhibited *Striga* emergence until the end of the season. This was reflected in a significantly increased sorghum plant height (by 80%) and sorghum shoot dry weight (400%) compared to the negative control. *Fusarium* species in alginate granules also delayed *Striga* emergence and reduced the total number of *Striga* throughout the growing season. The best efficacy was obtained by FA, which reduced the total number of *Striga* shoots by 71% (using 10% wheat flour) and 84% (6% sorghum flour or yeast extract) compared to the control. Likewise, it reduced the proportion of healthy *Striga* shoots by 71%, 88% and 84%, respectively, and *Striga* biomass by 50%, 81%, and 89%, respectively. Alginate formulations generally also significantly increased sorghum plant height by up to 80% and sorghum shoot dry weight by 200 to 400% compared to the control.

It can therefore be summarized that of the investigated fungal isolates and granular formulations FA formulated in “Pesta” granules showed the best efficacy in controlling *Striga* under field and controlled conditions.

The ability of FA to produce trichothecene mycotoxins that could be a hazard to humans or animals was assessed from samples of the fungus growing on autoclaved wheat grains. Additionally, samples of harvested sorghum seeds from various plots inoculated with the biocontrol agents were investigated for the content of trichothecene mycotoxins. None of the following toxins were either produced by FA or translocated to harvested sorghum seeds under field conditions: nivalenol, fusarenon X, deoxynivalenol, 15-actetyldeoxynivalenol, scirpentriol, monoacetoxyscirpenol, diacetoxyscirpenol, T-2 triol, HT-2 toxin, T-2 toxin and neosolaniol.

A simple seed coating treatment using fine “Pesta” granules and gum Arabic as adhesive material also showed a potential to control *Striga* in the first season experiment. It was able to reduce the total number of *Striga* shoots by more than 55% compared to the control. Consequently, sorghum biomass was increased by 54 – 67% and sorghum100-seed weight by 70%. The effects were comparable to that caused by chemical control using 2,4-D.

To improve the efficacy of seed coating under field conditions for the second season experiments, the effect of five types of adhesive materials (2 types of cellulose, 2 types of organic polymers and a clay) on growth and sporulation of FA and FN were tested in solid and liquid media. Cellulose 1 and 2 were found to enhance radial growth of both isolates but did not increase sporulation in liquid media. Organic polymer 2 was found to retard both radial growth and sporulation of the two isolates. Organic polymer 1 and clay significantly enhanced the production of chlamydospores, especially by FA, compared to Richard's solution alone or amended with the other tested materials. For this reason one of them was suggested to be used for seed coating in addition to Arabic gum. Sorghum seeds were coated by a private company (SUET Saat- und Erntetechnik GmbH, Eschwege, Germany), using air-dried fungal propagules fermented on 1.5 % (w/v) sorghum straw. Arabic gum was observed to give a better coverage of sorghum seeds and higher cfu per seed (4 x 10^4 [FA] and 19 x10^4 cfu [FN]) compared to the second adhesive material. Coating sorghum seeds with the biocontrol agents did generally not result in a significant reduction of *Striga* shoots in the field, but some of the seed-coating treatments were very efficient in inducing disease symptoms on *Striga* shoots. Especially FN coated with Arabic gum and FA applied to the seeds in fine "Pesta" granules increased the proportion of diseased *Striga* shoots significantly compared to the control by 79%.

In the pot experiment, the fungal isolates applied as a seed coating neither resulted in a significant reduction of *Striga* shoots. Nevertheless, FA applied to sorghum seeds using the adhesive material provided by SUET reduced the total number of *Striga* plants by 52% compared to the control at the end of the season. FN in the same treatment significantly increased the proportion of diseased *Striga* by 77% at the end of the season. The effect of seed coating on increasing sorghum plant height and dry matter was lower than that caused by the granular formulations and not statistically significant compared to the control.

From the obtained results it can be concluded that both granular formulations applied to the planting holes and seed coating can be used as effective delivery systems for biocontrol fungi and can be adopted under field conditions to reduce *Striga* infestation. However, the granular formulations showed a higher efficacy in controlling *Striga*. Inoculum type and concentration as well as nutritional amendments to the formulations should be further optimized in future investigations.

