# EVALUATION OF SOME PESTICIDES ON *FUSARIUM OXYSPORUM* F. SP. *STRIGAE,* A BIOCONTROL AGENT FOR MANAGING *STRIGA HERMONTHICA*

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**MAY, 2015**

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**BY**

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**DEPARTMENT OF CROP PROTECTION,**
**FACULTY OF AGRICULTURE,**
**AHMADU BELLO UNIVERSITY, ZARIA**
**NIGERIA.**

**MAY, 2015**

## DECLARATION

I declare that the work in this thesis entitled ‘‘Evaluation of some pesticides on *Fusarium oxysporum* f. sp. *strigae*, a biocontrol agent for managing *Striga hermonthica*’’ has been carried out by me in the Department of Crop Protection, Faculty of Agriculture, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree at this or any other Institution.

| Ali PETER | _____ | _____ |
|---|---|---|
| Name of Student | Signature | Date |

## CERTIFICATION

This thesis entitled ‘‘EVALUATION OF SOME PESTICIDES ON *FUSARIUM OXYSPORUM* F. SP. *STRIGAE,* A BIOCONTROL AGENT FOR MANAGING *STRIGA HERMONTHICA*’’ by ALI PETER meets the regulations governing the award of the Degree of Master of Science in Crop Protection of the Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

| | | |
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## DEDICATION

This work is dedicated to the glory of God, the author and finisher of all faith, and to my guardian, Mr. and Mrs. S. P. Simon. You remain role models to generations and to memory of my beloved late father Mr. Aaron Inde Bwala (Dad, I know you will surely be proud of me).

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## ABSTRACT

Five rates of benomyl, carbendazim, mancozeb, hexaconazole, carbendazim + mancozeb, imidacloprid + metalaxyl-M + tebuconazole and metsulfuron methyl were incorporated into molten potato dextrose agar to determine growth and sporulation of PSM 197 and Foxy 2, which were isolates of *Fusarium oxysporum* f. sp. *strigae* used against *Striga hermonthica*. Two hundred and eighty Petri dishes containing amended and un-amended media were inoculated with 14 day-old cultures of PSM 197 and Foxy 2 using flame-sterile 5 mm cork borer. Petri dishes were arranged on laboratory bench in a Completely Randomized Design (CRD) and observations taken at 24 hours interval. Mycelia growth and development of conidia were completely inhibited in media amended with benomyl, carbendazim, and carbendazim + mancozeb. Increasing mancozeb rate from 6 to 24 g/L resulted in lower mycelia diameter and conidia counts for PSM 197 and Foxy 2. Fourteen days after incubation, mycelia diameter of both isolates were low on media amended with highest rates of hexaconazole (1 ml/L), metsulfuron methyl (4.6 g/L) and imidacloprid + metalaxyl-M + tebuconazole (8 g/L). Metsulfuron methyl was adjudged most compatible with the two isolates of *Fusarium oxysporum* f. sp. *strigae* on the basis of their mycelial growth, micro-conidial number and conidia germination. With respect to counts macro-conidia, hexaconazole treatments resulted in least variations while viability of conidia was higher with mancozeb treatments followed by metsulfuron methyl. With respect to all parameters considered, imidacloprid + metalaxyl-M + tebuconazole had the highest percent variations than hexaconazole, mancozeb and metsulfuron methyl. The study therefore shows that metsulfuron methyl at 2.3 g/L is compatible with PSM 197 and Foxy 2 and should be used for the management of *S. hermonthica*.

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# CHAPTER ONE

## 1.0 INTRODUCTION

Parasitic weeds of the genus *Striga* (Orobanchaceae) pose a major threat to cereal production in the African savannahs. To this effect, these weeds are important limitations to the attainment of regional food security (Parker and Riches, 1993). Kuchinda *et al*. (2003), Marley *et al.* (2004), Marley and Shebayan (2005) and Ejeta (2007) reported independently, that *Striga hermonthica* (Del.) Benth is the most economically important parasitic weed of cereals, especially maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* (L.) Moench) in the African Savannah, causing 40 – 100 % grain yield losses.

Management approaches for Striga control in Nigeria are generally based on cultural practices such as land preparation, hand pulling, hoe weeding, use of trap and catch-crops, seed treatment, application of appropriate rate of nitrogen fertilizer, herbicide spray, use of biological control and host plant resistance (Lagoke *et al*., 1988; Marley *et al*., 1999; Nekoum and Marley, 2002; Menkir *et al.*, 2006). Control of Striga is particularly difficult due to its biology and intimate physiological interaction with its hosts. The parasite attaches its roots to the host, thereby withdrawing water, mineral nutrients, carbohydrates and amino-acids for its growth and development. These consequently cause stunted shoot growth, leaf chlorosis and reduced photosynthesis in the host (Ejeta and Butler, 2000; Frost *et al*., 1997).

Damage is done to the host before parasite shoots emerge above soil level. So far, effective control of Striga has not been achieved through any single measure, and therefore, the integrated approach of which bio-control is a crucial component, is the

most promising strategy for reducing Striga infestations. The potential of using fungi for biological control of Striga was proposed by Musselman and Hepper (1983) and has since generated research interest. Adeoti (1993) and Weber *et al*. (1995) showed that *Fusarium* spp., *Cercospora* spp., *Phoma* spp., *Alternaria* spp. and *Macrophomina* spp. were associated with *Striga* spp. Abbasher *et al.* (1995) first reported *Fusarium oxysporum* f. sp. *strigae* as pathogenic on *Striga* spp. This fungus infects Striga at all developmental stages from seeds to flowering (Fravel *et al.,* 2003). From diseased *Striga hermonthica* in northern Ghana, Foxy 2 was isolated (Abbasher *et al.,* 1999). Elzein *et al.* (2006) reported between 75 and 81 % reduction of healthy emerged Striga shoots when Sorghum seeds were coated with Foxy 2 using 40 % gum Arabic. PSM 197 isolated from diseased *Striga hermonthica* plants in northern Nigeria, is aggressive isolate which reduce *S. hermonthica* emergence by 89.1 and 100 % through foliar application and soil incorporation of the fungus respectively (Marley *et al*., 1999).

### 1.1 Justification

The application of biocontrol agent (*Fusarium oxysporum* f. sp. *strigae*) coated with maize seeds has been found effective in controlling *Striga hermonthica* (Ciotola *et a.*, 2000; Ndambi, 2011). However, many farmers use imidacloprid + metalaxyl-M + tebuconazole, benomyl dressed seeds to manage seedling diseases, metsulfuron methyl to manage weeds and application of carbendazim, mancozeb and hexaconazole for managing fungal diseases. The efficacy of PSM 197 and Foxy 2 as a biological control agent (BCA) can be affected by pesticides usage. Therefore, the need to test some of these pesticides on growth and conidia of *F. oxysporum* isolates obtained in West Africa *in vitro* became imperative.

## 1.2 Objectives of the Study

The objectives of the study were to:

1. evaluate effect of pesticides on the two *Fusarium oxysporum* f. sp. *strigae* isolates
2. determine the most appropriate rate of pesticides for application with *Fusarium oxysporum* f. sp. *strigae* isolates
3. determine the compatibility of pesticides with *Fusarium oxysporum* f. sp. *strigae* isolates

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 *Striga hermonthica* (Del.) Benth.

#### 2.1.1 Taxonomy and distribution

The root hemiparasite *Striga hermonthica* is closely related to approximately 40 *Striga* species. This genus has been identified as a member of the Orobanchaceae family (previously classified in the Scrophulariaceae family) (Olmstead *et al*., 2001; Riches *et al*., 2004). The genus comprises of several other species of economic importance such as *S. asiatica, S. gesnerioides, S. densiflora, S. euphrasioides and S. forbesii* (Rezaul *et al*., 1995). These weeds have spread to several regions of the world, including the United States and Asia, but to a larger extent in Africa (Ejeta, 2007). *Striga hermonthica* is mainly distributed in tropical arid and semi-arid zones with 400 to 1000 mm of annual rainfall. The most severely affected countries are Mali, Upper Volta, Niger, Nigeria, Cameroon, Chad, Sudan, Ethiopia and India (Julien *et al*., 2009). This hemiparasite can develop everywhere except in humid forests, where they are apparently unable to colonize probably due to the absence of suitable hosts and the prevailing moist conditions (Ejeta, 2007).

The main vector of Striga dispersal is human activity and this is directly linked with agricultural practices (Julien *et al*., 2009). The seeds are also readily moved to surrounding fields via livestock, machinery or even more prominently, with actual crop seeds. Hence, movement of *Striga* spp. mostly occurs when farmers from different villages exchange seeds. This is still a tangible problem as the private seed industries have not yet reached successful quality seeds and accessibility (Ejeta, 2007). The reduction in soil fertility due to land use intensification and the lack of

proper crop rotations are agronomic aspects that have also contributed to Striga's expansion on the African continent (Watson *et al*., 2007).

### 2.1.2 Biology of *Striga hermonthica*

*Striga hermonthica* is a hemiparasite, and it requires the host plant for food to complete its life cycle, even though it is able to form chlorophyll and obtain energy through photosynthesis after emergence above soil level.

*S. hermonthica* achieves heights averaging 80 cm but ranging from 30 to 100 cm. The erect *S. hermonthica* possesses shoots that are circular at the base while becoming quadrangular as racemes are formed (Parker and Riches, 1993). Although, plant organ proportions depend on various environment conditions, leaves of *S. hermonthica* commonly measure 3 to 10 cm long and 5 to 20 mm wide and flowers are often 1 to 2 cm in diameter (Parker and Riches, 1993). The flowers of *S. hermonthica* are generally pink, bell shaped, bent in its upper section and is characterized by two tips at the other extremity (Ramaiah *et al*., 1983). The corolla generally measures more than 5 mm in length, but its similar counterpart *S. aspera* measures less than 5 mm (Parker and Riches, 1993).

Flowering generally occurs in *S. hermonthica* before the sixth week after the plant has emerged from the soil. *S. hermonthica* is an allogamous species, typically cross-fertilized by insects. Seeds weigh an average of $10^{-5}$ g, while seed size is 0.3 mm x 0.15 mm and produced at the rate of about 700 per capsule. Generally, 60 to 70 capsules can be counted per plant (Parker and Riches, 1993; Babiker, 2007). *Striga* seeds are known to remain viable in soil for more than 10 years (Gbehounou *et al*.,

2003). After being released from the mother plant, seeds enter a period of dormancy that inhibits them from germinating during the same growing season in which they have been produced (Riches and Ejeta, 2007). The dormancy is broken when a suitable germination condition is met, which consists of a 10 – 15 day period under an appropriate moisture level and when soil temperatures are sustained around 25 – 30 $^{0}$C (Parker and Riches, 1993). In the presence of chemicals such as strigol, strigolactone (analogue of strigol), ethylene, dihydrosorgoleone, methionine, inositol, as well as specific cytokinins or jasmonates, the seed will germinate (Parker and Riches, 1993; Riches and Ejeta, 2007). Otherwise, it will enter a state of secondary dormancy (Babiker, 2007). After germination, the radicle of the weed responds to chemical signals and bends towards the host root network before it forms a primary haustorium, which attaches to host stele (Kroschel *et al*., 1999). Haustorial structures are covered by trichome – like protuberances that have rough papillate surfaces. Moreover, they are able to produce a substance made primarily of hemicellulose that serves to hook the Striga radicle onto crop roots (Riches and Ejeta, 2007). Secondary roots are then formed by the parasite, also attaching to host roots by forming 1 to 2 mm long secondary haustoria (Kroschel *et al*., 1999). Cotyledons of *S. hermonthica* emerge from the seed coat less than 24 hours after the vascular connections with the host are set. For a period of about six weeks, scale leaves are produced in pairs on the elongating stem as the plant remains in the soil. After this period, emergence of *S. hermonthica* can be observed. By this time, the host has suffered an estimated 70 % of productivity reduction (Berner *et al*., 1997).

After Striga has germinated, attached and initiated development on the host's root system, signs of parasitism are observable on the host's above and below ground

parts. Stunted plants, an increased root to shoot ratio, reduction in internode space, chlorosis, wilting and reduced photosynthesis activity coupled with an increased photorespiration response are all commonly reported observations from Striga parasitism. These symptoms are ultimately linked with decreases in plant performance and in the worst-case scenario, plant death (Babiker, 2007).

