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RESEARCH ARTICLE **(Open Access)**

Efficacy of freshly prepared pesta granular formulations from the multi-combination of wild and mutant strain of *Lasiodiplodia pseudotheobromae and Pseudomonas aeruginosa*

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Abstract:

This study was carried out to determined the efficacy of freshly prepared pestal granules containing the wild and mutant strains of *Pseudomonas aeruginosa* and *Lasiodiplodia pseudotheobromae* in controlling weeds in a potted experiment. Six multi-combination formulations of the wild and mutant strains of *Pseudomonas aeruginosa* and *Lasiodiplodia pseudotheobromae* were combined with a semolina kaolin granular formulation which were represented as follows: BHI, BH2, BH3, BH4, BH5, and BH6. The effect of each formulated pesta granules were supplemented with an adjuvants (glycerol, glucose, sucrose, fructose, dextrose, lactose sugar, peptone) and they were evaluated on *Tridax procumbens* and sorghum plant. The efficacy of the bioherbicides were in the following orders: BH4>BH2>BH6>BH3>BH1>BH5. BH4 showed the highest activity compared to other formulations by reducing *Tridax procumbens* to the lowest dry weights and showing the highest percentage disease severity on *Tridax procumbens* compared to other formulations. BH4 among other formulations also lead to increase in the amount of grain yield and 100-grain weight of sorghum plants compared to uninoculated plants. Therefore, the use of pesta granules containing wild and mutant strains of *Pseudomonas aeruginosa* and *Lasiodiplodia pseudotheobromae* exposed to U.V for 1 hour 30 minutes against weeds using *Tridax procumbens* as a study case could help in achieving a sustainable agriculture through the application of bioherbicide produced during this study .

Keywords: bioherbicide; deleterious rhizobacteria; pesta; *Lasiodiplodia pseudotheobromae*; *Pseudomonas aeruginosa;* multi-combination.

1. Introduction

The pesta granular formulation is an extruded product composed of grain flour and the microbial agent. Pesta technology is adaptable to many different microorganisms and ingredients [1]. The formulation is usually a wheat gluten matrix derived from inexpensive wheat flour, kaolin, nutritional adjuvants, and water, which house the microbial agent [2]. Once these ingredients are combined, the dough is extruded through a small pasta-making machine into thin sheets. These sheets are air-dried and sieved to a specific size. The granules are usually between 0.6 mm and 1.4 mm in diameter [3]. High-speed extrusion machinery used to make foods such as spaghetti can be used to produce pesta in large-scale operations [1,4]. In addition to the ability to be produce pesta on a large scale, there are several other advantages to the pesta formulation. It is non-toxic, cost effective, convenient to store, simple to use, and can be applied with agricultural machinery [5].

Deleterious rhizobacteria (DRB) are among the micro-organisms that have been reported to have a potential as biocontrol agents in controlling weeds [6]. However, successful application of live bacteria in a field setting depends upon many factors including environmental conditions and soil survival. Therefore, there is a need to formulate these biocontrol agents to enhance their field potential and facilitate their storage and application.

Many weed biological control agents have been formulated into liquid, solid and powder substrates [7],because of the low bacterial survival in liquid inoculants [9,10] and impractical use of cell suspensions for large scale application due to the difficult handling, transport and storage of the inoculum[11,12]. A formulated bioherbicide can be defined as a mixture of the active ingredient (the biological agent) within a carrier or solvent that delivers the active ingredient to the target weed, and the adjuvants that improve the survival and effectiveness of the product in adverse environmental

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conditions [13]. Among the different possible formulations, the dry solid or powder formulations provide several advantages. Bacterial cells immobilized in dry carrier are protected from the external environmental factors and their survival and efficacy are preserved in adverse environmental conditions[13,14,15]. Dry carriers also allow efficient and easy delivering of bacteria to the target weed [16]. For instance, the wheat-gluten matrix known as Pesta has been used to formulate granular biocontrol agents. This matrix is adaptable to many different micro-organisms and ingredients [1] and is nontoxic, cost-effective and easy to store and use [5]. Pesta formulations have been widely used to deliver mycoherbicides such as *Colletotrichum truncatum* against hemp sesbania (*Sesbania exaltata*) [3] and *Fusarium oxysporum* against sunflower broomrape (*Orobanche cumana*) [14] and Striga spp. [5]. Pesta formulations for bacteria with bioherbicide activities have also been used with *Pseudomonas fluorescens* BRG100 against green foxtail (*Setaria viridis*) [2], *P. fluorescens* strain G2-11 against velvetleaf (Abutilon theophrasti) [18] and *P. fluorescens* LS102 and LS174 against leafy spurge (*Euphorbia esula*) [19].