8 Zusammenfassung

Striga hermonthica ist eine parasitische Blütenpflanze aus der Familie der Orobanchaceae. Sie ist ein Wurzelparasit und befällt Sorghum, Mais, Hirse und verschiedene Gräser in den semiariden Tropen. Im Sudan ist *Striga* im Bewässerungs- und Regenfeldbau weit verbreitet und gilt als wichtigster biotischer Begrenzungsfaktor bei der Produktion von Sorghum, dem Hauptnahrungsmittel für die Mehrheit der sudanesischen Bevölkerung. Mehr als 500.000 Hektar des im Regenfeldbau kultivierten Ackerlands sind stark mit *Striga* befallen, was in der Regel Ertragsverluste von 70 bis 100% zur Folge hat. Mittlerweile ist deutlich geworden, dass es für das *Striga*-Problem in Afrika keine einfache, schnelle und billige Lösung gibt.

Biologische Kontrolle gilt als ein potentiell kostengünstiges und umwelt- freundliches Instrument, um Unkrautpopulationen in Ackerkulturen, Wäldern oder Weideland, wo niedrige Gewinnspannen einen hohen Herbizideinsatz unmöglich machen, zu regulieren. Die biologische Kontrolle von S. *hermonthica* mit Mikro- organismen (v.a. phytopathogenen Pilzen) erwies sich in bisherigen Untersuchungen als effektiv. Bis jetzt wird allerdings kein derartiges Verfahren in der Praxis genutzt, was unter anderem auf den Mangel an geeigneten Formulierungs- und Applikationssystemen zurückzuführen ist. Die Pathogenität zweier *Fusarium*-Isolate aus dem Sudan (*Fusarium nygamai* (FN) und *Fusarium* “Abuharaz” (FA)-Isolat) gegenüber *Striga* wurde mit Hilfe infizierter Sorghumkörner oder einer Sporensuspension als Inokulum untersucht. Mit diesen Formulierungen wurde *Striga* sehr effektiv unter kontrollierten und Freilandbedingungen bekämpft, allerdings wurden für eine ausreichende Kontrolle sehr große Mengen an Inokulum (etwa 800 kg ha^{-1} infizierte Sorghumkörner) benötigt. Dies würde für einen großflächigen Einsatz beispielsweise hinsichtlich Sterilität und Transport sehr problematisch werden, daher ist die Entwicklung einer angepassten Formulierungstechnologie unumgänglich. Granuläre Formulierungen wie “Pesta” oder Alginat-Pellets können für die biologische Unkrautbekämpfung geeignet sein. “Pesta”-Granulate werden durch Einkapseln der biologischen Gegenspieler in eine Glutenmatrix hergestellt. Für die Produktion von Alginat-Pellets wird das Inokulum in eine Natriumalginatlösung eingebracht, die in Calciumchlorid oder -gluconat ein- getropft wird. Die Pellets enstehen dann durch ionotrophe Gelbildung.

Die Hauptziele der vorliegenden Arbeit waren: a) die Effizienz der zwei *Fusarium*-Isolate formuliert als “Pesta”-Granulate oder Alginat-Pellets hinsichtlich ihrer Wirkung auf *Striga* unter Feldbedingungen zu untersuchen; (b) den Effekt auf den Sorghumertrag zu evaluieren; (c) die optimale Dosis des formulierten Materials zu bestimmen; (d) die Persistenz der formulierten pilzlichen Isolate im Boden zu beobachten; und (e) die Effizienz von Saatgutbehandlungen als alternative Applikationsform zu untersuchen. Hinsichtlich der Umweltverträglichkeit der potentiellen Produkte wurde das erst kürzlich isolierte *F*. “Abuharaz” auf seine Eigenschaft getestet, einige der wichtigsten Mykotoxine zu produzieren. Geerntete Sorghumkörner aus den *Fusarium*-Behandlungen im Feldversuch wurden ebenfalls hinsichtlich ihres Mykotoxingehaltes überprüft.

Eine Voraussetzung für die Formulierung von biologischen Gegenspielern ist die Entwicklung einer preiswerten Methode zur Produktion von Inokulum, die es ermöglicht, ausreichend pilzliche Biomasse mit lebensfähigen, virulenten Vermehrungseinheiten zu gewinnen. Chlamydosporen sind die Überdauerungsformen vieler *Fusarium*-Arten und werden als ideale Sporenform für die Herstellung von Festformulierungen betrachtet. Daher war eines der speziellen Ziele dieser Arbeit, ein Medium zu finden, das die beiden untersuchten *Fusarium*-Isolate zur Chlamydosporenproduktion anregt. Verschiedene Flüssigmedien wurden getestet, von denen SNB (Special Nutrient-poor Broth) plus Hefe bei beiden Isolaten die besten Ergebnisse erzielte (10^5 Chlamydosporen ml^{-1}). Diese Anzahl war jedoch noch zu gering, um für die Weiterverarbeitung in einer Formulierung in Frage zu kommen. Die höchste Anzahl an Mikrokonidien nach 5 Tagen Inkubationszeit (10^8 ml^{-1}) wurde in Richard’s Medium gebildet, daher wurde dieses während der gesamten Arbeit als Wachstumsmedium für die Pilze verwendet.