### 2.1.3 Management practices against *Striga hermonthica*

As the problems concerning Striga have been part of the agriculture's scheme of Africa for a long time, a diversified array of tools and techniques have been developed by farmers and agronomists to limit damage (Ejeta, 2007). However, it is very hard to control its adverse effects on crops because about half of the parasite's life cycle occurs underground and the accumulated Striga seeds within soil are abundant. Weed management practices against *S. hermonthica* can be grouped basically into two categories: preventive and active control measures.

#### 2.1.3.1 Preventive control measures

The basic elements of management that are available to farmers are of cultural nature. Crop rotations using non-host plants and trap crops are perhaps the best methods available to farmers. On-farm yields, using resistant crops may allow farmers to produce enough food for a living while limiting Striga's seed bank replenishment and its level of infestation. It is often recommended to adopt a rotation that does not include any Striga-susceptible crops for three subsequent years (Parker and Riches, 1993). One year of trap cropping actually allows farmer to decrease the amount of Striga seeds in their fields by provoking suicidal germination of the seeds (Ejeta,

2007). Trap crops include cotton (*Gossypium* spp.), groundnut (*Arachis hypogaea*), sun hemp (*Crotalaria juncea* L.), pigeonpea (*Cajanus cajan* L.), alfalfa (*Medicago sativa* L.), sunflower (*Helianthus annuus* L.) and sesame (*Sesamum indicum*), all are able to stimulate parasitic germination and preventing further haustorial development (Babiker, 2007). However, due to climatic, social and economic reasons, crop rotation and use of trap crops are not practices widely adopted. Other field-based techniques such as intercropping or use of a multi-year fallow have been proven to be effective against *S. hermonthica* (Parker and Riches, 1993). These methods are not extensively being pursued since farmers view them a labour intensive (Ejeta, 2007).

Few numbers of farmers have access to *S. hermonthica* tolerant or resistant varieties. These cultivars have been bred so that one of the biological phases of the parasitism is broken or at least weakened. Hence, breeders have generated lines that are able to stimulate *S. hermonthica* germination, limit haustorial formation and development or inhibit infiltration into crop's vascular system (Babiker, 2007). Some maize cultivars developed by the International Institute of Tropical Agriculture (IITA, Ibadan) have been released for farmers use in West Africa (Menkir *et al*., 2006). Amendment of soils with nitrogen fertilizers such as ammonium sulphate $(NH_4)_2SO_4$ and ammonium phosphate ($NH_4H_2PO_4$) have been found to reduce Striga seed germination and inhibit Striga shoot development (Babiker, 2007). Studies have shown that, no-tillage systems and delayed planting operations are advantageous in the fight against Striga (Parker and Riches, 1993).

### 2.1.3.2 Active control measures

The common active method available to all growers is the hand-pulling of *S. hermonthica* plants once they have emerged from the soil. Although, most of the damage done by Striga in terms of yield loss is done prior to the plant's emergence, losses are still occurring at later stages of Striga development (Parker and Riches, 1993). The main advantage of pursuing hand weeding is related to the prevention of the seed bank's rejuvenation, other efficient physical method of Striga repression is hoeing, as it allows destruction of the plants grown above soil level (Ejeta, 2007). The most important constraint of these two techniques is by far the extensive demand for labour. For this reason, hand-pulling and hoeing are generally only encouraged and undertaken under low or least-infested fields (Parker and Riches, 1993). Other tillage operations are mostly ineffective since they are usually shallow and in the inter-rows, far from the actual areas of parasitism.

Germination stimulants can be employed in the absence of a Striga host crop in order to force suicidal germination of *S. hermonthica* seeds and consequently to achieve a partial depletion of the seed bank. Foliar applied phenoxy herbicides such as 2, 4-D [(2, 4-dichlorophenoxy) acetic acid] and MCPA [(4-chloro-2-methylphenoxy) acetic acid] are able to destroy Striga (Kanampiu *et al*., 2007). They however, require high rates of application, specific spraying apparatus and some elementary knowledge. In addition, they are applied as post-emergence when the current season's damage has already been done. Dicamba and paraquat are other *S. hermonthica* repressing herbicides but their utilization necessitates the same requirements as mentioned above (Babiker, 2007). Hence, it is now recognized that selective herbicides cannot be used efficiently underground except if the crop has an element of resistance to the

herbicide (Kanampiu *et al*., 2007). Vurro *et al*. (2006) stated that, one of the most recent chemical approach developed to control Striga involves the utilization of amino acids. He mentioned that several amino acids caused physiological disorders during seed germination of broomrapes (*Orobanche ramosa*) when they were applied at high enough quantities. Trials were also conducted on Striga seeds and showed that, germination inhibition and germ tube elongation were altered by the presence of leucine and threonine Vurro *et al*. (2006).

### 2.1.4 Biological control of weeds

Biological weed control concern intentional utilization of natural enemies of an undesired plant species in order to reduce its development and ultimately to decrease its density in a given ecosystem (Watson, 1993). The two main categories under which biocontrol initiatives are classified include the "classical approach" and the "inundative strategy". In the first case, also called the inoculative approach, natural enemies of a pest are brought from its center of origin to an area where the weed has been transported and where it developed extensively. In the second category, endemic weed species are controlled with their natural enemies in their native habitats. The biocontrol agents are first mass-produced and mass-released as part of specific and optimized formulations. This method is especially effective when a weed is in an area where the population of its enemy is insufficient for proper control, or when an agent is slowly disseminated from the environment into the weed-infected zones (Watson, 1993).

A tremendous array of weed biocontrol agents remained at research stage and not made available commercially. This is explained, in part, because biocontrol agents

require several years of study to be formulated cost-efficiently. Furthermore, safety issues need to be addressed and the required analyses are often complex and fastidious.

### 2.2 Description of *Fusarium* Species

*Fusarium* spp. are soil-borne fungi and are distributed worldwide in every type of soil (Fravel *et al*., 2003; Kroschel and Muller-Stover, 2004). The life cycle of *F. oxysporum* has been described to include a saprophytic and parasitic phase in which it grows into host plants following entry into the xylem elements (Beckman and Roberts, 1995).

In the saprophytic phase, these fungi grow and survive for long periods on soil organic matter and in the rhizosphere of many plant species (Garrett, 1970). Many strains of *Fusarium oxysporum* are pathogenic on crops infecting roots through wounds or lateral roots causing either root rots or penetrating through the cortex to the xylem and upwards to the stem causing tracheomycosis (Fravel *et al*., 2003). This affects plant water supply, in a severe condition, leading to death (Agrios, 2005). However, non pathogenic *Fusarium* spp. has been used as biocontrol agents against *Fusarium* wilt and has been reported for tomato, watermelon and cucumber amongst others in both greenhouse and field trials (Larkin and Fravel, 1999). It was reported that, in some cases the biocontrol agent induced host plant resistance to *Fusarium* wilt and in other cases there is competition for nutrients, root colonization and infection sites (Benhamou *et al*., 2002). Some isolates proposed for use as biocontrol agents for their pathogenic and specificity on parasitic weeds include *Fusarium oxysporum* isolates M12-4A, Foxy 2, PSM 197 and 4-3-B against *S. hermonthica* (Beed *et al*.,

2007), *F. oxysporum* f. sp. *Orthoceras* against *Orobanche cumana* (Bedi and Donchev, 1991).

*Fusarium oxysporum* f. sp. *strigae* are known to be highly host specific, and are not pathogenic on economically important crops or other wild plants (Elzein and Kroschel, 2006; Leslie and Summerell, 2006). Widespread use of *Fusarium* spp. as biocontrol agents is however limited because of their ability to produce toxins (Nelson *et al*., 1992). A wide variety of fungal metabolites are both mycotoxic (toxic to animals and can also affect humans) and phytotoxic (toxic to plants). Phytotoxic effects include necrosis, chlorosis, growth inhibition, wilting and inhibition of seed germination (Zonnon and Vurro, 1999).

### 2.2.1 Biology of *Fusarium oxysporum*

Based on the structure in or on which conidiogenous hyphae are borne, *Fusarium* spp. are classified under the Hyphomycetidae subclass of the Deuteromycetes. It is an anamorphic (lacks asexual reproductive stage) species that includes numerous plant pathogenic strains causing wilt diseases of a broad range of agricultural and ornamental host plant species (Appel and Gordon, 1996). *Fusarium oxysporum* produces three types of asexual spore: macro-conidia, micro-conidia and chlamydospores (Nelson *et al*., 1983). Conidia are produced on monophialides and in sporodochia and are scattered loosely over the surface of a mycelium (Griffin, 1994). Microconidia are one or two cells, most frequently and abundantly produced conidia under all conditions (Agrios, 2005). The macro-conidia are produced abundantly, multinucleate, and germinate rapidly multiplying the fungus efficiently. Chlamydospores are viable, asexually produced accessory spores resulting from the structural modification of a vegetative hyphal segments or conidial cell possessing

thick wall and function primarily for survival in soil. Morphological characterization of *F*. *oxysporum* is based on the shapes of macro and micro-conidia, structure of conidiophores, formation and disposition of chlamydospores (Beckman, 1987). Plant pathogenic forms of *F*. *oxysporum* are divided into formae speciales based on the host they attack (Armstrong and Armstrong, 1981). Further subdivisions of formae speciales into races are made on their virulence to a set of differential host cultivar (Correll, 1991).

### 2.2.2 Life cycle of *Fusarium oxysporum*

Species of the genus *Fusarium* possess the ability to colonize crop residue and other organic materials incorporated in the soil (Ciotola *et al*., 2000). *Fusarium oxysporum* survives in infected plant debris in the soil as mycelium and spore forms, but most commonly as chlamydospores in the cooler temperate regions (Agrios, 2005). This confers them the advantage of surviving for long periods of time without being in direct contact with their living hosts. In addition to being saprophytes, the chlamydospores produced by this kind of fungus have the advantage of being able to survive extreme environmental events while still remaining viable (Leslie and Summerell, 2006). Chlamydospores remain dormant and immobile in the remains of decayed plant tissues until stimulated to germinate by utilizing nutrients that are released from extending roots of many different plants (Beckman and Roberts, 1995). The chlamydospores germinate when they reach the 0.5 mm region surrounding the roots of their hosts (Beckman, 1987).

When germinating, chlamydospores and micro-conidia typically produce a single germ tube, while macro-conidia characteristically produce two (Leslie and

Summerell, 2006). During germ tube elongation, macro-conidia do not require exogenous carbon as they use their own energy, at the opposite of the smaller chlamydospores and micro-conidia. Germ tubes of each type of spores as well as mycelia produced from them all have the ability to enter the host by direct penetration, via wounds or at entry points located at junctions where lateral roots are formed (Agrios, 2005). Invasion of the roots is followed by penetration of host epidermal cells. In advanced stages of the disease, *F. oxysporum* grows out of the vascular system into adjacent parenchyma cells, producing vast quantities of conidia and chlamydospores.

### 2.2.3 Formation and germination of conidia

Micro-conidia are formed from phialides in false heads by basipetal division. Macro-conidia are generally produced from phialides on conidiophores by basipetal division. Chlamydospores are formed by the modification of hyphal cells (Nelson *et al.*, 1983). These asexual spores play important roles in the disease cycle of *F. oxysporum*: micro-conidia and macro-conidia are important as secondary inocula, and chlamydospores are endurance organs and act as primary inocula (Rekah *et al*., 2000). Chlamydospores formation in pathogenic *Fusarium species* commonly takes place in hyphae in the infected and decaying host tissue (Christou and Snyder, 1962). Schippers and Van-Eck (1981) proposed that chlamydospore formation depends on the nutrients status of the inoculums. Under field conditions, fungal inoculums may be subjected to much lower nutrients level when compared to the 'well-fed' macro-conidia produced on rich sugar media. Once carbohydrates are released from decaying plant tissue or from roots, chlamydospore germination is stimulated (Schippers and Van-Eck, 1981). Qureshi and Page (1970) further suggested that chlamydospore is

formed with the addition of organic or inorganic carbon source. Hsu and Lockwood (1973) reported that, an environment deficient in energy, but with an appropriate weak salt solution, was required for chlamydospore formation. Chlamydospore germination in nature appears to be dependent on exogenous energy sources (e.g carbon and nitrogen) (Griffin, 1970). Spore density is the single most important factor affecting the nutritional requirements for germination of conidia and chlamydospore in pure culture (Griffin, 1981). Exogenous carbon and nitrogen were required for high or complete chlamydospore germination at high spore density in axenic culture (but not at low spore density) and in soil (Griffin, 1970). At high conidia densities, macro-conidia do not germinate, but every conidia is converted into a chlamydospore (Schneider and Seaman, 1974). According to Griffin (1981) the inability of macro-conidia to germinate at high conidia densities resulted from the presence of self-inhibitors. Self-inhibitors are substances accumulating in growth medium suppressing germination of macro-conidia at high spore densities in the soil (Griffin, 1970).