Kayode and Ige [20], discovered that *C. odorata* (L) and *T. procumbens L*. are one of the major weeds that constitute a major impediment to agricultural and natural ecosystem in Nigeria. *T. procumbens* L. belongs to the family Asteraceae whose method of dispersal is by wind. It is a semi prostrate perennial herb which occurs throughout the tropics and subtropics. It is frequently found in annual crops, fallow land and occasionally in perennial crops. Its wide distribution is attributed to its spreading stems and abundant seed production which is put at between 50- 1500 per plant [21].

The main objectives of this work was to develop an appropriate formulations from the multi-combination of the wild and mutant strains of deleterious rhizobacterium *Pseudomonas aeruginosa* and *Lasiodiplodia pseudotheobromae* and to evaluate their efficacy in inhibiting weeds using *Tridax procumbens* in a potted experiment.

2. Material and Methods

2.1. Source and maintenance of Fungal Isolates

Fungal strains were isolated from small chlorotic and necrotic lesions on leaves of *Tridax procumben* weeds collected from Ogbomoso and Ilorin environment. The weed leaves were surfaced sterilized for 2 minute in 0.5% sodium hypochlorite, rinsed in sterile distilled water, and placed on Difco PDA(Potatoes Dextrose Agar) plates at 24^{o}C with 12 hours light, for 3-7days . Fungal identification was carried out according to the procedure described by Samson and Van Reenen-Hoekstra [22]. Cultural and microscopic morphology was used to confirm *Lasiodiplodia pseudotheobromae* isolates. Koch's postulates was applied to establish the pathogenic status of pure isolates of *Lasiodiplodia pseudotheobromae* on weed from which they where isolated. Purified *Lasiodiplodia pseudotheobromae* isolates were then preserved by storing a hyphal fragment and spore suspension in a 1:1 skim milk (10% v/v) to glycerol (40% w/v) solution and then stored at 4 ^{o}C. Isolates were revived by thawing a vial containing the fungus to room temperature. The content was aseptically spread on the surface of 15-cm diameter petri dishes containing Difco potato dextrose agar (PDA). The plates were then incubated at room temperature with natural light for 1–2 weeks.

2.2. Exposure of Lasiodiplodia pseudotheobromae to UV light to induce Mutation

This experiment was carried out in order to see whether mutation can improve the amount of phytotoxic metabolites in the medium. This was carried out by preparation of fresh PDA plate to grow the organisms. After the growth of the organisms, cork borer was used to obtain several mycelia plugs from the culture into a sterile PDA plate. The sterile plate containing several mycelia plugs were placed under UV lamp at 300 nm wavelength at a distance of 30 cm to the plates. At different time interval (30, 60, and 90 minutes), 5 mycelia plugs were withdrawn and used as inoculants for potatoes dextrose medium on the rice bran. The mycelia plugs from the domesticated type culture serve as the control.

2.3. Isolation and characterization of pure cultures from the bacteria

1 gramm of soil was remove from the wheat rhizosphere with the help of sterilized spatula, and the soil sample was placed inside an aluminum foil from the farm of Nigerian stored product research institutes .It was then placed in a sterile test tube and dissolved with 10 ml of distilled water to make the stock. The suspension was filtered through sterile glass wool. Serial dilution was done to the necessary dilution factors and pour-plated. The plates were left to gel and then incubated. The bacteria plates were incubated at 37^{o}C for 48 hours on Kings agar. At the end of each incubation period, the colonies were

counted and sub-cultured onto fresh media maintained on slants from Kings agar and preserved at 4°C in the refrigerator according to Fawole and Oso

[23]. Tentative identification of bacterial isolates was done using the Bergey's Manual of Determinative Bacteriology [24].

2.4. Exposure of Pseudomonas aeruginosa to UV light to induce Mutation

This experiment was carried out in order to see whether mutation can improve the amount of phytotoxic metabolites in the medium . *Pseudomonas aeruginosa* was cultured for 3 days at 35℃ in a moist air atmosphere supplemented with 2.5% CO_2. Plates were then be flooded with 10 ml of 3 mM potassium phosphate buffer, pH 7.0, and bacteria was scraped off the agar with a bent glass rod. Ten ml culture suspensions was adjusted to 1 x 10^7 colony forming units (CFU/ ml) was placed in the bottom half of 100 mm diameter petri dishes and exposed to U.V. The sterile plate containing the bacteria culture were placed under UV lamp at 300 nm wavelength at a distance of 30 cm to the plates .The suspensions were mechanically rocked during irradiation to ensure even dose distribution. Samples was removed at different time interval (30, 60, and 90 minutes) intervals and kept in the dark at 4°C to prevent photo-reactivation effects as described by Carson and Peterson [25].

