# The Use of Micro-Biological Agents at Different Pairing Times in the Control of *Fusarium verticillioides* Pathogen of Maize (*Zea mays*)

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**Abstract:** *Fusarium verticillioides* is a widely distributed mitosporic pathogen of maize, able to cause corn seedling blight, root rot, stalk rot and kernel or ear rot. Synthetic fungicides and some crop management practices are also not advisable in the control of this pathogen because chemical fungicide result in environmental pollution or hazards. Antagonistic micro-biological agents (bioagents) can be recommended to farmers because it is cheaper and environmental friendly. This aim of this study was to assess the efficiency of antagonistic micro organisms in the control of *Fusarium verticillioides* of maize. The efficacy of micro-biological agents: *Trichoderma viride, T. pseudokoningii, T. harzianum* and *Bacillus subtilis* were assessed *in vitro*. Laid in the laboratory in a Completely Randomized Design (CRD) and subjected to analysis of variance using SAS, 2001. The four antagonistic bioagents showed different inhibitory effect in the control of *F.verticillioides*. *T. viride* and *T. pseudokoningii* were the most effective antagonists; they caused significant inhibitory effect on the growth of *F.verticillioides* by 0.75cm and 0. 72cm compared to the control which was 2.57cm respectively at 120 hours of incubation. *T. harzianum* and *B. subtilis* had the least inhibitory effect against the pathogen. There was a significant inhibition in the growth of *F. verticillioides* at < 0.05 when paired with all the micro-biological agents used. The introduction of the antagonist before the pathogen *in vitro* was observed to be the best followed by the simultaneous pairing, and the least inhibition was when the introduction of the antagonist 24 hours after the pathogen. It was observed that all the antagonists tested had good inhibitory potentials on the pathogen, *F. verticillioides*.

**Keywords:** Maize, *Trichoderma spp*, Bioagents, *Bacillus spp*

## 1. Introduction

Maize is highly susceptible to both pests and parasites that cause pre- and post harvest losses which lead to great economic losses in Nigeria [23]. *Fusarium* ear rot of maize is among the destructive diseases in many areas of the country and most maize growing areas in the world. A report gave range of 5 – 40% infected fields in the tropics [3]. Ear rot reduces crop yield directly by destroying the grains, and the mycotoxin produced due to this disease causes food contamination which is poisonous to human and livestock. Maize is most commonly colonized crop by *Fusarium*

*verticillioides* (Sacc.) Nirenberg (synonym *Fusarium moniliforme* Sheldon) [14].

This fungus is common in African countries and Nigeria is not an exception [14]. The pathogen causes ear and stalk rot of maize. Stalk rot causes stalk breakage and lodging thereby making harvesting difficult and consequently reducing grain yield, while ear rot reduces plant yield directly by destroying the grain quality. It was reported that *F. verticillioides* colonizing maize tissues are resident in roots, stalks, ear and most other plant parts [15]. Symptomless (latent) infection can exist throughout the plant, and seed-transmitted strains of the fungus can develop systemically to infect the kernels [19].

Diseases of maize associated with *F. verticillioides* include seed rot, root rot, stalk rot, kernel or ear rot, and seedling blight [5, 6]. It is an important contributor to maize seedling disease, and for the induction of leaf lesions indicative of foliar maize diseases [26]. Fumonisin is a mycotoxin produced by this pathogen in the grains.

The fungus can exist as a saprobe or an endophyte. The grains from the symptomless infected plants enter the food chain of animal and man [5] with the potential to produce a mycotoxin that adversely affects the consumers [14, 16]. The mycotoxin occurs anywhere this fungus proliferates, causing chemical pollution of biological origin and setting [4, 17, and 18]. [8] Reported that *Fusarium* infection is generally a problem in grain crops. The high incidence of *Fusarium* species can be attributed to favorable weather conditions (during the rainy season when relative humidity is high) [10], for the fungus to develop and this occurs during grain filling, at harvest, and not in the store. In Nigeria, the public is generally unaware of the hazard posed by this fungus on any infected grains either symptomatic or asymptomatic [4]. There is little information at farmers' level on how to reduce or eradicate its presence in maize either at pre- and /or post-harvest.