### 2.2.4 Infection of Striga seedlings by *Fusarium oxysporum*

Fungal infection commences when hyphae adhere to host root surface (Bishop and Cooper, 1983). Adhesion of fungi to the surface is not a specific process, as they adhere to surfaces of both host and non-host plants (Videhyasekaran, 1997). Site-specific binding are important in anchoring propagules to root surface (Recorbet and Albouvette, 1997). *Fusarium* spp. as several other Deuteromycetes, are able to initiate vascular wilts of their hosts (Agrios, 2005). The pathogen invades and fills the host xylem with mycelia and mitospores including macro-conidia, micro-conidia and chlamydospores. In addition to fungal parts causing the xylem to be clogged, gums, gels and various polysaccharides accumulate as a result of fungal activity and tissue

maceration. This contributes further to restricting the xylem to play its role of moving water and nutrient upwards (Agrios, 2005). As a result, leaves of infected plants lose their turgidity, appear flaccid and ultimately wilt and desiccate. Other symptoms can include leaf chlorosis and general plant stunting (Parker and Riches, 1993). In the most severe cases, wilting achieves such important dimensions that infected hosts die (Ciotola *et al*., 2000).

### 2.2.5 Efficacy of *Fusarium oxysporum* f. sp. *strigae* isolates as biocontrol agents of *Striga hermonthica*

Striga poses a serious problem to cereal crops in West Africa. *Striga hermonthica* seed banks are extremely rich and a partial reduction in the amount of seed input would only have a minimal effect on the population dynamics of the parasitic plant. Thus, the option of using micro-organisms affecting *Striga* spp. at earlier growth stages was then pursued by researchers, since repression effects are seen in both short and long terms (Ciotola *et al*., 1995). Several field surveys in Mali, Niger, Nigeria, Ghana, Sudan and Burkina Faso have led to the conclusion that *Fusarium oxysporum* f. sp. *strigae* is the most virulent pathogen affecting the development of different *Striga* species (Ciotola *et al*., 2000; Elzein and Kroschel, 2004).

This fungus is preferred to other micro-organisms as biological control agent to manage Striga (bioherbicides) because it is host-specific, highly aggressive and diverse in terms of number of isolates (Ciotola *et al*., 2000). Different authors reported that seed coating technology improves the performance of a fungal biocontrol agent, give uniform inoculum distribution and maximizes time. It simplifies the transport of the *Fusarium* chlamydospores and it ensures the availability of the agent where it is actually needed. In addition, the utilization of

Arabic gum has been shown to increase the rate of mycelial development and enhanced sporulation of *Fusarium oxysporum* significantly (Ciotola *et al*., 2000; Elzein *et al*., 2006). The fungus can saprophytically colonize the root system of the cereal host and subsequently infect *S. hermonthica* at the seed or later stages of growth (Elzein *et al*., 2006).

Isolates M12-4A, PSM 197 and Foxy 2 of *F. oxysporum* from *Striga hermonthica* are pathogenic and highly aggressive against all developmental stages of *S. hermonthica* (Marley *et al*., 2005; Elzein and Kroschel, 2006). In previous works, *Fusarium oxysporum* strain PSM 197 decreased *S. hermonthica* emergence by 89.7 % (Marley and Shebayan, 2005). Ciotola *et al*. (2000), observed 92 % reduction in *Striga* emergence when $10^7$ chlamydospores of M12-4A were soil applied as a dry powder formulation in addition to 10 g of ground Sorghum straw amendment. Foxy 2 was effective in controlling Striga by causing 86 % disease in *S. hermonthica* seedlings when coated on susceptible Sorghum cultivar seeds (Ndambi, 2011). Striga seedlings were considered diseased when their colour changed to dark brown or black. The infected seedlings browning start primarily at host root, extending to growing points, until the Striga wilted (Ndambi, 2011). Furthermore, a complete reduction in *S. hermonthica* above ground biomass was recorded when using seed coating technology with 2 x $10^5$ spores of PSM 197 per Sorghum seed. Julien *et al*. (2009) reported that the isolates PSM 197 and Foxy 2 were effective in *S. hermonthica* repression, especially when applied as pesta granules. Result has demonstrated that when mycoherbicide pesta formulation was spot applied at 2 g per hill, Striga count was significantly reduced and number of wilted Striga plant increased (Zarafi and Marley, 2008). Application of 40 kg of pesta formulation per hectare showed a higher level of

Striga control when applied directly into planting hole (Schuab *et al*., 2006). This way of application is supposed to be visible as local farmers do sowing manually and the amount of inoculums are not too high.

Parker and Riches (1993) reported that significant reduction in Striga emergence and flowering as a result of combining mycoherbicide and host plant resistance is an important feature to prevent further Striga distribution and infestation in fields as Striga seeds can remain viable in the soil for at least up to 14 years. However, Abbasher *et al*. (1999) conducted a pathogenicity tests and reported that *Fusarium* spp. was able to control more than one *Striga* species; *F. nygamai*, *Fusarium. oxysporum* and *F. solani* isolated from infected *S. hermonthica* plants from Sudan and Ghana reduced emergence of *S. asiatica* and *S. gesnerioides*, thereby improving the performance of the host crops, maize and cowpea.

**2.3 Effects of Fungicides on Fungal Pathogens**

In order to prevent plant diseases of fungal origin and to protect the crop plants against pathogens, fungicide control measures are in practice. Inhibitory effect of benomyl against many plant pathogenic fungi has been reported by many researchers. Marley and Gbenga (2004) demonstrated the efficacy of benomyl at 5, 10 and 20 g to control *Stenocarpella maydis* (on maize) *in vitro.* Similarly, Karaoglanidis *et al.* (2003) reported the effectiveness of benomyl in controlling leaf-spot caused by *Cercospora beticola* on sugar beet. The use of benomyl, carbendazim and mancozeb significantly inhibited *Physoderma maydis* on maize (Brown spot) and 100 % inhibition of sporangia germination at 10,000 ppm a.i. of the fungicides. However, carbendazim at 50,000 ppm a.i was effective in the control of the disease (Brown spot) in the field and resulted in highest grain yield (Osunlaja and Alamutu, 1999).

Metalaxyl and benzoid fungicides have been effective in the inhibition of mycelia growth of *Pythium deliense* and *Pythium oligandrum* isolated from damped-off maize (Hani *et al.,* 2004). Research has also shown that, the fungicides benomyl, benomyl + thiram, tricyclazole, metalaxyl-M + thiomethozan + difenconozole, carbendazim and mancozeb, evaluated *in vitro* inhibited mycelial growth of *F. pallidoroseum* at varying rate (Mamza *et al.,* 2010). Mukhtar (2007), in his work compared varying rates of benomyl, carbendazim and captan and reported that, different doses applied significantly reduced mycelia growth of *Fusarium oxysporum* at 0.2 % rate. Carbendazim and benomyl were most effective against *Fusarium oxysporum.* Similarly, Cother (1977) obtained effective control of gram wilt by seed treatment with benomyl, captan and thiram. Gupta *et al*. (1997) screened 6 fungicides against *F. oxysporum* f. sp. *ciceri* and reported carbendazim at 100 mg/ml as most effective in inhibiting the growth of the fungus *in vitro*. However, at low rate, test fungicides also showed equally effective behaviour. Christian *et al*. (2007) also reported significant effect of five fungicides, benomyl (1 mg/L), dodine (50 mg/L), manzate (100 mg/L), cupric sulphate (200 mg/L) and thiabendazole (4 mg/L) under *in vitro* conditions on growth of 28 isolates of pathogenic fungi. Chemical seed treatment with thiram (0.15 %) + carbendazim (0.1 %) were found most effective against *F. oxysporum* f. sp. *ciceri* (Nikum *et al*., 2007). Obagwu (2003) reported that, half recommended dosage of benomyl (0.3 g/L) combined with 1% (w/v) garlic extract was as effective as the full dosage of the fungicide, which gave complete control of *Colletotrichum capsici*.

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 Preparation of Potato Dextrose Agar Media

Two hundred grams of peeled and diced potato was boiled in one litre of tap water for 30 minutes and filtered through double layer muslin cloth. Dextrose (20 g) and agar powder (15 g) were dissolved in the filtrate. The volume was raised to one litre and autoclaved at 121 $^{o}$C and 1.1 kg/cm$^2$ for 15 minutes. Five millilitre streptomycin sulphate was added after cooling to a temperature of 40 $^{o}$C.

### 3.1.1 Determining the effect of pesticides and pesticide rates on isolates of *Fusarium oxysporum* f. sp. *strigae*

Three factors: fungal isolates (2), pesticides (7) and their rates (5) were studied using a factorial experiment with 4 repetitions was carried out. Seven pesticides: benomyl (Benomyl® 50 % WP; Shanghai Shenglian Chemical Co., Limited), carbendazim (Forcelet® 50 % WP; Sino Agro Chemical Industry Limited), mancozeb (Sarozeb® 80 % WP; Forward (BEIHAI) Hepu Pesticide Co., Limited), carbendazim + mancozeb (Funguforce® 75.5 % WP; Sino Agro Chemical Industry Limited), hexaconazole (Control Total® 5 % SC; Meghmani Industries Limited), imidacloprid + metalaxyl-M + tebuconazole (Dress force® 42 % WS; Sino Agro Chemical Industry Limited) and metsulfuron methyl (MSM® 20 % WP; Du Pont) at 0.0x, 0.5x, 1.0x, 1.5x and 2.0x where x is the manufacturer's recommended rate and 0.0x is control. These were incorporated into freshly prepared Potato Dextrose Agar with Streptomycin (PDAs). Required quantities of each pesticide for each rate (Table 1) were dissolved in 20 ml sterile distilled water (SDW) in 250 ml conical flasks and the volume raised to 200 ml with molten PDAs. Two isolates of *Fusarium oxysporum* f. sp. *strigae*: PSM 197 and

Table 1: Pesticides used and their rates

| Pesticide trade name | Type of pesticide | Active ingredient | Formulation | Pesticide rates | Quantity used per litre |
|---|---|---|---|---|---|
| Benomyl® | Fungicide | Benomyl | 50 % WP | 0.0x | 0.0 g |
| | | | | 0.5x | 1.25 g |
| | | | | 1.0x | 2.50 g |
| | | | | 1.5x | 3.75 g |
| | | | | 2.0x | 5.00 g |
| Funguforce® | Fungicide | Carbendazim + mancozeb | 75.5 % WP | 0.0x | 0.0 g |
| | | | | 0.5x | 3.13 g |
| | | | | 1.0x | 6.25 g |
| | | | | 1.5x | 9.38 g |
| | | | | 2.0x | 12.50 g |
| Forcelet® | Fungicide | Carbendazim | 50 % WP | 0.0x | 0.0 g |
| | | | | 0.5x | 3.75 g |
| | | | | 1.0x | 7.50 g |
| | | | | 1.5x | 11.25 g |
| | | | | 2.0x | 15.00 g |
| Sarozeb® | Fungicide | Mancozeb | 80 % WP | 0.0x | 0.0 g |
| | | | | 0.5x | 6.00 g |
| | | | | 1.0x | 12.00 g |
| | | | | 1.5x | 18.00 g |
| | | | | 2.0x | 24.00 g |
| Control Total® | Fungicide | Hexaconazole | 5 % SC | 0.0x | 0.0 ml |
| | | | | 0.5x | 0.25 ml |
| | | | | 1.0x | 0.50 ml |
| | | | | 1.5x | 0.75 ml |
| | | | | 2.0x | 1.00 ml |
| Dress Force® | I + f + f | IMT | 42 % WS | 0.0x | 0.0 g |
| | | | | 0.5x | 2.00 g |
| | | | | 1.0x | 4.00 g |
| | | | | 1.5x | 6.00 g |
| | | | | 2.0x | 8.00 g |
| MSM® | Herbicide | Metsulfuron methyl | 20 % WP | 0.0x | 0.0 g |
| | | | | 0.5x | 1.15 g |
| | | | | 1.0x | 2.30 g |
| | | | | 1.5x | 3.44 g |
| | | | | 2.0x | 4.59 g |

x = manufacturer's recommended rate, I + f + f = Insecticide + fungicide + fungicide,
IMT = imidacloprid + metalaxyl-M + tebuconazole

Foxy 2 were used. The isolates were obtained from culture room of Department of Crop Protection, Ahmadu Bello University, Zaria and cultured in PDAs for 14 days at room temperature (28 ± 2 $^{o}$C) and transferred using 5 mm flame-sterile cork borer to the centre of amended and control PDAs. The experiment was laid out in completely randomized design (CRD) with four repetitions on laboratory bench.