2.5. Preparation of pesta granules

The procedures developed by Connick et al [3] and optimized by Elzen et al. [5] were used. Thirty-two g semolina, a coarse durum wheat flour, 6 g kaolin, 2 g sucrose, 20 ml of fungal and bacteria inoculum serving as active ingredient and 3 ml of deionized water were thoroughly mixed in a dish. The dough was then rolled through a small, hand-operated pasta machine into sheets, which were folded and extruded 10–15 times at different roller gap settings until it became homogeneous. The dough sheets were then extruded, without refolding, at a narrow setting to yield a 1-mm thick sheet. The sheets were then placed on aluminum foil and air-dried at ambient laboratory conditions [28±2 °C, 33±2 % relative humidity (RH)]. The dried sheets were ground in a grinder into granules and sieved to specific sizes (501– 2000 µm). Their initial viability were determined by serial dilution method by plating the different dilution on nutrient agar and potatoes dextrose agar. The end-product Pesta granules was packed into transparent polyethylene bags (120 × 200 mm, 0.025 mm).

The various pestal granules formulated into various bioherbicides were designated as followed:

(a) BH1= 32 gramms of semolina +6 g kaolin + 20 ml of glycerol + wild strain of *Lasiodiplodia pseudotheobromae* (Lp) + *Pseudomonas aeruginosa* (Pa 30 minutes) + glucose + sucrose + fructose + dextrose + lactose sugar + peptone.

(b) BH2= 32 gramms of semolina + 6 g kaolin + 20ml of glycerol + mutant strain of *Lasiodiplodia pseudotheobromae* (Lp 1 hour 30 minutes) + mutant strain of *Pseudomonas aeruginosa* (Pa 1hour 30 minutes).

(c) BH3 = 32 gramms of semolina + 6 g kaolin + 20ml of glycerol + *Lasiodiplodia pseudotheobromae* (Lp 30 minutes) + glucose + sucrose + fructose + dextrose + lactose sugar + peptone + *Pseudomonas aeruginosa* (Pa 30 minutes).

(d) BH4 = 32 gramms of semolina +6 g kaolin + 20ml of glycerol + mutant strain of *Lasiodiplodia pseudotheobromae* (Lp 1 hour 30 minutes) + glucose+ sucrose + fructose + dextrose + lactose sugar + peptone + mutant strain of *Pseudomonas aeruginosa* (Pa 1hour 30 minutes).

(e) BH5= 32 gramms of semolina + 6 g kaolin +20ml of glycerol + wild strain of *Lasiodiplodia pseudotheobromae* (Lp) + wild strain of *Pseudomonas aeruginosa* (Lp).

(f) BH6= 32 gramms of semolina + 6 g kaolin +20ml of glycerol + mutant of *Lasiodiplodia pseudotheobromae* (Lp 1 hour) + mutant strain of *Pseudomonas aeruginosa* (Pa 1 hour) + glucose+ sucrose + fructose + dextrose + lactose sugar + peptone.

2.6 Evaluation of the phytotoxic activity of the freshly prepared Pesta granules

The efficacy of the 'Pesta' granules containing wild and mutant strain of *Lasiodiplodia pseudotheobromae* and *Pseudomonas aeruginosa* that were freshly prepared were evaluated in potted experiments .The experiment was performed in buckets filled with 12 kg of sterilized mixture of soil, sand and peat (1 : 1 : 1, v/v/v). Five seeds from *Tridax procumbens* and sorghum were placed in each bucket. Ten grams of the stored granules from each of the different formulations was incorporated into the sterilized soil in different pots. Granules containing no bacteria and fungus were used as

controls. The experimental design was a completely randomized block with five replications. The dry weights of *Tridax procumbens* was determined by drying them in overnight at 70°C, and the dry weights of each plant were recorded. Data regarding yield parameters of sorghum such as the weight of hundred grains, sorghum height, sorghum straw yield were also determined. Disease severity was rated using a modified 0 to 5 scale from Smith [26]: 0 = no visible symptoms, 1 = leaves with small necrotic flecks, but no stem lesions (0-5%), 2 = discrete lesions on leaves and/or stem, some plant wilting (6-25%); 3 = lesions 40.5 cm of stem's circumference and leaf tissue with necrotic lesions, more severe wilting of plant and top leaves (27-75%); 4 = girdling stem lesions and total leaf necrosis (76-99%), and 5 = plant death or girdled and falling over.