The necessity of quality maize for cultivation and storage for later use, has led to various efforts aimed at controlling this disease of maize, which will subsequently reduce the incidence of mycotoxin accumulation in maize seeds thereby acting as a way to minimize the hazard due to mycotoxin contamination. The approaches to the control of pathogens of maize have taken various forms such as the use of chemicals, cultural control strategies and integrated methods [16]. These control measures sometimes have various setbacks even at the farmer's level of operation in Nigeria. The use of chemicals has long-term effects on the health of the populace, the natural environment, sometimes; pathogens build up of resistance to it. The World Health Organization linked several deaths of people and those that suffer acute health conditions to pesticide poisoning [11].

This has raised the interest in developing alternative methods of control from nature. Nature has a lot of plants and microorganisms for use in natural crop protection for a cleaner and safer environment. This is the use of micro-biological agents (bioagents) control methods. The development of environmentally friendly strategies for disease management requires an understanding of suitable antagonistic micro-organisms for better management of the disease, reduce contamination thereby producing cleaner, safer maize seeds, enhance higher yields and invariably increase the farmers' income. The aim of this study was to evaluate the antagonistic ability of four (4) micro-biological agents on *Fusarium* ear rot pathogen of maize.

## 2. Materials and Methods

The antagonistic microorganisms (*Trichoderma harzianum, T. pseudokoningii, Pseudomonas fluorescens* and *Bacillus subtilis*) used in this study were isolated from soil in maize growing areas and kept in the stock culture. The pathogen and bioagents were cultured on potato dextrose agar (PDA) and nutrient agar (NA) as needed depending on the type of organism to grow. For the preparation of PDA, 39 g of PDA powder was suspended in 1 L of distilled water. These were prepared routinely. Similarly, 28 g Nutrient agar (NA) powder was suspended in 1litre of distilled water. The solutions were dispensed into 250 ml conical flasks. Shortly before it was poured into the Petri plates, 1.25 g of streptomycin powder was released in 1 litre of molten PDA to make an antibacterial medium for non-bacterial growth. The cooling agar was dispensed in into sterile glass Petri dishes.

Maize plants showing symptoms of ear rot from the field and thoroughly washed the diseased parts with running tap water, small pieces (3 mm) of the infected parts were cut at the boundary of the healthy and infected tissues. These were later surface sterilized for 1 min in 10% NaOCl solution (with 5.25% chlorine content), rinsed in 5 changes of sterilized distilled water and dried with sterile paper towel. These were then placed on solidified PDA in Petri dishes and incubated at room temperature (28 + 2) °C for 7 days. Observation was made daily for mycelial growth. Pure cultures were obtained by subculturing into fresh plates. Stock cultures were maintained on agar slants in McCartney bottles and stored at 4°C in the refrigerator.

Test biocontrol organisms (*Trichoderma harzianum, T. pseudokoningii, Pseudomonas fluorescens* and *Bacillus subtilis*) used in this study (i.e. the antagonists) were from soil collected in maize growing areas (rhizosphere). A flame-sterilized 5 mm diameter cork borer was used to cut mycelia discs randomly from 7 day old pure cultures of fungi. The mycelial discs were aseptically transferred to the centre of cooled PDA contained in Petri dishes with the aid of a flame-sterilized mounted inoculating needle, the bottom of the Petri dishes were marked with 2 perpendicular lines passing through the centre. The plates were incubated at (28 + 2) °C for 7 days, and the radial growth was measured along the perpendicular lines and the mean calculated for each isolate.

The pathogen and antagonists were placed at two peripheral locations in opposite direction, about 1cm from the plate wall to prevent contamination and were done in this order:

- The pathogen was introduced 24 hours before the antagonist
- The pathogen was introduced simultaneously with the antagonist
- The pathogen was introduced 24 hours after the antagonist

The dixenic cultures of 3 replicates were incubated at 28-30°C for one week and observations on the growth of the organisms were taken from 48 hours of inoculation and at 24 hours interval. The interaction of antagonists and the pathogen was observed and recorded. The experiment was laid out in a Completely Randomized Design (CRD) and statistical analysis was done using SAS to test for significant differences in treatment means of each of the parameters.

# 3. Results and Discussion

On the third day after inoculation, mycelia growth of *F. verticillioides* was 3.1 cm in diameter and by the 7th day the whole plate was covered. Mycelia were fluffy and pinkish-white viewed from the top of the plate whilst the underside of the plate was brown in color. Repeated sub-culturing did not affect its cultural characteristics.