FN und FA wurden erfolgreich sowohl in “Pesta”-Granulate als auch in Alginat-Pellets (angereichert mit 10% Weizenmehl oder 6% Sorghummehl oder Hefeextrakt) formuliert. Alginat-Pellets zeigten durchweg eine höhere Anzahl von „colony forming units“ (cfu) pro g formuliertem Material verglichen mit den “Pesta”-Granulaten. Die Alginat-Formulierungen mit 6% Sorghummehl oder Hefeextrakt hatten signifikant höhere cfu verglichen mit den Pellets mit Zusatz von 10% Weizenmehl. Der Zusatz von Hefeextrakt erhöhte die Anzahl der cfu pro g nochmals um 38% (FN) und 32% (FA) gegenüber dem Zusatz von Sorghummehl.

Im ersten Feldversuch (2003/2004) wurde ein Screening für eine effektive Dosis der “Pesta”-Granulate durchgeführt, zusätzlich wurde die Saatgutbehandlung als alternative Applikationsmethode der biologischen Gegenspieler getestet. Die “Pesta”-Formulierung erwies sich als geeignet für die Applikation der *Fusarium*-Isolate. In dieser Formulierung verzögerten beide Isolate den Auflauf der *Striga*-Pflanzen, verringerten die Gesamtanzahl der Sprosse und induzierten Krankheitssymptome an allen Stadien der Parasiten, unabhängig von der eingesetzten Dosis. Die höchste Kontrolleffizienz wurde nach der Applikation von FA mit 1.5 g pro Pflanzloch erzielt: die Gesamtanzahl der *Striga*-Sprosse gegenüber der unbehandelten Kontrolle wurde um 82% und die Anzahl der gesunden *Striga*-Sprosse um 88 % reduziert. Dies hatte eine Erhöhung der Sorghum-Biomasse und des 100-Korn-Gewichts von Sorghum um 86% bzw. 110% zur Folge. FN und die Kombination beider *Fusarium*-Isolate kontrollierten das parasitische Unkraut mit einer leicht geringeren Wirksamkeit. 1.5 g “Pesta”- Granulat pro Pflanzloch erwies sich als ausreichende Dosis für das *Striga*-Management, da durch Erhöhung der Dosierung keine weitere Wirksamkeitssteigerung erzielt werden konnte.

In der zweiten Feldversuchsperiode (2004/2005) wurde zusätzlich zu den “Pesta”-Granulaten die Effizienz der Alginatformulierungen mit 10 % Weizenmehl in einer Dosis von 1.5 g pro Pflanzloch untersucht. Die Alginat-Pellets konnten den *Striga*-Befall signifikant verzögern und reduzierten die Anzahl der Sprosse anfangs um 64-78% gegenüber der unbehandelten Kontrolle. Im Gegensatz zum ersten Versuch verzögerten die pilzlichen Isolate in “Pesta”-Granulaten das Auflaufen von *Striga* nicht signifikant, allerdings konnten sie die Anzahl der Sprosse anfangs um 42-55% gegenüber der Kontrolle reduzieren. Am Ende der Vegetationsperiode wurde der Effekt beider Formulierungen auf die Anzahl der *Striga*-Sprosse vernachlässigbar gering, aber sie erhöhten signifikant den Anteil erkrankter Sprosse im Vergleich zur unbehandelten Kontrolle. FA in Alginat-Pellets oder als “Pesta” war in dieser Hinsicht besonders effektiv, das Isolat verursachte bei 74 und 80% der Sprosse Krankheitssymptome und konnte so die Anzahl gesunder Sprosse um 55 und 60% gegenüber der Kontrolle reduzieren. FA in “Pesta” war auch die effektivste Behandlung hinsichtlich der Reduktion der *Striga*-Biomasse (58% im Vergleich zur Kontrolle), was sich positiv in einem erhöhten Korn- (63%) und Strohertrag (73%) bei der Wirtspflanze auswirkte.