3. Results and Discussion

The efficacy of the pestal granules that was freshly prepared were examined in a potted experiment with six different formulated bioherbicides, which were BHI, BH2, BH3,BH4, BH5, and BH6. The efficacy of the various formulated bioherbicides in term of biomass and height are in the followingorder:
BH4>BH2>BH6>BH3>BH1>BH5>Control. The greatest height of sorghum was recorded from BH4 among all the formulated bioherbicides with a height of 109.3 ± 4.2 cm/plant compare to the control that had the lowest height of 70.3 ± 5.2 cm/plant.(Figure 2)

The rate of disease severity were assessed from the formulated bioherbicides on the *Tridax procumbens* plant. BH4 had the largest necrotic area on the shoot *Tridax procumbens* with a disease severity of 78.0 ± 10.9 % compared to the control that had had the lowest necrotic area after applying the various formulated bioherbicides with 12.03 ± 1.7. (Figure 5)

After the evaluation of the phytotoxic activity of the freshly prepared 'Pesta' granular formulations, BH4 showed the highest activity compared to other formulations by reducing the wet biomass of *Tridax procumbens* with a dry weights of 0.1 ± 0.1 gramm per pot, while the un-inoculated plants had 0.9 ± 0.1 gramm per pot (Figure 1).

Moreover, 'Pesta' granules from BH4 formulation containing *Lasiodiplodia pseudotheobromae* and *Pseudomonas aeruginosa* increased the plant growth of sorghum plant with a straw yield of 6.8 ± 1.4gramm per pot compared to the control that had 1.3 ± 0.2 gramm per pot (Figure 3). BH4 also had the highest increase in the amount of 100-grain weight of 2.8 ±0.3 gramm were recorded in sorghum plants compared to uninoculated plants which had 100-grain weight of 1.1 ± 0.2 per pot (Table 4).

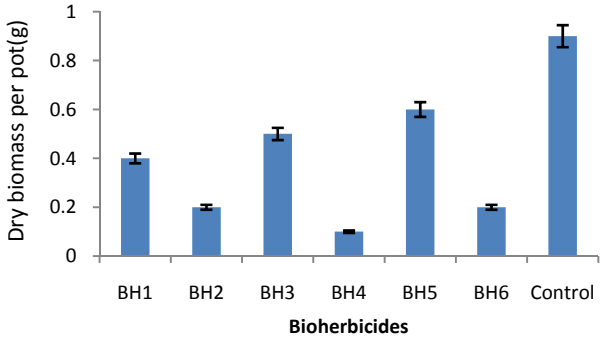


Figure1: Effect of freshly prepare pestal granules containing wild and mutant strains of *Lasiodiplodia pseudotheobromae* and *Pseudomonas aeruginosa* on *Tridax* procumbens dry biomass per pot. Error bar =standard error of means

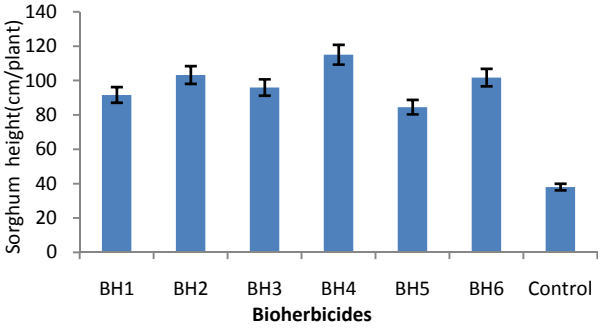


Figure 2: Effect of freshly prepare pestal granules containing wild and mutant strains of *Lasiodiplodia pseudotheobromae* and *Pseudomonas aeruginosa* on sorghum height (cm/plant). Error bar =standard error of means

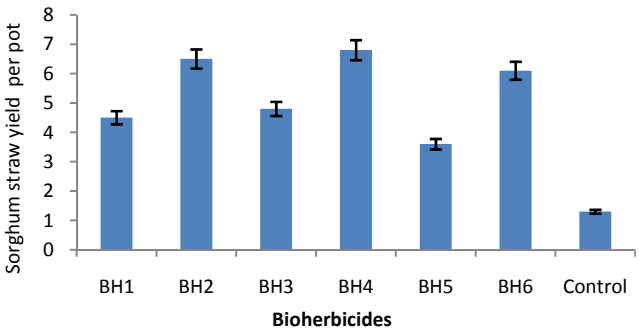


Figure 3: Effect of freshly prepare pestal granules containing wild and mutant strains of *Lasiodiplodia pseudotheobromae* and *Pseudomonas aeruginosa* on sorghum straw yield per pot. Error bar =standard error of means