## 3.1. Effect of Different Pairing Times of the Antagonists with the Pathogen

*a. Pairing of the antagonists 24 hours before the pathogen*

When *T. pseudokoningii* was inoculated 24 hours earlier than *F. verticillioides,* it grew very fast covering the entire 90 mm Petri plate 5 days after pairing. The pathogen, *F. verticillioides* grew at an average of 0.7 cm (7mm) diameter at the early stage of incubation. The pathogen's mycelia were overgrown completely by the antagonist by the 7th day. The pathogen had mycelial extension of 3.00 cm at the end of the

observation period (Table 1a &b). The antagonist, *T. harzianum* grew luxuriously and restricted the growth of the pathogen to its point of inoculation with mycelial growth mean of 1.3 cm. By the 5th day, the antagonist had suppressed the growth of the pathogen. The pathogen's growth on the 7th day of incubation was 2.2 cm (Table 1). When *P. fluorescens* was inoculated earlier than *F. verticillioides,* the bacterium grew very slowly covering 1.1 cm of the Petri plate seven days after pairing. The pathogen *F. verticillioides* grew to 2.8 cm diameter on the Petri plates. The pathogen's mycelia were pinkish to light purple color towards the antagonist by the 7th day. There were zones of inhibition by the antagonist on the pathogen. The inhibition zone was 5.2 cm (Table 1a & b). When *B. subtilis* was introduced 24 hours before the pathogen, *F. verticillioides,* the bacterium grew fast covering 1.30 cm within seven days of pairing. The pathogen *F. verticillioides* grew to 3.40 cm diameter in the Petri plates. The pathogen's mycelia were pinkish white, not as fluffy as those of the control at the 7th day (Table 1a & b).

***Table 1.** Pairing of each antagonist 24 hours before the pathogen.*

1St Trial(a) Time of incubation (hrs) of F. verticillioides*

| Antagonists | 48 | 72 | 96 | 120 | 144 | 168 |
|---|---|---|---|---|---|---|
| *T. harzianum* | 0.70b | 0.90b | 1.30c | 1.60d | 1.90e | 2.20d |
| *T. pseudokoningii* | 0.70b | 1.20b | 1.80b | 2.60b | 2.80c | 3.00c |
| *B. subtilis* | 0.80b | 1.10b | 1.90b | 2.80b | 3.10b | 3.40b |
| *P. fluorescens* | 0.70b | 1.30b | 1.70b | 2.20c | 2.50d | 2.80c |
| Control | 2.40a | 4.50a | 6.90a | 8.86a | 8.86a | 9.00a |
| Mean | 1.06 | 1.80 | 2.72 | 3.61 | 3.83 | 4.08 |
| LSD | 0.304 | 0.488 | 0.269 | 0.308 | 0.285 | 0.281 |
| 2nd Trial(b) *T. harzianum* | 0.50b | 0.90b | 1.10b | 1.40e | 1.73e | 2.03c |
| *T. pseudokoningii* | 0.90b | 1.10b | 1.86b | 2.33c | 2.70c | 3.00c |
| *B. subtilis* | 0.70b | 1.00b | 1.70b | 3.00b | 3.30b | 3.60b |
| *P. fluorescens* | 0.50b | 1.10b | 1.50b | 1.86d | 2.16d | 2.46d |
| Control | 2.16a | 4.30a | 6.36a | 8.70a | 8.86a | 9.00a |
| Mean | 0.95 | 1.68 | 2.50 | 3.45 | 3.74 | 4.01 |
| LSD | 0.448 | 0.853 | 1.259 | 0.420 | 0.257 | 0.225 |

*means of 3 replicates (cm). Means in the same column followed by the same letter are not significantly different at 0.05 level (LSD).

***Table 2.** Growth of F. verticillioides when paired with the antagonists simultaneously.*

1st trial Time of incubation (hrs) of F. Verticillioides (cm)