### 3.1.2 Data collection

**Mycelial growth measurement**

Colony formation and colour of *Fusarium oxysporum* f. sp. *strigae* isolates PSM 197 and Foxy 2 on PDAs was observed visually. Mycelial diameter was measured at 24 hours interval for fourteen days, starting from 2 days after inoculation (DAI) along two perpendicular lines drawn at bottom of the Petri dishes. Mycelia diameter measurement was terminated at 14 DAI. Percent inhibition (PI) of mycelial diameter for each treatment was calculated using the formula described by Ashwani *et al*. (2012)

$$PI = \frac{A - B}{A} \; x \; 100$$

where, A = mycelial diameter in control plate,

B = mycelial diameter in treated plate.

**Sporulation**

At 14 day after incubation, mycelia were harvested from 4 Petri dishes per treatment into 250 ml beakers using scalpel, macerated in 80 ml SDW, using Binatone Turbo blender (Model No. HM-350S), filtered through sterile double layer muslin cloth. Conidia suspension obtained was homogenized, divided into four portions each representing a repetition. Micro and macro-conidia were counted per repetition using

haemocytometer under electrically powered binocular microscope at x400 magnification. The values obtained were substituted into the formula below to calculate the number of conidia per millilitre (ml) of each repetition

$$C = \frac{n}{256} 4\ x\ 10^6$$

where, C = number of conidia/ml

n = number of conidia counted/ chamber

256 = constant (16 x 16 square grids)

**Conidia size**

Conidia sizes (length and width) measurement were made for 50 randomly selected micro and macro-conidia from conidia suspension prepared per repetition. Length and width measurements (µm) were obtained using an ocular micrometer at x400 magnification on electrically mounted microscope. Length and width of conidia measured were then averaged by dividing total length or width to 50.

**Conidia germination**

Conidia germination was determined using conidia harvested from 14 day-old culture. Conidia suspension taken from the culture was adjusted to 7.8 x $10^5$/ml and 10 ml of each treatment solution was introduced into 50 ml potato dextrose broth and incubated for 24 hours at room temperature (28 ± 2 $^oC$). Number of germinating conidia was counted using haemocytometer under magnification ×400. Viability percentage of conidia was calculated using the formula described by Taskeen *et al.* (2011)

$$A(\%) = \frac{B}{C} \times 100$$

where, A = percent conidia viability

B = number of conidia germinated

C = Total number of conidia counted.

**Compatibility of pesticides with *Fusarium oxysporum* f. sp. *strigae* isolates**

Compatibility of pesticides to isolates PSM 197 and Foxy 2 was obtained from percent variations of mycelia growth, conidia number and viability from the arithmetic calculations. The higher the percent variation, the higher the incompatibility. The lower the variation the more the compatibility of the pesticide with the test isolates parameter.

$$VP(\%) = \frac{t_i - t_o}{t_o} \times 100$$

where, VP = percent variation of development parameters

$t_i$ = parameter of treated

$t_o$ = parameter of control.

**3.2 Data Analysis**

Data collected were subjected to analysis of variance (ANOVA) using SAS (2002) software version 9 and means were separated using Student-Newman-Keuls (SNK) test at 5 % level of significance.

# CHAPTER FOUR

## 4.0 RESULTS

### 4.1 Cultural and Morphological Characteristics of *Fusarium oxysporum* f. sp. *strigae* Isolates PSM 197 and Foxy 2 on PDAs.

PSM 197 and Foxy 2 grown on PDAs were initially cottony white but later became purple, well branched with uniform growth. The isolates grew slowly covering 9 cm Petri dish in 12 – 14 days. The two isolates of the fungus had conidiophores, phialides, micro and macro-conidia. The micro-conidia were smooth, hyaline, ovoid to cylindrical and abundant 2,947.59 x $10^5$ and 2,719.71 x $10^5$/ml for PSM 197 and Foxy 2 respectively. The macro-conidia were smooth, hyaline and straight to curve shaped. Isolates PSM 197 and Foxy 2 produced macro-conidia 78.64 x $10^5$ and 76.39 x $10^5$/ml respectively.

#### 4.1.1 Main effect of pesticides and rate on morphological characteristics and conidia germination of *Fusarium oxysporum* f. sp. *strigae* isolates in Zaria, Nigeria.

The reactions of Foxy 2 and PSM 197 isolates of *F. oxysporum* f. sp. *strigae* to the tested pesticides were not significantly ($P \leq 0.05$) different from each other in respect of percent mycelia growth inhibition, micro-conidial length and width, as well as macro-conidial width (Table 2). Isolate PSM 197 produced significantly ($P \leq 0.05$) higher number of micro and macro-conidia than Foxy 2. Similarly, the macro-conidia produced by PSM 197 were longer and had higher percent germination than those of Foxy 2.

There was significant ($P \leq 0.05$) difference among the pesticides treatment on mycelial growth, conidia count, size and germination of *Fusarium oxysporum* f. sp.

*strigae*. The fungicides benomyl, carbendazim, carbendazim + mancozeb completely inhibited mycelia growth, followed by imidacloprid + metalaxyl-M + tebuconazole (IMT), mancozeb, hexaconazole and metsulfuron methy1 recorded the lowest 19.65 % inhibition. Highest ($5{,}248.56 \times 10^5$) number of micro-conidia was obtained from metsulfuron methyl treated media compared to other pesticides. This was followed by hexaconazole, mancozeb and IMT, while there was no micro or macro-conidia produced on benomyl, carbendazim and carbendazim + mancozeb amended media. For macro-conidia, isolates cultured on hexaconazole incorporated media yielded the highest ($137.31 \times 10^5$) number compared with other pesticides treatment. This was followed by metsulfuron methyl, while mancozeb and IMT did not differ significantly ($P \leq 0.05$). Micro-conidia produced on metsulfuron methy1 and mancozeb amended media did not differ significantly from each other although were longer than those on IMT and hexaconazole treated media. IMT amended media recorded narrower micro-conidia but was not significantly ($P \leq 0.05$) different from those produced on mancozeb, hexaconazole and metsulfuron methyl. The macro-conidia produced on mancozeb and hexaconazole treated media were similar, although, they were significantly ($P \leq 0.05$) longer than those produced on metsulfuron methyl and IMT. Macro-conidia produced on mancozeb treated media was significantly wider than those on other pesticides tested. Narrower macro-conidia was recorded on IMT. Conidia germination recorded when media was amended with mancozeb and metsulfuron methyl were not different from each other, though, they were significantly ($P \leq 0.05$) higher than those produced on hexaconazole and IMT respectively.

The effect of pesticide rates was significantly ($P \leq 0.05$) different on mycelial growth, conidia count, size and germination of *Fusarium oxysporum* f. sp. *strigae*. Mycelia

growth inhibition in *Fusarium oxysporum* f. sp. *strigae* was increased proportionally with increased rates of pesticides used. Highest (81.44 %) inhibition was observed at twice recommended rate used. Number of micro-conidia produced was higher ($8{,}363.91 \times 10^5$) on an un-amended media. When pesticide rate was increased to half, number of micro-conidia was reduced but with increased rates at recommended and one and half rate, there was no significant ($P \leq 0.05$) difference in number of micro-conidia. Macro-conidia recorded on un-amended media was significantly ($P \leq 0.05$) higher than any pesticide rates amended media. There was no significant ($P \leq 0.05$) difference in macro-conidia count between half and recommended rate as well as one and half and twice rate respectively. Micro-conidia produced on an un-amended media was significantly ($P \leq 0.05$) longer than those on media with recommended and twice rate. Micro-conidia recorded at half, recommended rate and un-amended media were similar but were wider than when the pesticide rate was increased above one and half. Macro-conidia recorded was significantly ($P \leq 0.05$) longer (11.23 μm) at half rate than any other rates. When pesticide rate was increased at recommended, macro-conidial length was reduced but with further increase in pesticide rate to one and half and twice recommended rate, conidial length was not different from each other. Wider macro-conidia was observed at half rate but did not differ significantly ($P \leq 0.05$) with the other rates. Conidia germination was significantly ($P \leq 0.05$) reduced with every increased pesticide rate by half.

Significant interactions between isolate and pesticide on mycelia growth, micro-conidia count and conidia germination, between isolate and rate on mycelia growth and conidia germination, between pesticide and rate on mycelia growth, conidia count, size and germination, and between isolate and pesticide and rate on mycelia

Table 2: Main effect of pesticides and rates on morphological characteristics and conidia germination of *Fusarium oxysporum* f. sp. *strigae* isolates in Zaria, Nigeria

| Treatment | Mycelia inhibition (%) | Conidial count (x$10^5$) | | Conidial size (µm) | | | | Conidia germination (%) |
|---|---|---|---|---|---|---|---|---|
| | | Micro-conidia | Macro-conidia | Micro-conidia | | Macro-conidia | | |
| | | | | Length | Width | Length | Width | |
| **Isolate (I)** | | | | | | | | |
| PSM 197 | 73.48$^{NS}$ | 2,947.59$^{a}$ | 78.64$^{a}$ | 4.31$^{NS}$ | 2.51$^{NS}$ | 10.72$^{a}$ | 2.57$^{NS}$ | 33.78$^{a}$ |
| Foxy 2 | 73.45$^{NS}$ | 2,719.71$^{b}$ | 76.39$^{b}$ | 4.39$^{NS}$ | 2.49$^{NS}$ | 10.48$^{b}$ | 2.55$^{NS}$ | 29.50$^{b}$ |
| S. E. ± | 0.106 | 2.803 | 0.691 | 1.081 | 0.500 | 0.174 | 0.435 | 3.021 |
| **Pesticide (P)** | | | | | | | | |
| Benomyl | 100.00$^{a}$ | 0.00$^{e}$ | 0.00$^{d}$ | 0.00$^{c}$ | 0.00$^{c}$ | 0.00$^{d}$ | 0.00$^{d}$ | 0.00$^{d}$ |
| Carbendazim | 100.00$^{a}$ | 0.00$^{e}$ | 0.00$^{d}$ | 0.00$^{c}$ | 0.00$^{c}$ | 0.00$^{d}$ | 0.00$^{d}$ | 0.00$^{d}$ |
| Carbendazim+mancozeb | 100.00$^{a}$ | 0.00$^{e}$ | 0.00$^{d}$ | 0.00$^{c}$ | 0.00$^{c}$ | 0.00$^{d}$ | 0.00$^{d}$ | 0.00$^{d}$ |
| IMT | 85.51$^{b}$ | 112.06$^{d}$ | 3.30$^{c}$ | 4.29$^{ab}$ | 2.46$^{ab}$ | 9.03$^{c}$ | 2.48$^{c}$ | 19.08$^{c}$ |
| Mancozeb | 82.06$^{c}$ | 305.87$^{c}$ | 6.34$^{c}$ | 4.38$^{a}$ | 2.53$^{a}$ | 12.38$^{a}$ | 2.70$^{a}$ | 39.98$^{c}$ |
| Hexaconazole | 45.42$^{d}$ | 3,021.65$^{b}$ | 137.31$^{a}$ | 4.34$^{ab}$ | 2.50$^{a}$ | 12.24$^{a}$ | 2.59$^{b}$ | 25.37$^{b}$ |
| Metsulfuron methyl | 19.65$^{e}$ | 5,248.56$^{a}$ | 87.94$^{b}$ | 4.37$^{a}$ | 2.51$^{a}$ | 9.60$^{b}$ | 2.55$^{bc}$ | 37.66$^{a}$ |
| S. E. ± | 0.189 | 39.059 | 1.565 | 0.036 | 0.019 | 0.084 | 0.022 | 0.680 |
| **Rates (R) g/L** | | | | | | | | |
| 0.0x | 0.00$^{e}$ | 8,363.91$^{a}$ | 235.85$^{a}$ | 4.47$^{a}$ | 2.57$^{a}$ | 10.77$^{b}$ | 2.60$^{ab}$ | 66.22$^{a}$ |
| 0.5x | 68.46$^{d}$ | 2,944.84$^{b}$ | 73.28$^{b}$ | 4.37$^{ab}$ | 2.53$^{ab}$ | 11.23$^{a}$ | 2.62$^{a}$ | 36.81$^{b}$ |
| 1.0x | 74.93$^{c}$ | 2,281.53$^{c}$ | 70.28$^{b}$ | 4.32$^{b}$ | 2.52$^{ab}$ | 10.66$^{b}$ | 2.58$^{ab}$ | 31.37$^{c}$ |
| 1.5x | 79.55$^{b}$ | 2,363.02$^{c}$ | 62.13$^{c}$ | 4.37$^{ab}$ | 2.45$^{b}$ | 10.13$^{c}$ | 2.50$^{b}$ | 25.06$^{d}$ |
| 2.0x | 81.44$^{a}$ | 2,048.77$^{d}$ | 58.12$^{c}$ | 4.31$^{b}$ | 2.47$^{b}$ | 10.10$^{c}$ | 2.52$^{ab}$ | 20.16$^{e}$ |
| S. E.± | 0.143 | 39.059 | 1.565 | 0.036 | 0.019 | 0.084 | 0.022 | 0.680 |
| Interaction | | | | | | | | |
| I*P | ** | ** | Ns | Ns | Ns | Ns | Ns | ** |
| I*R | * | Ns | Ns | Ns | Ns | Ns | Ns | ** |
| P*R | ** | ** | ** | Ns | Ns | ** | Ns | ** |
| I*P*R | ** | Ns | Ns | Ns | Ns | Ns | Ns | Ns |