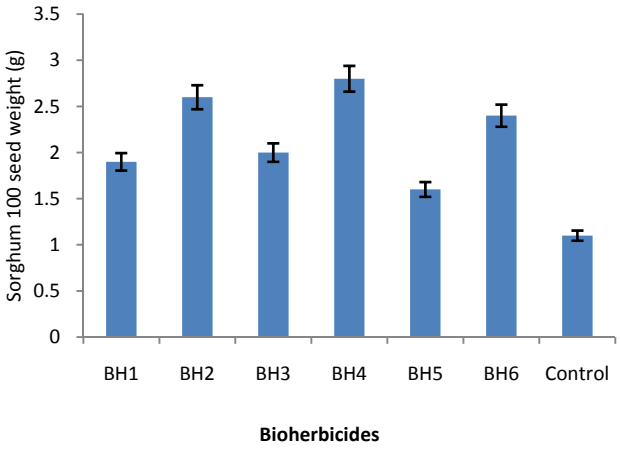
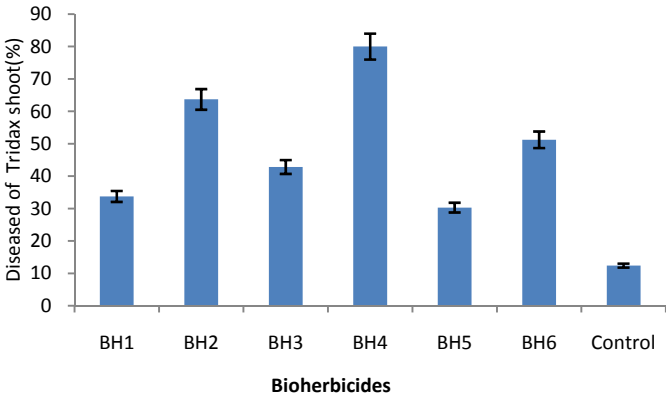


Figure 4: Effect of freshly prepare pestal granules containing wild and mutant strains of *Lasiodiplodia pseudotheobromae* and *Pseudomonas aeruginosa* on sorghum 100 seed weight Error bar =standard error of means

The formulation of a bioherbicide is a tool with which the storability and ease of application of a biocontrol agent can be improved, and to some extend the negative influence of environmental factors can be reduced as well. Understanding the needs of a biological control agent for survival during processing and storage as well as for proliferation and effectiveness in performing its biological mission are the basis for the development of an appropriate formulation [27]. The capability of bioherhicidal agent to reduce the competitive ability and reproductive capacity of weeds by reducing emergence, causing root decay or wilting, make them more suitable for pre-emergence bioherbicidal application. Thus, the bioherbicidal pathogens (e.g. *Lasiodiplodia pseudotheobromae and Pseudomonas aeruginosa*) have the advantages of being effective antagonists for root parasitic plants, because they can infect the parasite at all developmental stages. There is a growing belief that innovations in formulation will be a vital component to the success of the next generation of bioherbicides, especially for foliar-applied products [28]. For best results, formulations should predispose weeds to infection by pathogens



and buffer pathogen propagules against environmental extremes while promoting disease development.

Figure 5: Effect of freshly prepare pestal granules containing wild and mutant strains of *Lasiodiplodia pseudotheobromae* and *Pseudomonas aeruginosa* on diseased of tridax shoot. Error bar =standard error of means

Lack of appropriate delivery systems is one of the main limitations that constrain biological control to come to practical field application [29,30]. Therefore, formulation of a bioherbicide is the key for a successful biological product that can be effectively delivered to the target weed [27,13,30]. Solid or granular formulations are quite suitable for microorganisms that infect their target weed at or below the soil level and are hence suitable for pre-emergence application [31,13,32]. Furthermore, a solid formulation can buffer environmental extremes [13,31,1], often more easily than a liquid formulation. Moreover, granules can be produced on a large scale and applied using the existing agricultural machinery[34,1]. During this study “Pesta” formulations proved to be effective delivery systems for *Lasiodiplodia pseudotheobromae* and *Pseudomonas aeruginosa*, potential biocontrol agents for controlling *Tridax procumbens*. Pestal granules formulated for this study

containing different substrates (glucose + sucrose + fructose + dextrose + lactose sugar + peptone) as additives gave a better performance than those that does not have. This could be due to higher losses of viable propagules during the "Pesta" formulation process, which is often reported to reduce the viability of the formulated inoculum: losses varying considerably depending mainly on the type of inoculum used [35,36,37,2,5]. The ingredients of Pesta, especially wheat flour "semolina" with its high gluten proteins content (gliadin and glutenin), may have served as a suitable nutrient source for the fungus and the bacteria used, thereby providing faster growth and sporulation of the fungus or enhancing their biological activity. A sustainable and continuous production and dispersal of the bioherbicidal inoculum in Pesta can be expected when conditions are favorable. The importance of sucrose in the formulation for extending survival of the biological agents has been reported [38]. Sucrose protects microbes against desiccation [39] and functions as a stabilizer for the membranes during the drying process by replacing water molecules in the lipid bilayer. It also protects protein structure in the dry state [40,41]. Through the control of water activity during storage (moisture content of the granules) and the sucrose content of the formulation, viability of the microbial agents in Pesta granules can be significantly improved [17].

The presence or availability of various nutrient also contribute to the greater survival and better performace of the *Lasiodiplodia pseudotheobromae* and *Pseudomonas aeruginosa* in the pestal granules. Nutrient supplements, including simple sugars, amino acids, pectins, salts, and plant extracts have been added to formulations to stimulate the infection process and protect germinating propagules, but these nutritional effects are often agent specific [42,43,44]. Exogenous nutrients may stimulate germination and growth of many fungi, but frequently appressorial initiation is even more important to plant penetration and infection.