Die Unterschiede in der Kontrolleffizienz der “Pesta”-Formulierung zwischen den beiden Versuchsjahren sind möglicherweise auf drei Gründe zurückzuführen. Zum einen unterschieden sich die Klimabedingungen zwischen beiden Jahren, im zweiten Versuchsjahr herrschten deutlich höhere Temperaturen in Verbindung mit weniger Niederschlägen und einer geringeren relativen Luftfeuchtigkeit. Zum anderen traten im Feld im zweiten Versuchsjahr größere Probleme mit hoher Natriumsättigung des Bodens auf, was die Vermehrung der Pilze im Boden beeinträchtigt haben könnte. Zum dritten könnten sich die Abbauprodukte des applizierten Insektizids Sevin (Carbaryl (1-naphthyl N-methylcarbamat)) negativ auf die Virulenz der Isolate ausgewirkt haben.

Zusätzlich wurde ein Freiland-Topfversuch durchgeführt, um die Effizienz der Alginat-Formulierungen mit verschiedenen Zusätzen (Weizenmehl, Sorghummehl und Hefeextrakt) hinsichtlich der *Striga*-Kontrolle im Vergleich mit der “Pesta”-Formulierung und den Saatgutbehandlungen zu untersuchen. In diesem Versuch war die Applikation von FA in “Pesta”-Granulaten die effektivste Behandlung und verhinderte das Auflaufen von *Striga* bis zum Ende der Vegetationsperiode. Dies spiegelte sich in signifikant höheren Wirtspflanzen (um 80%) und erhöhtem Sorghum-Sprosstrockengewicht (400%) gegenüber der negativen Kontrolle wider. Die *Fusarium*-Isolate in Alginat-Pellets verzögerten ebenfalls das Auflaufen von *Striga* und reduzierten die Anzahl der *Striga*-Sprosse während der gesamten Vegetationsperiode. Die höchste Effizienz wurde dabei wiederum von FA erzielt, das die Anzahl der Sprosse um 71% (mit 10% Weizenmehl) oder 84% (6% Sorghummehl oder Hefeextrakt) verringerte. Desgleichen wurde der Anteil gesunder Sprosse um 71%, 88% und 84% und die *Striga*-Biomasse um 50%, 81% und 89% reduziert. Die Applikation der Alginat-Formulierungen erhöhte grundsätzlich auch die Wirtspflanzenhöhe um bis zu 80% und die Sprosstrockenmasse um 200 bis 400% gegenüber der Kontrolle.

Es kann zusammenfassend festgestellt werden, dass von allen getesteten Isolaten und Formulierungen FA in “Pesta”-Granulaten die höchste Kontrolleffizienz sowohl im Freiland als auch unter kontrollierten Bedingungen aufwies.

Die Fähigkeit von FA, Mykotoxine der Klasse der Trichothecene zu bilden, die eine Gefahr für Mensch oder Tier darstellen, wurde anhand von Proben des Pilzes, der dazu auf autoklavierten Weizenkörnern angezogen wurde, überprüft. Zusätzlich wurden Proben des geernteten Sorghums aus verschiedenen Parzellen im Feld, die

mit *Fusarium* spp. inokuliert worden waren, hinsichtlich ihres Trichothecen-Gehalts überprüft. FA produzierte keine der im folgenden aufgelisteten Toxine und sie wurden ebenfalls nicht in den im Feld produzierten Körnern nachgewiesen: Nivalenol, Fusarenon X, Deoxynivalenol, 15-actetyldeoxynivalenol, Scirpentriol, Monoacetoxyscirpenol, Diacetoxyscirpenol, T-2 Triol, HT-2 Toxin, T-2 Toxin und Neosolaniol.

Eine einfache Behandlung des Sorghumsaatgutes mit feinen “Pesta”-Granulaten und Gummi arabicum als Kleber zeigte in der ersten Feldsaison ebenfalls vielversprechende Ergebnisse hinsichtlich der *Striga*-Kontrolle. Die Anzahl der *Striga*-Sprosse wurde dadurch um mehr als 55% gegenüber der Kontrolle reduziert, was ein Ansteigen der Sorghum-Biomasse um 54 bis 67% und des 100-Korn-Gewichtes um 70% zur Folge hatte. Diese Effekte waren vergleichbar mit denen der chemischen Bekämpfung mit 2,4-D.