Means with the same superscript in each column under each factor are not significantly different at P ≤ 0.05, using the students-Newman-Keuls (SNK) Test. IMT = imidacloprid+metalaxyl-M+tebuconazole, x = manufacturer's recommended rate, Ns = not significant at 0.05 % level of significance, * = significant at 0.05 % and ** = highly significant at 0.05 % level of significance

growth was observed.

The isolates PSM 197 and Foxy 2 cultured on benomyl, carbendazim, carbendazim + mancozeb, IMT and hexaconazole did not interact significantly ($P \leq 0.05$) on mycelial growth except mancozeb and metsulfuron methyl (Table 3). Holding mancozeb constant, mycelial growth inhibition in Foxy 2 was significantly ($P \leq 0.05$) higher than PSM 197. However, mycelial growth inhibition with metsulfuron methyl in PSM 197 was significantly higher than Foxy 2. Lower mycelial growth inhibition was observed in Foxy 2 with metsulfuron methyl.

The interaction between pesticide and isolate on micro-conidia number of *Fusarium oxysporum* f. sp. *strigae* is presented in (Table 4). There was no significant ($P \leq 0.05$) of pesticides across the isolates except hexaconazole and metsulfuron methyl. The isolate PSM 197 significantly ($P \leq 0.05$) produced higher number of micro-conidia when cultured on hexaconazole and metsulfuron methyl amended media than Foxy 2. Highest number of micro-conidia was observed in PSM 197 cultured on metsulfuron methyl.

The pesticides imidacloprid + metalaxyl-M + tebuconazole (IMT), hexaconazole and metsulfuron methyl interacted significantly ($P \leq 0.05$) with the isolates (Table 5). The number of conidia of Foxy 2 germinated when cultured on media amended with metsulfuron methyl, hexaconazole and IMT was significantly ($P \leq 0.05$) higher than those germinated in PSM 197. The highest conidia germination was recorded with metsulfuron methyl in Foxy 2, although it was not significantly ($P \leq 0.05$) different with

Table 3: Interaction between isolate and pesticide on mycelia growth of *Fusarium oxysporum* f. sp. *strigae* in Zaria, Nigeria.

| Treatment | Mycelia growth inhibition (%) | |
|---|---|---|
| | Isolate | |
| Pesticide | PSM 197 | Foxy 2 |
| Benomyl | 100.00[a] | 100.00[a] |
| Carbendazim | 100.00[a] | 100.00[a] |
| Carbendazim + mancozeb | 100.00[a] | 100.00[a] |
| IMT | 85.77[b] | 85.24[b] |
| Mancozeb | 81.04[d] | 83.09[c] |
| Hexaconazole | 45.36[e] | 45.49[e] |
| Metsulfuron methyl | 20.58[f] | 18.73[g] |
| S. E. ± | 0.267 | 0.267 |

Means with same superscript in each column are not significantly different at ($P \leq 0.05$) using Students-Newman-Keuls (SNK) Test.
IMT = imidacloprid + metalaxyl-M + tebuconazole

Table 4: Interaction between isolate and pesticide on micro-conidia number of *Fusarium oxysporum* f. sp. *strigae* in Zaria, Nigeria.

| Treatment | Micro-conidia count ($x10^5$) | |
|---|---|---|
| | Isolate | |
| Pesticide | PSM 197 | Foxy 2 |
| Benomyl | 0.00[f] | 0.00[f] |
| Carbendazim | 0.00[f] | 0.00[f] |
| Carbendazim + mancozeb | 0.00[f] | 0.00[f] |
| IMT | 109.46[f] | 114.65[f] |
| Mancozeb | 318.31[e] | 293.44[c] |
| Hexaconazole | 3,165.23[c] | 2,878.07[d] |
| Metsulfuron methyl | 5,520.75[a] | 4,976.37[b] |
| S. E. ± | 55.238 | 55.238 |

Means with same superscript in each column are not significantly different at ($P \leq 0.05$) using Students-Newman-Keuls (SNK) Test.
IMT = imidacloprid + metalaxyl-M + tebuconazole

mancozeb. While lower conidia germination was obtained with IMT in PSM 197.

The interaction between isolate and rate on mycelia growth of *F. oxysporum* f. sp. *strigae* isolate is presented in (Table 6). Comparing the isolates across different rates, at 0.0x, 0.5x and 1.0x, mycelial growth inhibition did not differ significantly except at 1.5x and 2.0x. At 1.5x, mycelia inhibition in PSM 197 was significantly ($P \leq 0.05$) higher than Foxy 2. But when pesticide rate was increased to 2.0x, mycelial growth inhibition was observed higher in Foxy 2 than was obtained in PSM 197. Highest mycelial growth inhibition was recorded at 2.0x with Foxy 2.

Table 7 shows the interaction between isolate and rate on conidia germination of *F. oxysporum* f. sp. *strigae*. There was significant ($P \leq 0.05$) difference in conidia germination of the isolates when pesticide rates were varied. Higher conidia germination was recorded at 0.0x in the two isolates but when pesticide at 0.5x was used, conidia germination was decreased with every increased rate of pesticide. High number of conidia germinated was recorded in Foxy 2 at 0.0x while lower germination was observed in PSM 197 at 2.0x.

There was significant ($P \leq 0.05$) interaction between pesticide and rate on mycelia growth of *F. oxysporum* f. sp. *strigae* (Table 8). Mycelial growth inhibition at 0.0x for all the pesticides did not differ significantly ($P \leq 0.05$). The fungicides benomyl, carbendazim and carbendazim + mancozeb as the rates were increased from 0.5x to 2.0x, they were significantly ($P \leq 0.05$) similar in mycelia growth inhibition, although, they were higher

Table 5: Interaction between isolate and pesticide on conidia germination of *Fusarium oxysporum* f. sp. *strigae* in Zaria, Nigeria.

| Treatment | Conidia germination (%) | |
|---|---|---|
| | Isolate | |
| Pesticide | PSM 197 | Foxy 2 |
| Benomyl | 0.00[g] | 0.00[g] |
| Carbendazim | 0.00[g] | 0.00[g] |
| Carbendazim + mancozeb | 0.00[g] | 0.00[g] |
| IMT | 17.41[f] | 20.75[e] |
| Mancozeb | 39.82[a] | 40.14[a] |
| Hexaconazole | 23.72[d] | 27.02[c] |
| Metsulfuron methyl | 33.60[b] | 41.71[a] |
| S. E. ± | 0.962 | 0.962 |

Means with same superscript in each column are not significantly different at ($P \leq 0.05$) using Students-Newman-Keuls (SNK) Test.
IMT = imidacloprid + metalaxyl-M + tebuconazole

Table 6: Interaction between isolate and rate on mycelia growth of *Fusarium oxysporum* f. sp. *strigae* in Zaria, Nigeria.

| Isolate | Pesticide rate (g/L) | | | | |
|---|---|---|---|---|---|
| | 0.0x | 0.5x | 1.0x | 1.5x | 2.0x |
| Foxy 2 | 0.00g | 68.38f | 74.81e | 79.24d | 81.88a |
| PSM 197 | 0.00g | 68.53f | 75.06e | 79.85c | 80.99b |
| S. E. ± | 0.184 | 0.184 | 0.184 | 0.184 | 0.184 |

Means with same superscript in each column are not significantly different at ($P \leq 0.05$) using Students-Newman-Keuls (SNK) Test.

Table 7: Interaction between isolate and rate on conidia germination of *Fusarium oxysporum* f. sp. *strigae* in Zaria, Nigeria.

| Isolate | Pesticide rate (g/L) | | | | |
|---|---|---|---|---|---|
| | 0.0x | 0.5x | 1.0x | 1.5x | 2.0x |
| Foxy 2 | 68.47a | 38.42c | 33.64d | 26.45ef | 23.53g |
| PSM 197 | 63.98b | 35.20d | 29.11e | 23.66fg | 16.78h |
| S. E. ± | 0.983 | 0.983 | 0.983 | 0.983 | 0.983 |

Means with same superscript in each column are not significantly different at ($P \leq 0.05$) using Students-Newman-Keuls (SNK) Test.

than any other pesticide. When pesticide rates of IMT, mancozeb, hexaconazole and metsulfuron methyl were increased from 0.5x to 2.0x, it was observed that mycelial growth inhibition was also increased except for IMT and mancozeb. Mycelial growth did not differ significantly ($P \leq 0.05$) when rates of IMT and mancozeb were increased from 1.5x to 2.0x. Lower mycelial growth inhibition was recorded with metsulfuron methyl at 0.5x.

Interaction between pesticide and rate on number of micro-conidia of *F. oxysporum* f. sp. *strigae* is presented in (Table 9). There was no significant ($P \leq 0.05$) difference among the pesticides at 0.0x with respect to number of micro-conidia produced but was higher than all the pesticide rates tested. The number of micro-conidia produced on IMT at all rate did not differ significantly ($P \leq 0.05$) from each other but were similar to those produced on mancozeb at 1.0x. It was observed that, when rates of hexaconazole and metsulfuron methyl were increased from 0.5x to 2.0x, there was decreased number of micro-conidia produced.