Mutation was also induced on *Lasiodiplodia pseudotheobromae* by exposing the wild type of the fungus to 1 hour 30 minute, 1 hour, 30 minutes of Ultraviolent ray so as to produced the different type of mutant strains. It was observed that Mutant strain of *Lasiodiplodia pseudotheobromae* and *Pseudomonas aeruginosa* in the BH4 bioherhicidal formulation exposed for 1 hours 30 minutes had the greatest activities against the tested weed.

Mutation induction has become an established tool in strain improvement to supplement existing strains and to improve species in certain specific traits. Therefore, several approaches including chemical mutation, UV irradiation and genetic engineering to obtain high yield strains have been given a priority in the last decades [45].

BH4 had the largest percentage of disease severity on the *Tridax procumbens* weed showing the capability of *Lasiodiplodia pseudotheobromae* and *Pseudomonas aeruginosa* as a potential bioherbicidal agent. The effectiveness of pesta formulations made with different fungal inocula was compared using their calculated efficacy. The results of the experiments showed that the efficacy of BH4 (i.e. the percent reduction in healthy emerged *Tridax procumbens* shoots compared to the control). Furthermore, bioherbicide BH4 containing [32 gramms of semolina + 6 g kaolin +20 ml of glycerol + mutant strain of *Lasiodiplodia pseudotheobromae* (Lp 1hour 30 minutes) + glucose + sucrose + fructose + dextrose + lactose sugar + peptone + mutant strain of *Pseudomonas aeruginosa* (Pa 1hour 30 minutes)] reduced the wet biomass of *Tridax procumbens* weed greatly while it lead to the increased in sorghum plant.The development of biocontrol products that are easily delivered such as pesta formulation could accelerate the acceptance of these products. Wheat flour-kaolin granules pesta can be modified easily to accommodate ingredients from indigenous agricultural resources of Africans countries such as other cereal grain flours which may give a more effective product. The cost-effectiveness of the naturally occurring ingredients in Africa may improve the economic feasibility of pesta formulations. More detailed investigations on this area need to be carried out. Additional advantages of pesta formulations are: non-toxic, relatively cost effective, can be produced on a large scale [1], convenient to store [5], simple to use, compatible with agricultural machinery, and can be easily integrated with existing weed control methods, e.g. cultural, mechanical and use of resistant varieties.

4. Conclusions

This study has demonstrated the successful use of strain improvement of the bioherbicidal agents used and their multi-combination in different 'Pesta' granules as a bioherbicide against *Tridax procumbens* used as a study case. The use and application of such bioformulations in the field can result in the reduction of application of harmful chemicals, protect the

environment and biological resources and be an important component of integrated pest management in sustainable agriculture. This investigation is only the first step in order to obtain a suitable formulation for application in the field after storage. Other research needs to be addressed before large-scale applications. In particular, further work will be performed to (i) assess the survival, effects on other weeds and the efficacy of the different formulated 'Pesta' containing multi-combination of wild and mutant strains of the deleterious rhizobacterium *Pseudomonas aeruginosa* and *Lasiodiplodia pseudotheobromae* in soils with different chemical and physical characteristics.