Um für die zweite Feldsaison die Effektivität der Saatgutbehandlung zu verbessern, wurde der Effekt von 5 verschiedenen Klebern (zwei Arten Cellulose, zwei organische Polymere und ein Ton) auf das Wachstum und die Sporulation von FA und FN auf festem und in flüssigem Medium getestet. Cellulose 1 und 2 wirkten sich beide positiv auf das radiale Wachstum der Isolate aus, hatten aber keinen Einfluss auf die Sporulation im Flüssigmedium. Eines der organischen Polymere reduzierte sowohl das radiale Wachstum als auch die Sporulation der beiden Pilze. Das andere Polymer und der Ton erhöhten dagegen signifikant die Produktion von Chlamydosporen besonders durch FA, verglichen mit der Produktion in Richard’s Medium allein oder unter Zusatz der anderen Klebematerialien. Aus diesem Grund wurde eines dieser Materialien zusätzlich zu Gummi arabicum als Kleber für die zweite Feldsaison verwendet. Die Behandlung des Saatguts wurde von der Firma SUET Saat- und Erntetechnik GmbH, Eschwege, Deutschland, mit luftgetrockneten pilzlichen Ver- mehrungseinheiten aus einer Fermentation auf 1.5 (w/v) Sorghumstroh durchgeführt. Gummi arabicum erzielte eine bessere Bedeckung der Samen und eine höhere An- zahl von cfu pro Samenkorn (4×10^4 (FA) und 19×10^4 (FN)) im Vergleich zum zweiten verwendeten Kleber. Die Saatgutbehandlungen führten in der zweiten Saison nicht zu einer signifikanten Reduktion der Anzahl der *Striga*-Sprosse im Feld, aber einige der Behandlungen erzeugten hohe pilzliche Befallsraten bei den Sprossen. Besonders FN plus Gummi arabicum und FA als

feine “Pesta”-Granulate auf den Samenkörnern erhöhten den Anteil der erkrankten Sprosse im Vergleich zur Kontrolle signifikant um 79%.

Im Topfversuch führten die Saatgutbehandlungen ebenfalls nicht zu einer signifikanten Reduktion der Sprossanzahl. FA mit dem Kleber der Firma SUET konnte aber die Anzahl der *Striga*-Sprosse zu Versuchsende um 52% gegenüber der Kontrolle verringern. FN in derselben Behandlung erhöhte den Anteil erkrankter Sprosse signifikant um 77%. Der Effekt der Saatgutbehandlungen auf die Höhe und Trockenmasse der Wirtspflanze war geringer als der von den Festformulierungen verursachte und nicht statistisch signifikant im Vergleich zur Kontrolle.

Aus den Ergebnissen kann man zusammenfassend schließen, dass sowohl das Ausbringen beider Festformulierungen ins Pflanzloch als auch die Saatgutbehandlung als Applikationssysteme für die biologischen Gegenspieler geeignet sind und für den Einsatz zur *Striga*-Kontrolle unter Feldbedingungen übernommen werden können. Allerdings zeigten die Festformulierungen eine bessere Effektivität als die Saatgutbehandlungen. Die Art und Konzentration des formulierten Inokulums sowie mögliche Zusätze zur Formulierung müssen in zukünftigen Untersuchungen weiter optimiert werden.

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Appendices

Appendix 1: Metrological data

Source: Metrological Station Wad Medani

First season metrological data (2003)

Month	Mean max. temp. (˚C)	Mean min. temp. (˚C)	R.H. %	Rainfall total (mms)	Wind speed 200cm(m/s)
June	40.1	25.6	51	38.4	04.47
July	35.7	23.4	75	101.9	03.98
August	33.4	22.5	83	152.1	02.91
September	36.6	22.3	76	51.0	01.88
October	39.4	22.9	56	11.8	01.37
November	38.1	20.2	45	Nil	01.90
Average range	33.4– 40.1	20.2– 25.6	45 - 83	354.3	01.37 – 04.47

Second season metrological data (2004)

Month	Mean max. temp. (˚C)	Mean min. temp. (˚C)	R.H. %	Rainfall total (mms)	Wind speed 200cm(m/s)
June	40.1	24.9	55	46.7	03.69
July	38.4	23.9	61	19.6	04.47
August	36.3	23.4	73	99.9	03.60
September	37.8	23.3	67	10.9	02.57
October	39.3	22.6	50	11.3	01.68
November	38.1	20.1	40	Nil	02.10
Average range	36.3 – 40.1	20.1 – 24.9	40 - 73	188.4	01.68 – 04.47

Curriculum Vitae

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1992 – 1995 **Degree** **Thesis**	Faculty of Agriculture, University of Khartoum Master of Science (M.Sc.) in Agriculture Identification and characterization of cowpea mild mottle virus (CMMV) Naturally infecting groundnut in Khartoum State
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Erklärung

Hiermit versichere ich an Eides statt, dass die vorliegende Arbeit von mir selbst, lediglich unter Verwendung der angegebenen Quellen und Hilfsmittel, durchgeführt und verfasst wurde. Wörtlich oder inhaltlich übernommene Stellen sind als solche kenntlich gemacht. Die Arbeit wurde in gleicher oder ähnlicher Form keiner anderen Prüfungsbehörde vorgelegt.

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