Table 10 shows interaction between pesticide and rate on macro-conidia number of *F. oxysporum* f. sp. *strigae*. There was no significant ($P \leq 0.05$) difference observe at 0.0x though was higher than any pesticide rate used. Similarly, benomyl, carbendazim, carbendazim + mancozeb, IMT and mancozeb at all their rates did not differ significantly ($P \leq 0.05$) with each other in macro-conidia number. In every increase in rate of hexaconazole and metsulfuron methyl from 0.5x to 2.0x, there was decreased number of macro-conidia produced. However, macro-conidia produced on hexaconazole at 2.0x and

Table 8: Interaction between pesticide and rate on mycelia growth of *Fusarium oxysporum* f. sp. *strigae* in Zaria, Nigeria.

| Treatment | Mycelia inhibition (%) | | | | |
|---|---|---|---|---|---|
| | Pesticide rate (g/L) | | | | |
| Pesticide | 0.0x | 0.5x | 1.0x | 1.5x | 2.0x |
| Benomyl | $0.00^{o}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ |
| Carbendazim | $0.00^{o}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ |
| Carbendazim+mancozeb | $0.00^{o}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ |
| IMT | $0.00^{o}$ | $80.35^{d}$ | $85.61^{c}$ | $87.96^{b}$ | $88.11^{b}$ |
| Mancozeb | $0.00^{o}$ | $49.31^{g}$ | $78.94^{e}$ | $100.00^{a}$ | $100.00^{a}$ |
| hexaconazole | $0.00^{o}$ | $37.93^{j}$ | $42.39^{i}$ | $48.22^{h}$ | $53.16^{f}$ |
| metsulfuron methyl | $0.00^{o}$ | $11.60^{n}$ | $17.59^{m}$ | $20.63^{l}$ | $28.79^{k}$ |
| S. E. ± | 0.344 | 0.344 | 0.344 | 0.344 | 0.344 |

Means with same superscript in each column are not significantly different at ($P \leq 0.05$) using Students-Newman-Keuls (SNK) Test.
IMT = imidacloprid + metalaxyl-M + tebuconazole

Table 9: Interaction between pesticide and rate on micro-conidia number of *Fusarium oxysporum* f. sp. *strigae* in Zaria, Nigeria.

| Treatment | Micro-conidia count ($x10^{6}$) | | | | |
|---|---|---|---|---|---|
| | Pesticide rate (g/L) | | | | |
| Pesticide | 0.0x | 0.5x | 1.0x | 1.5x | 2.0x |
| Benomyl | $836.39^{a}$ | $0.00^{l}$ | $0.00^{l}$ | $0.00^{l}$ | $0.00^{l}$ |
| Carbendazim | $836.39^{a}$ | $0.00^{l}$ | $0.00^{l}$ | $0.00^{l}$ | $0.00^{l}$ |
| Carbendazim+mancozeb | $836.39^{a}$ | $0.00^{l}$ | $0.00^{l}$ | $0.00^{l}$ | $0.00^{l}$ |
| IMT | $836.39^{a}$ | $23.99^{jk}$ | $9.50^{kl}$ | $6.47^{kl}$ | $4.87^{kl}$ |
| Mancozeb | $836.39^{a}$ | $37.99^{j}$ | $23.19^{jk}$ | $0.00^{l}$ | $0.00^{l}$ |
| hexaconazole | $836.39^{a}$ | $433.52^{e}$ | $335.03^{g}$ | $235.71^{h}$ | $204.4^{i}$ |
| metsulfuron methyl | $836.39^{a}$ | $682.44^{b}$ | $544.90^{c}$ | $466.73^{d}$ | $405.36^{f}$ |
| S. E. ± | 7.393 | 7.393 | 7.393 | 7.393 | 7.393 |

Means with same superscript in each column are not significantly different at ($P \leq 0.05$) using Students-Newman-Keuls (SNK) Test.
IMT = imidacloprid + metalaxyl-M + tebuconazole

metsulfuron methyl at 0.5x rates are significantly ($P \leq 0.05$) similar.

Interaction between pesticide and rate on macro-conidial length of *F. oxysporum* f. sp. *strigae* is presented in (Table 11). Macro-conidial length among the pesticides at 0.0x was not significantly ($P \leq 0.05$) different from each other. When rate of imidacloprid + metalaxyl-M + tebuconazole (IMT), mancozeb, hexaconazole and metsulfuron methyl was increased from 0.5x to 2.0x, macro-conidial length was significant. Holding IMT constant, macro-conidial length was statistically similar when the rate was increased from 0.5x to 2.0x but were shorter than other pesticides. Hexaconazole at 0.5x recorded significantly ($P \leq 0.05$) longer macro-conidia than other pesticides. However, when the rate was increased at 1.0x to 2.0x there was no significant ($P \leq 0.05$) difference in macro-conidial length but was statistically similar with mancozeb at 1.0x. When metsulfuron methyl rate was increased from 0.5x to 2.0x, it was observed that macro-conidial length was reduced. At 1.0x to 2.0x of metsulfuron methyl, macro-conidial length was observed similar with IMT at 0.5x.

There was significant interaction between pesticide and rate on conidia germination of *F. oxysporum* f. sp. *strigae* (Table 12). Comparing pesticides across their rates, there was no significant ($P \leq 0.05$) difference in conidia germinated among all the pesticides at 0.0x. However, IMT, mancozeb, hexaconazole and metsulfuron methyl across all their rates differ significantly ($P \leq 0.05$) from each other. Number of conidia germinated when *F. oxysporum* f. sp. *strigae* was cultured on metsulfuron methyl at 0.5x was statistically similar with mancozeb at 0.5x. Similarly, conidia germinated on metsulfuron methyl at

Table 10: Interaction between pesticide and rate on macro-conidia number of *Fusarium oxysporum* f. sp. *strigae* in Zaria, Nigeria.

| Treatment | Macro-conidia count ($x10^6$) | | | | |
|---|---|---|---|---|---|
| | Pesticide rate (g/L) | | | | |
| Pesticide | 0.0x | 0.5x | 1.0x | 1.5x | 2.0x |
| Benomyl | $235.85^{a}$ | $0.00^{i}$ | $0.00^{i}$ | $0.00^{i}$ | $0.00^{i}$ |
| Carbendazim | $235.85^{a}$ | $0.00^{i}$ | $0.00^{i}$ | $0.00^{i}$ | $0.00^{i}$ |
| Carbendazim+mancozeb | $235.85^{a}$ | $0.00^{i}$ | $0.00^{i}$ | $0.00^{i}$ | $0.00^{i}$ |
| IMT | $235.85^{a}$ | $4.70^{i}$ | $4.11^{i}$ | $2.59^{i}$ | $1.81^{i}$ |
| Mancozeb | $235.85^{a}$ | $7.73^{i}$ | $4.94^{i}$ | $0.00^{i}$ | $0.00^{i}$ |
| hexaconazole | $235.85^{a}$ | $172.48^{b}$ | $145.47^{c}$ | $124.65^{d}$ | $106.64^{e}$ |
| metsulfuron methyl | $235.85^{a}$ | $108.22^{e}$ | $93.98^{f}$ | $83.61^{g}$ | $65.92^{h}$ |
| S. E. ± | 3.628 | 3.628 | 3.628 | 3.628 | 3.628 |

Means with same superscript in each column are not significantly different at ($P \leq 0.05$) using Students-Newman-Keuls (SNK) Test.
IMT = imidacloprid + metalaxyl-M + tebuconazole

Table 11: Interaction between pesticide and rate on macro-conidial length of *Fusarium oxysporum* f. sp. *strigae* in Zaria, Nigeria.

| Treatment | Macro-conidial length (µm) | | | | |
|---|---|---|---|---|---|
| | Pesticide rate (g/L) | | | | |
| Pesticide | 0.0x | 0.5x | 1.0x | 1.5x | 2.0x |
| Benomyl | $10.77^{d}$ | $0.00^{i}$ | $0.00^{i}$ | $0.00^{i}$ | $0.00^{i}$ |
| Carbendazim | $10.77^{d}$ | $0.00^{i}$ | $0.00^{i}$ | $0.00^{i}$ | $0.00^{i}$ |
| Carbendazim+mancozeb | $10.77^{d}$ | $0.00^{i}$ | $0.00^{i}$ | $0.00^{i}$ | $0.00^{i}$ |
| IMT | $10.77^{d}$ | $9.30^{fgh}$ | $9.01^{gh}$ | $8.93^{h}$ | $8.90^{h}$ |
| Mancozeb | $10.77^{d}$ | $12.46^{b}$ | $12.30^{bc}$ | $0.00^{i}$ | $0.00^{i}$ |
| hexaconazole | $10.77^{d}$ | $13.21^{a}$ | $11.84^{c}$ | $11.95^{c}$ | $11.94^{c}$ |
| metsulfuron methyl | $10.77^{d}$ | $9.94^{e}$ | $9.52^{ef}$ | $9.50^{ef}$ | $9.46^{fg}$ |
| S. E. ± | 0.163 | 0.163 | 0.163 | 0.163 | 0.163 |

Means with same superscript in each column are not significantly different at ($P \leq 0.05$) using Students-Newman-Keuls (SNK) Test.
IMT = imidacloprid + metalaxyl-M + tebuconazole

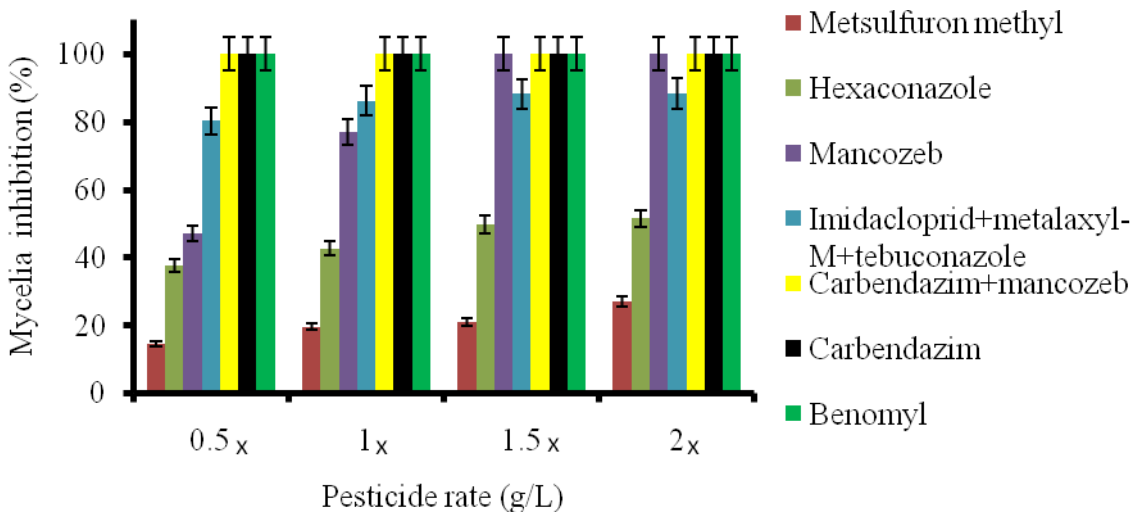
1.5x did not differ significantly ($P \leq 0.05$) from mancozeb at 1.0x and hexaconazole at 0.5x. In addition, hexaconazole at 1.0 and 1.5x was statistically similar with number of conidia germinated with IMT at 0.5x and 1.0x.

Interaction between isolate and pesticide and rate on mycelia growth of *F. oxysporum* f. sp. *strigae* is presented in (Figure 1 and 2). Varying the pesticides across their increasing rate, at 0.0x, there was no mycelia growth inhibition observed. When rate of benomyl, carbendazim and carbendazim + mancozeb was increased at 0.5x to 2.0x, they were significantly ($P \leq 0.05$) the same though mycelia growth of the isolates PSM 197 and Foxy 2 were completely inhibited. Mycelia growth was reduced significantly ($P \leq 0.05$) when IMT at 0.5x as increased to 1.0x, while further increase at 1.5x to 2.0x there was no significant ($P \leq 0.05$) difference in the two isolates. PSM 197 cultured on mancozeb amended media at 0.5x and 1.0x recorded lower reduction of mycelia growth than in Foxy 2 but at 1.5x and 2.0x both isolates were completely inhibited. Mycelia growth inhibition in PSM 197 and Foxy 2 cultured on hexaconazole amended media at 0.5x and 1.0x did not differ significantly ($P \leq 0.05$) from each other. Hexaconazole at 1.5x significantly ($P \leq 0.05$) recorded higher reduction of mycelia growth in PSM 197 than in Foxy 2. However, further increase in rate to 2.0x, showed that, mycelia growth in Foxy 2 was inhibited more than PSM 197. Mycelia growth in Foxy 2 on metsulfuron methyl was lower than PSM 197 except at 2.0x, PSM 197 was least inhibited.

Table 12: Interaction between pesticide and rate on conidia germination of *Fusarium oxysporum* f. sp. *strigae* in Zaria, Nigeria.