5. References

1. Daigle DJ, Connick WJ, Boyette CD, Lovisa MP, Williams KS, Watson M: **Twin-screw extrusion of "Pesta"-encapsulated biocontrol agents**. World of Microbiology and Biotechnology 1997, **13**: 671-676.
2. Daigle DJ, Connick WJ, Boyetchko SM: **Formulating a weed-suppressive bacterium in "Pesta"**. Weed Technology 2002, **16**: 407-413.
3. Connick WJ, Boyette CD, Mc Alpine JR: **Formulation of mycoherbicides using a pasta-like process**. Biological Control 1991,**1**: 281-287.
4. Boyetchko SM, Rosskopf EN, Caesar AJ, Charudattan R: **Biological weed control with pathogens: search for candidates to applications**. In: Khachatourians GG, Arora DK eds. Applied Mycology and Biotechnology, Vol. 2. Agriculture and Food Production. The Netherlands: Elsevier Science B.V; 2002, 239–274.
5. Elzein A, Kroschel J, Muller-Stover D: **Optimization of storage conditions for adequate shelf-life of a Pesta formulation of *Fusarium oxysporum* "Foxy 2", a potential mycoherbicide for Striga: effects of temperature, granule size and water activity**. Biocontrol Science and Technology 2004, **14**: 545-559.
6. Elliott LF, Lynch JM: **Plant growth-inhibiting pseudomonads colonizing winter wheat (*Triticum aestivum L.)* roots**. Plant Soil 1985, **84**: 57–65.
7. Green S, Stewart-Wade SM, Boland GJ, Teshler MP, Liu SH: **Formulating microorganisms for biological control of weeds**. In Plant-Microbe Interactions and Biological Control ed. Boland, G.J. and Kuykendall, L.D. New York: Marcel Dekker; 1998: 249–280,
8. Singleton P, Keyser H, Sande E: **Development and evaluation of liquid inoculants. In Inoculants and Nitrogen Fixation of Legumes in Vietnam ed. Herridge, D**: ACIAR Proceedings 109e. Australia: ACIAR Publishing. 2002: 52–66
9. Tittabutr P, Payakapong W, Teaumroong N, Singleton PW, Boonkerd N: **Growth, survival and field performance of bradyrhizobial liquid inoculants formulations with polymeric additives**. Science Asia 2007, **33**: 69–77.
10. Albareda M, Rodriguez-Navarro DN, Camacho M, Temprano FJ: **Alternatives to peat as a carrier for rhizobia inoculants: solid and liquid formulations**. Soil Biol Biochem 2008, 40: 2771–2779.
11. Rabindran R, Vidhyasekaran P: **Development of a formulation of *Pseudomonas fluorescens* PfALR2 for management of rice sheath blight**. Crop Prot 1996,**15**: 715–721.
12. Vidhyasekaran P, Sethuraman K, Rajappan K, Vasumathi K: **Powder formulation of *Pseudomonas fluorescens* to control Pigeon pea Wilt. Biol Control** 1997, **8**: 166–171.
13. Boyette CD, Quimby PC Jr, Daigle DJ, Fulgham F: **Progress in the production, formulation and application of mycoherbicides**. In Microbial Control of Weeds ed. Tebeest, D.O. New York: Chapman and Hall. 1991, 209–222.
14. Shabana YM, Mu¨ller-Stover D, Sauerbornb J: **Granular Pesta formulation of *Fusarium oxysporum f. sp. orthoceras* for biological control of sunflower broomrape: efficacy and shelf-life**. Biol Control 2003,**26**:1989–1201.
15. Kinay P, Yildiz M: **The shelf life and effectiveness of granular formulations of *Metschnikowia pulcherrima* and *Pichia guilliermondii* yeast isolates that control postharvest decay of citrus fruit**. Biol Control 2008, **1**(45): 433–440.

16. Sabaratnam S, Traiquair JA: **Formulation of a Streptomyces Biocontrol agent for the suppression of Rhizoctonia damping-off in Tomato Transplants**. Biol Control 2002, **23**: 245–253.

17. Connick WJ Jr, Daigle DJ, Boyette C.D, Williams KS, Vinyard BT, Quimby PC Jr: **Water activity and others factors that affect the viability of *Colletotrichum truncatum* Conidia in wheat flour-kaolin granules ('Pesta')**. Biocontrol Sci Technol 1996, **6**: 277–284.

18. Zdor RE, Alexander CM, Kremer RJ: **Weed suppression by deleterious rhizobacteria is affected by formulation and soil properties**. Commun Soil Sci Plant Anal 2005,**36**: 1289–1299.

19. Brinkman MA, Clay SA, Kremer RJ**: Influence of rhizobacteria on leafy spurge (*Euphorbia esula*) roots**. Weed Technol 1999,**13**: 835–839.

20. Kayode J, Ige OE: **A Quantitative study of weed flora in three Abandoned Farmlands in Ekiti State**. Biosci. Res. Comm 1998,**3**: 169- 178.

21. Holm L, Doll J, Holm E, Pancho J, Herberger J: World weeds: **Natural histories and distribution**. John Wiley and Sons. 1997,1129.

22. Samson RA, Van Reenen-Hoekstra ES: **Introduction to foodborne fungi**. 3rd edition. Centraal bureauvoor Schimmdoultur Baarn. 1982, 2-89.

23. Fawole MO, Oso BA: **Biochemical Test. Laboratory manual of Microbiology**, Spectrum books limited, Ibadan. 2004,14-17.

24. Bucchanans RE, Gibbons NE: **Bergey's manual of determinative bacteriology**. Baltimore, Williams and Wilkin Co 8th edition. 1974.

25. Carson LA, Peterson NJ: **Photo reactivation of *Pseudomonas cepacia* after ultraviolet exposure: a potential source of contamination in ultraviolet-treated waters**. J. clin. Microbiol 1975,**1**: 462—464.

26. Smith DA: **Evaluation of *Microsphaeropsis amaranthi* as a bioherbicide for the control of waterhemp (*Amaranthus tuberculatus*)**. M.S. Thesis, Purdue University, West Lafayette, IN. 47907. USA. 2003.

27. Connick WJ Jr, Lewis JA, Quimby PC Jr: **Formulation of biocontrol agents for use in plant pathology**. In: Baker RR, Dunn PE eds. New Directions in Biological Control: Alternatives for Suppressing Agricultural Pests and Diseases. New York: Alan R. Liss Inc. 1990,345–372.

28. Greaves MP, Holloway PJ, Auld BA: **Formulation of microbial herbicides**. In: Burges HD ed. Formulation of Microbial Biopesticides: Beneficial Microorganisms, Nematodes, and Seed Treatments. Dordrecht: Kluwer Academic Publishers. 1998, 203–233.

29. Baker CA, Henis JMS: **Commercial production and formulation of microbial biocontrol agents**. In: R.R. Baker, P.E. Dunn (eds.): New Directions in Biological Control:Alternatives for Suppressing Agricultural Pests and Diseases. Alan R. Liss, New York. 1990, 333-334.

30. Rhodes DJ: **Formulation of biological control agents**. In: Jones DG ed. Exploitation of Microorganisms. London: Chapman & Hall. 1993,411–439.

31. Connick WJ Jr: **Formulation of living biocontrol agents with alginate**. In: Cross, B. Scher, H.B. (Eds.). Pesticide Formulations: Innovations and Developments. ACS Symposium Series No. 371, Amer. Chem. Soc., Washington DC, USA. 1988, 241-250.

32. Daigle DJ, Cotty PJ: **Production of conidia of *Alternaria cassiae* with alginate pellets**. Biol Control 1992, **1**(2):278–281.

33. Weidemann GJ, Boyette CD, Templeton GE: **Utilization criteria for mycoherbicides**. In: Hall FR, Barry JW, editors. Biorational pest control agents: formulation and delivery. ACS Symposium Series, No. 595. American Chemical Society, Washington, DC. 1995, 238-251.

34. Fravel DR, Marois J, Lumsden R, Connick WJ: **Encapsulation of potential biocontrol agents in an alginate-clay matrix**. Phytopathology 1985,**75**:774 -777.

35. Hebbar KP, Lumsden RD, Lewis JA, Poch SM, Bailey BA: **Formulation of**

mycoherbicidal strains of *Fusarium oxysporum*. Weed 1998, **46**: 501-507.

36. Lawrie J, Down VM, Greaves MP: **'Effects of Storage on Viability and Efficacy of Granular Formulations of the Microbial Herbicides *Alternaria alternata* and *Trematophoma lignicola'*,** Biocontrol Science and Technology 2001, **11**:283-295.

37. Müller-stöver D: **Possibilites of Biological Control of *Orobanche crenata* and *O. cumana* with *Ulocladium botrytis* and *Fusarium oxysporum* f. sp. *orthoceras***. Agroecology 3, Apia-Verlag, Laubach, Germany. 2001.

38. Caesar AJ, Burr TJ: **Effect of conditioning, betaine, and sucrose on survival of rhizobacteria in powder formulations**. Appl Environ Microbiol 1991: **57**:168–172.

39. Quimby PC Jr, Birdsall JL, Caesar AJ, Connick WJ Jr, Boyette CD, Caesar TC, Sands DC: **Oil and absorbent coated granules containing encapsulated living organisms for controlling agricultural pests**. US Patent. 1994, **5**:358–863.

40. Leslie SB, Israelli E, Lighthart B, Crowe JH, Crowe LM: **Trehalose and sucrose protect both membranes and proteins in intact bacteria during drying**. Appl Environ Microbiol 1995,61: 3592–3597.

41. Zidack NK, Quimby PC: **Formulation and application of plant pathogens for biological weed control**. In: Hall, F.R. Menn, J.J: Methods In Biotechnology, volume 5: Uses and Delivery. Humana Press Inc., Totowa,NJ, USA, 1999,371-381.

42. Bothast RJ, Schisler DA, Jackson MA, VanCauwenberge, JE, Slininger PJ: **Use of pregelatinized starch and other polysaccharides for improved storage and efficacy of biocontrol agents**. In: Berger PD, Devisetty BN, Hall FR eds. Pesticides Formulation and Application Systems. Vol. 12. Philadelphia: ASTM. 1993, 45–56.

43. Schisler DA, Jackson MA, McGuire MR, Bothast RJ: **Use of pregelatinized starch and casamino acids to improve the efficacy of *Colletotrichum truncatum* conidia produced in differing nutritional environments**. In: Delfosse ES, Scott RR eds. Proceedings of the VIII International Symposium on Biological Control of Weeds, Lincoln University, Canterbury, New Zealand. Melbourne: DSIR/CSIRO. 1995, 659–664.

44. Womack JG, Burge MN: **Mycoherbicide formulation and the potential for bracken control**. Pestic Sci 1993, **37**:337–341.

45. Kotchoni SO, Shonukan OO: **Regualtory mutations affecting the synthesis of cellulase**. World J Microbiol Biotechnol 2002,**18**: 487-491.