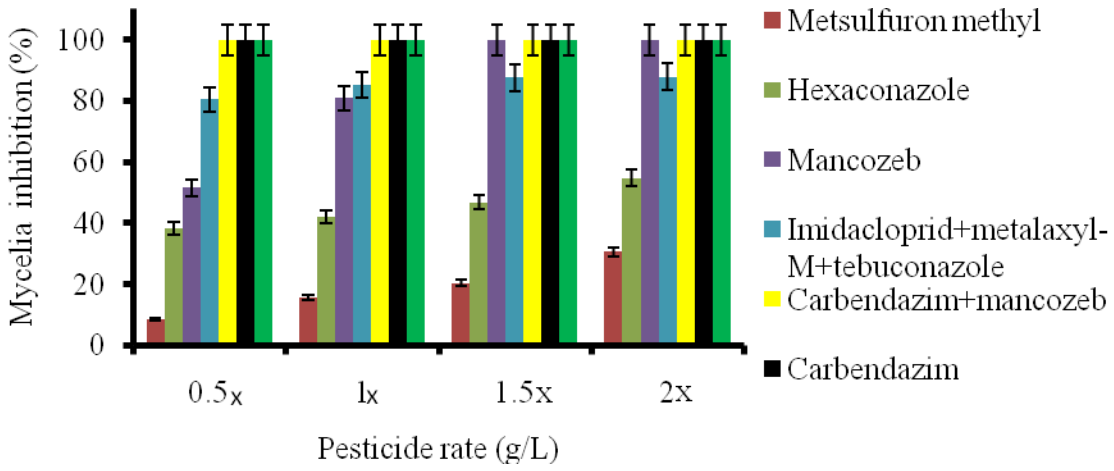
| Treatment | Conidia germination (%) | | | | |
|---|---|---|---|---|---|
| | Pesticide rate (g/L) | | | | |
| Pesticide | 0.0x | 0.5x | 1.0x | 1.5x | 2.0x |
| Benomyl | 66.22$^{a}$ | 0.00$^{j}$ | 0.00$^{j}$ | 0.00$^{j}$ | 0.00$^{j}$ |
| Carbendazim | 66.22$^{a}$ | 0.00$^{j}$ | 0.00$^{j}$ | 0.00$^{j}$ | 0.00$^{j}$ |
| Carbendazim+mancozeb | 66.22$^{a}$ | 0.00$^{j}$ | 0.00$^{j}$ | 0.00$^{j}$ | 0.00$^{j}$ |
| IMT | 66.22$^{a}$ | 24.23$^{ef}$ | 22.08$^{fg}$ | 17.11$^{h}$ | 12.90$^{i}$ |
| Mancozeb | 66.22$^{a}$ | 44.29$^{bc}$ | 35.67$^{d}$ | 0.00$^{j}$ | 0.00$^{j}$ |
| hexaconazole | 66.22$^{a}$ | 32.40$^{d}$ | 25.66$^{ef}$ | 23.91$^{f}$ | 19.50$^{gh}$ |
| metsulfuron methyl | 66.22$^{a}$ | 46.32$^{b}$ | 42.09$^{c}$ | 34.15$^{d}$ | 28.07$^{e}$ |
| S. E. ± | 1.391 | 1.391 | 1.391 | 1.391 | 1.391 |

Means with same superscript in each column are not significantly different at ($P \leq 0.05$) using Students-Newman-Keuls (SNK) Test.
IMT = imidacloprid + metalaxyl-M + tebuconazole




X = manufacturer's recommended rate for the different pesticides

Figure 1. Mycelia inhibition of PSM 197 by different pesticides at varying rates




X = manufacturer's recommended rate for the different pesticides

Figure 2. Mycelia inhibition of Foxy 2 by different pesticides at varying rates

### 4.3 Compatibility variation of Pesticides with *Fusarium oxysporum* f.sp. *strigae* Isolates.

The compatibility variation of pesticides with isolates PSM 197 and Foxy 2 is presented in (Table 13). The most important parameters for determining compatibility are mycelia growth, sporulation and conidia germination. Compatibility variation of imidacloprid + metalaxyl-M + tebuconazole (IMT) with PSM 197 mycelia growth was higher than that of mancozeb and hexaconazole, while the least 20.58 % was with metsulfuron methyl. Higher compatibility percent variation was observed with IMT and mycelia growth of Foxy 2. This was followed by mancozeb, hexaconazole and metsulfuron methyl respectively. Micro-conidia count of PSM 197 varied with IMT showed higher compatibility variation compared to other pesticides tested. In Foxy 2, compatibility variation of IMT with micro-conidia count was 98.62 %, while least variation was observed with metsulfuron methyl. Lower (38.62 %) variation was recorded with hexaconazole and macro-conidia count of PSM 197. It was followed by metsulfuron methyl, mancozeb and IMT in that order. Foxy 2 macro-conidia count variation with IMT was higher compared to mancozeb and metsulfuron methyl while the least was with hexaconazole. Compatibility variation of conidia germination of PSM 197 with mancozeb was lower (37.76 %), followed by metsulfuron methyl, hexaconazole and IMT respectively. In Foxy 2, the least (39.08 %) variation of conidia germination was with metsulfuron methyl while the highest (69.70 %) was with imidacloprid + metalaxyl-M + tebuconazole.

Table 13. Compatibility variation of Pesticides with *Fusarium oxysporum* f.sp. *strigae* isolates.

| Pesticide | Compatibility variation (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Mycelia growth | | Micro-conidia count | | Macro-conidia count | | Conidia germination | |
| | PSM 197 | Foxy 2 | PSM 197 | Foxy 2 | PSM 197 | Foxy 2 | PSM 197 | Foxy 2 |
| IMT | 85.77 | 85.24 | 98.70 | 98.62 | 98.67 | 98.53 | 72.79 | 69.70 |
| Mancozeb | 62.07 | 66.17 | 96.21 | 96.48 | 97.26 | 97.36 | 37.76 | 41.38 |
| Hexaconazole | 45.36 | 45.49 | 62.30 | 65.46 | 38.62 | 44.96 | 62.93 | 60.54 |
| Metsulfuron methyl | 20.58 | 18.73 | 34.24 | 40.28 | 64.39 | 61.03 | 47.48 | 39.08 |

IMT = imidacloprid + metalaxyl-M + tebuconazole

(NB: Mycelia was completely inhibited hence, no conidia was produced on benomyl, carbendazim and carbendazim + mancozeb amended media)

# CHAPTER FIVE

## 5.0 DISCUSSION

The result of this study has shown that, *Fusarium oxysporum* f. sp. *strigae* isolates (PSM 197 and Foxy 2) did not differ significantly from each other in their response to the different pesticides in term of mycelia growth inhibition and conidia sizes. This could probably be because they are of the same genera and species. PSM 197 was isolated from diseased *S. hermonthica* in northern Nigeria (Marley *et al.*, 1999) and Foxy 2 in northern Ghana (Abbasher *et al.*, 1999). Mycelial growths on PDAs were cotton-like, well branched, initially white but later became purple. Conidiophores were slender and simple; micro-conidia were hyaline, one-celled and ovoid; macro-conidia were hyaline, multi - celled and slightly curved. This is similar to the description of *Fusarium oxysporum* by Booth (1971), Leslie and Summerell (2006) and Barnett and Hunter (2006).

The effects of seven pesticides, benomyl, carbendazim, carbendazim + mancozeb, mancozeb, imidacloprid + metalaxyl-M + tebuconazole, hexaconazole and metsulfuron methyl on PSM 197 and Foxy 2 isolates of *Fusarium oxysporum* f. sp. *strigae* was studied *in vitro*. The result showed that benomyl, carbendazim and carbendazim + mancozeb completely inhibited mycelia growth, sporulation, conidia sizes and conidia germination of *Fusarium oxysporum* f. sp. *strigae* isolates PSM 197 and Foxy 2. Yang *et al*. (2011) and Koo *et al*. (2009) showed that, benomyl and carbendazim specifically binds to protein subunits called tubulin inhibiting their assembly to form microtubules which functions in various cellular processes, including mitosis, intracellular transport of molecules and maintenance of cell shape. The observed effect of benomyl on both PSM 197 and Foxy 2 isolates of the

biocontrol agents against *S. hermonthica* could be due to inhibition of cell division and transport of molecules in the two isolates as reported by Yang *et al.* (2011) and Koo *et al.* (2009). Similar inhibitory effect of fungicides on other *Fusarium* spp. both *in vitro* and *in vivo* have been reported. Sultana and Ghaffar (2010) reported inhibition of *Fusarium solani* by benomyl and carbendazim *in vitro*. Similarly, Chavan *et al*. (2009) reported that 0.2 % and 0.3 % carbendazim and carbendazim + mancozeb resulted in 100 % inhibition of *F. solani* mycelia.

In this study, inhibitory effect of pesticides on mycelia growth, sporulation, conidia size and conidia germination increased significantly with increase in pesticide from 0.5x to 2.0x rates. Mamza *et al.* (2010) reported mycelial growth inhibition of *Fusarium pallidoroseum* with benomyl used at the rate of 2.25, 1.5 and 0.75 g/L. The effect of pesticides observed in this study due to increased pesticide rate could be due to increase in toxicity of the pesticides chemicals.

This study showed that, mancozeb, imidacloprid + metalaxyl-M + tebuconazole (IMT), hexaconazole and metsulfuron methyl at all rates tested supported sporulation and conidia germination of the two *Fusarium oxysporum* f. sp. *strigae* isolates (PSM 197 and Foxy 2). Growth inhibition increased proportionally as rate of mancozeb, IMT, hexaconazole and metsulfuron methyl was increased from 0.5x to 2.0x indicating a positive dosage response curve relationship. With mancozeb alone, however, mycelia growth and sporulation recorded at 1.5x and 2.0x (18.0 and 24.0 g/L) was 100 %. In a report by Cycon *et al.* (2010), mancozeb was found to inhibit energy production within *Fusarium oxysporum* and inactivated the – SH groups in amino acids, proteins and enzymes. Similarly, the effect of mancozeb observed in this

study could be attributed to the depletion of energy production and protein utilization in *Fusarium oxysporum* f. sp. *strigae* isolates. Result obtained showed that, macro-conidial length on mancozeb were longer than those obtained on control. This was similar to Mamza *et al.* (2010) who reported that, mycelia growth on mancozeb treated PDA had longer and wider conidia of *Fusarium pallidoroseum* than the control. Singh and Singh (2006) reported that mancozeb at 2500, 2000, 1000, 500 and 250 ppm against *Alternaria alternata* causing blight of tomato did not completely inhibit mycelial growth of the fungus. Similarly, Mamza *et al*. (2012), reported that the use of mancozeb at 3.13, 6.25 and 9.38 g/L against *Fusarium pallidoroseum* partially inhibited sporulation of the fungus. It was also reported that, mancozeb used in the control of *Physoderma maydis* (Brown spot) on maize significantly inhibited mycelia growth and there was complete inhibition of sporangia germination at 10,000 ppm a.i. (Osunlaja and Alamutu, 1999).

In this study, imidacloprid + metalaxyl-M + tebuconazole at all rates had high inhibitory effect on mycelia growth, sporulation, conidia length and width, and conidia germination of the two isolates PSM 197 and Foxy 2. Buchenauer (1990) reported that, metalaxyl-M inhibited nucleic acids synthesis on fungal growth. The poor mycelia growth and conidia produced could be attributed to the effect of metalaxyl-M inhibiting nucleic acids synthesis in both PSM 197 and Foxy 2 isolates. Mamza *et al*. (2012) reported that thiomethoxan + metalaxyl–M + difenoconazole gave high inhibitory effect on sporulation of *Fusarium pallidoroseum* at 0.06 g/ml. It was reported that tebuconazole inhibit demethylase enzyme involved in sterol biosynthesis which is essential component of fungal cell membranes, hence their biosynthesis inhibition could hinder fungal growth (Singh *et al*., 2009). However, in

tebuconazole, a component in Dress force (imidacloprid + metalaxyl-M + tebuconazole), the observed effect may possibly be due to synergistic effect or be attributed to inhibition of sterol biosynthesis in PSM 197 and Foxy 2 isolates. In their study, Singh *et al*. (2009) also reported the use of tebuconazole at recommended rates supported mycelia growth of *Paenibacillus* sp. *in vitro*. The results showed that, hexaconazole at 0.5x (0.25 ml/L) significantly produced high mycelia growth and sporulation in the two isolates PSM 197 and Foxy 2 of *Fusarium oxysporum* f. sp. *strigae*. Sarkar *et al.* (2010) reported that, hexaconazole at 5 ppm least inhibited *Trichoderma harzianum* but induced inhibition when fungicide rate was increased to 10 ppm.

In this study, metsulfuron methyl, a herbicide against *S. hermonthica* supported highest mycelia growth, sporulation, conidia germination of both isolates at 0.5x (1.15 g/L) and at 1.0x (2.30 g/L). Milhomme and Bastide (1990) reported that, Metsulfuron methyl inhibited cellular division in meristerms in higher plants and hence, stopped growth. It also blocked normal function of the enzyme acetolactate also known as acetohydroxy acid in plants. *Fusarium oxysporum* f. sp. *strigae* isolate PSM 197 and Foxy 2 are heterotrophic organisms which have the ability of growth and sporulation better on metsulfuron methyl. Yu *et al*. (2005) reported fungal degradation of metsulfuron methyl in liquid culture, 79 % of metsulfuron methyl at rates of 0.1, 1 and 10 mg/L was degraded by *Curvularia* sp. after 7 days of incubation. Saha and Rao (2010) demonstrated the efficacy of metsulfuron methyl at 8 g/ha applied 10 days after transplanting, controlled pre-dominant weeds effectively (87.9 %) resulting to increase of 72 % yield over uncheck.

From the calculations made for compatibility of pesticide with the isolates PSM 197 and Foxy 2, variations in percentage of mycelial growth, sporulation and conidia germination were used to determine the level of compatibility. Imidacloprid + metalaxyl-M + tebuconazole had highest percent variations for all the parameters. This means that Imidacloprid + metalaxyl-M + tebuconazole was not compatible with the isolates. In a situation where Imidacloprid + metalaxyl-M + tebuconazole, a seed dressing chemical is used together with PSM 197 or Foxy 2, development of the isolates will be affected. Similarly, subsequent inoculum will also be affected hence the aim of using the isolates as a biocontrol agent against *Striga hermonthica* will not be achieved. Metsulfuron methyl had lower percent variation in term of mycelial growth, sporulation and conidia germination. This implies that, metsulfuron methyl a seed dressing and foliar spray herbicide for Striga control is most compatible with the isolates. In addition, metsulfuron methyl and PSM 197 or Foxy 2 can be used together as integrated management strategy against *Striga hermonthica*. The fungicides mancozeb and hexaconazole had average percent variations. These fungicides are moderately compatible with the isolates. In determining compatibility of pesticide with biocontrol agent, safety of the biocontrol agent will be guaranteed.

# CHAPTER SIX

## 6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

### 6.1 Summary

The effect of benomyl, carbendazim, hexaconazole, mancozeb, carbendazim + mancozeb, imidacloprid + metalaxyl-M + tebuconazole and metsulfuron methyl on PSM 197 and Foxy 2, isolates of *Fusarium oxysporum* f. sp. *strigae* was studied *in vitro*. Mycelial diameter and number of conidia/ml were suppressed in media amended with benomyl, carbendazim, and carbendazim + mancozeb. Mycelia diameter and number of conidia/ml of PSM 197 and Foxy 2 on media amended with mancozeb decreased as rate increased from 0.5x to 2.0x (6 to 24 g/L). Fourteen days after incubation, mycelia diameter of both PSM 197 and Foxy 2 was not affected in media amended with highest rates of hexaconazole (1 ml/L), metsulfuron methyl at 2.0x (4.6 g/L) and imidacloprid + metalaxyl-M + tebuconazole at 2.0x (8 g/L).

Imidacloprid + metalaxyl-M + tebuconazole had higher variation, was not compatible with PSM 197 and Foxy 2 in term of mycelia growth, sporulation and conidia germination. Metsulfuron methyl had lower percent variation in term of mycelial growth, micro-conidia count and conidia germination of the two isolates PSM 197 and Foxy 2. Hence, Metsulfuron methyl was most compatible with the isolates. The fungicides mancozeb and hexaconazole had average percent variations with respect to mycelia growth, sporulation and conidia germination. These fungicides are moderately compatible with the isolates.

## 6.2 Conclusion

Metsulfuron methyl was most compatible with the two isolates of *Fusarium oxysporum* f. sp. *strigae* using mycelia growth, micro-conidia and conidia germination. Hexaconazole was better on macro-conidia number. Mancozeb was only better in conidia viability. Imidacloprid + metalaxyl-M + tebuconazole did not out perform metsulfuron methyl, mancozeb nor hexaconazole in all parameters measured. The study indicates that metsulfuron methyl should be used with PSM 197 and Foxy 2 for the management of *S. hermonthica*.

## 6.3 Recommendation

Based on the findings of this study, the fungicides benomyl at 0.5x (1.25 g/L), carbendazim at 0.5x (3.75 g/L) and carbendazim + mancozeb at 0.5x (3.13 g/L) did not allow any form of mycelia growth and sporulation of *Fusarium oxysporum* f. sp. *strigae* isolates *in vitro*. Therefore, these fungicides should not be used in situations where either PSM 197 or Foxy 2 is part of *S. hermonthica* management options.

Metsulfuron methyl at 1.0x (2.3 g/L) and *Fusarium oxysporum* f. sp. *strigae* isolates may be combined and be formulated as seed dressing technology against *S. hermonthica.* In addition, the different formulations can be applied together at the same time.

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## APPENDICES

### Appendix 1. ANOVA Tables

A: ANOVA Table for mycelia growth inhibition of *Fusarium oxysporum* f. sp. *strigae* isolates

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Isolate | 1 | 1.8457 | 1.8457 | 1 | 0.3173 |
| Pesticide | 6 | 148777 | 24796.1 | 13500.3 | <.0001 |
| Rate | 4 | 267080 | 66769.9 | 36353.1 | <.0001 |
| Isolate*Pesticide | 6 | 103.836 | 17.3059 | 9.42 | <.0001 |
| Isolate*Rate | 4 | 22.4997 | 5.6249 | 3.06 | 0.0176 |
| Pesticide*Rate | 24 | 47015.6 | 1958.99 | 1066.58 | <.0001 |
| Isolate*Pesticide*Rate | 24 | 227.602 | 9.4834 | 5.16 | <.0001 |
| Error | 210 | 385.708 | 1.8367 | | |
| Total | 279 | 463613 | | | |

B: ANOVA Table for micro-conidia count of *Fusarium oxysporum* f. sp. *strigae* isolates

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Isolate | 1 | $1.80 \times 10^{15}$ | $1.80 \times 10^{15}$ | 10.17 | 0.0016 |
| Pesticide | 6 | $7.69 \times 10^{18}$ | $1.28 \times 10^{18}$ | 7262.42 | <.0001 |
| Rate | 4 | $2.25 \times 10^{19}$ | $5.61 \times 10^{18}$ | 31810.5 | <.0001 |
| Isolate*Pesticide | 6 | $4.81 \times 10^{15}$ | $8.02 \times 10^{14}$ | 4.54 | 0.0002 |
| Isolate*Rate | 4 | $1.29 \times 10^{15}$ | $3.24 \times 10^{14}$ | 1.83 | 0.1236 |
| Pesticide*Rate | 24 | $2.29 \times 10^{18}$ | $9.56 \times 10^{16}$ | 541.64 | <.0001 |
| Isolate*Pesticide*Rate | 24 | $5.98 \times 10^{15}$ | $2.49 \times 10^{14}$ | 1.41 | 0.1034 |
| Error | 210 | $3.71 \times 10^{16}$ | $1.77 \times 10^{14}$ | | |
| Total | 279 | $3.25 \times 10^{19}$ | | | |

C: ANOVA Table for macro-conidia count of *Fusarium oxysporum* f. sp. *strigae* isolates

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Isolate | 1 | $6.09 \times 10^{11}$ | $6.091 \times 10^{11}$ | 0.34 | 0.5593 |
| Pesticide | 6 | $5.14 \times 10^{15}$ | $8.57 \times 10^{14}$ | 481.14 | <.0001 |
| Rate | 4 | $1.98 \times 10^{16}$ | $4.96 \times 10^{15}$ | 2783.04 | <.0001 |
| Isolate*Pesticide | 6 | $7.79 \times 10^{11}$ | $1.298 \times 10^{11}$ | 0.07 | 0.9985 |
| Isolate*Rate | 4 | $6 \times 10^{10}$ | $1.5 \times 10^{10}$ | 0.01 | 0.9999 |
| Pesticide*Rate | 24 | $1.46 \times 10^{15}$ | $6.09 \times 10^{13}$ | 34.22 | <.0001 |
| Isolate*Pesticide*Rate | 24 | $7.27 \times 10^{11}$ | $3.031 \times 10^{10}$ | 0.02 | 1 |
| Error | 210 | $3.74 \times 10^{14}$ | $1.78 \times 10^{12}$ | | |
| Total | 279 | $2.68 \times 10^{16}$ | | | |

D: ANOVA Table for micro-conidia length of *Fusarium oxysporum* f. sp. *strigae* isolates

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Isolate | 1 | 0.08964 | 0.08964 | 2.25 | 0.1348 |
| Pesticide | 6 | 724.927 | 120.821 | 3037.72 | <.0001 |
| Rate | 4 | 286.602 | 71.6506 | 1801.46 | <.0001 |
| Isolate*Pesticide | 6 | 0.11775 | 0.01962 | 0.49 | 0.8129 |
| Isolate*Rate | 4 | 0.12048 | 0.03012 | 0.76 | 0.5542 |
| Pesticide*Rate | 24 | 318.48 | 13.27 | 333.64 | <.0001 |
| Isolate*Pesticide*Rate | 24 | 0.23072 | 0.00961 | 0.24 | 0.9999 |
| Error | 210 | 8.35248 | 0.03977 | | |
| Total | 279 | 1338.92 | | | |

E: ANOVA Table for micro-conidia width of *Fusarium oxysporum* f. sp. *strigae* isolates

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Isolate | 1 | 0.00079 | 0.00079 | 0.05 | 0.8237 |
| Pesticide | 6 | 239.031 | 39.8384 | 2512.93 | <.0001 |
| Rate | 4 | 86.8595 | 21.7149 | 1369.73 | <.0001 |
| Isolate*Pesticide | 6 | 0.03914 | 0.00652 | 0.41 | 0.8709 |
| Isolate*Rate | 4 | 0.05922 | 0.01481 | 0.93 | 0.4452 |
| Pesticide*Rate | 24 | 103.395 | 4.30813 | 271.75 | <.0001 |
| Isolate*Pesticide*Rate | 24 | 0.08514 | 0.00355 | 0.22 | 1 |
| Error | 210 | 3.32921 | 0.01585 | | |
| Total | 279 | 432.799 | | | |

F: ANOVA Table for macro-conidia length of *Fusarium oxysporum* f. sp. *strigae* solates

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Isolate | 1 | 1.58251 | 1.58251 | 5.22 | 0.0233 |
| Pesticide | 6 | 4231.72 | 705.287 | 2328.53 | <.0001 |
| Rate | 4 | 1613.76 | 403.44 | 1331.97 | <.0001 |
| Isolate*Pesticide | 6 | 2.18335 | 0.36389 | 1.2 | 0.3068 |
| Isolate*Rate | 4 | 1.14983 | 0.28746 | 0.95 | 0.4366 |
| Pesticide*Rate | 24 | 2108.41 | 87.8506 | 290.04 | <.0001 |
| Isolate*Pesticide*Rate | 24 | 5.25009 | 0.21875 | 0.72 | 0.826 |
| Error | 210 | 63.6067 | 0.30289 | | |
| Total | 279 | 8027.67 | | | |

G: ANOVA Table for macro-conidia width of *Fusarium oxysporum* f. sp. *strigae* isolates

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Isolate | 1 | 0.00836 | 0.00836 | 0.43 | 0.5146 |
| Pesticide | 6 | 249.291 | 41.5486 | 2117.75 | <.0001 |
| Rate | 4 | 89.0901 | 22.2725 | 1135.24 | <.0001 |
| Isolate*Pesticide | 6 | 0.07421 | 0.01237 | 0.63 | 0.7058 |
| Isolate*Rate | 4 | 0.06138 | 0.01535 | 0.78 | 0.5379 |
| Pesticide*Rate | 24 | 112.713 | 4.69639 | 239.38 | <.0001 |
| Isolate*Pesticide*Rate | 24 | 0.23106 | 0.00963 | 0.49 | 0.9793 |
| Error | 210 | 4.12003 | 0.01962 | | |
| Total | 279 | 455.59 | | | |

H: ANOVA Table for conidia germination of *Fusarium oxysporum* f. sp. *strigae* isolates

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Isolate | 1 | 708.328 | 708.328 | 50.95 | <.0001 |
| Pesticide | 6 | 33356.4 | 5559.41 | 399.87 | <.0001 |
| Rate | 4 | 129811 | 32452.7 | 2334.22 | <.0001 |
| Isolate*Pesticide | 6 | 398.988 | 66.498 | 4.78 | 0.0001 |
| Isolate*Rate | 4 | 277.854 | 69.4635 | 5 | 0.0007 |
| Pesticide*Rate | 24 | 18570.9 | 773.786 | 55.66 | <.0001 |
| Isolate*Pesticide*Rate | 24 | 215.459 | 8.9774 | 0.65 | 0.8978 |
| Error | 210 | 2919.63 | 13.903 | | |
| Total | 279 | 186258 | | | |