| Antagonists | 48 | 72 | 96 | 120 | 144 | 168 |
|---|---|---|---|---|---|---|
| *T. harzianum* | 1.10b | 2.30b | 2.70bc | 3.10b | 3.40c | 3.80c |
| *T. pseudokoningii* | 0.90b | 1.40c | 2.90b | 3.46b | 3.76b | 4.16b |
| *B. subtilis* | 1.00b | 1.30c | 2.03d | 3.50b | 3.83b | 4.23b |
| *P. fluorescens* | 0.90b | 1.43c | 2.50c | 3.60b | 3.90b | 4.26b |
| Control | 2.00a | 3.40a | 5.10a | 7.03a | 8.90a | 9.00a |
| Mean | 1.18 | 1.96 | 3.04 | 4.13 | 4.75 | 5.09 |
| LSD | 0.269 | 0.357 | 0.328 | 2.374 | 0.322 | 0.325 |
| 2nd Trial | | | | | | |
| *T. harzianum* | 1.00b | 2.00b | 2.60b | 3.20b | 3.50b | 3.93b |
| *T. pseudokoningii* | 0.70c | 1.20c | 2.70b | 3.10b | 3.40b | 3.80b |
| *B. subtilis* | 0.90bc | 1.10c | 1.80c | 3.20b | 3.50b | 3.90b |
| *P. fluorescens* | 1.00b | 2.10b | 2.70b | 3.30b | 3.60b | 3.40b |
| Control | 2.10a | 3.10a | 4.70a | 7.20a | 7.50a | 7.86a |
| Mean | 1.14 | 1.90 | 2.90 | 4.00 | 4.30 | 4.57 |
| LSD | 0.281 | 0.453 | 0.438 | 0.430 | 0.430 | 0.420 |

*Means of 3 replicates (cm). Means in the same column followed by the same letter are not significantly different at 0.05 level (LSD).

*b. Pairing of the antagonists simultaneously with the pathogen*

*T. pseudokoningii* grew fast, stopped the pathogen's growth

at an average of 2.90 cm diameter by the third day of incubation. The antagonist sporulated on mycelia of *F.*

*verticillioides*. The pathogen later grew to 4.16 cm at later hours of incubation (Table 2). When both pathogen and antagonist were simultaneously introduced, *T. harzianum* grew fast, almost touching the *F. verticillioides*. Fifth day of incubation there was no clear zone of inhibition. By 7th day of pairing, *T. harzianum* had completely grown over *F. verticillioides,* and the pathogen's growth was 3.8 cm (Table 2). When *F. verticillioides* was simultaneously inoculated with *Pseudomonas fluorescens,* the antagonist grew to 0.70 cm in diameter and stopped the pathogen's growth which was 2.50 cm in diameter by the third day of incubation. This was maintained throughout the period of observation. The deep purple coloration of the pathogen mycelial was observed at the termination of the experiment. There was 4.26 cm zone of inhibition (Table 2). During simultaneous inoculation of *F. verticillioides* with *B. subtilis, B. subtilis* grew fast and stopped the pathogen's growth at 2.03 cm diameter by the third day of incubation. The pathogen grew to 4.23 cm with a thin zone of inhibition 7 days after pairing (Table 2).

*c. Pairing of the antagonists 24 hours after the pathogen*

With the introduction of the pathogen before the antagonist, *T. pseudokoningii* grew much faster than the pathogen in all Petri plates. By the third day after pairing, radial growth of *F. verticillioides* was 2.10 cm diameter which was overgrown by *T. pseudokoningii*. By the 7th day of pairing, *T. pseudokoningii* had filled the plate, sporulating on the pathogen's mycelia. The mycelia growth of *F. verticillioides* was 4.53 cm after a week of observation (Table 3). The earlier introduction of *F. verticillioides* than *T. harzianum* into the plate resulted in *T.*

*harzianum* growing fast, making contact with the pathogen when it was at 2.43 cm. *T. harzianum* significantly inhibited the growth of *F. verticillioides* to 3.90 cm (Table 3). *Pseudomonas fluorescens* grew much slower than when it was introduced before the pathogen and simultaneously with the pathogen in all Petri plates. By the third day after pairing, mycelial growth of *F. verticillioides* was 2.40 cm diameter. The pathogen's mycelia sporulated much well than when the other two methods of pairing used. There were zones of inhibition by this antagonist. The fungus growth was limited to 3.53 cm. The inhibition zone was 3.07 cm. The pathogen, *F. verticillioides* introduced 24 hours before the antagonist, *B. subtitles* grew to 1.00 cm in all Petri plates. By the third day of pairing, growth of *F. verticillioides* was 2.46 cm diameter. By the 7th day after pairing, *F. verticillioides* grew to 4.06 cm on the average in diameter.

In the first trial (Table 3) of the pairing with the antagonists introduction 24 hours after the pathogen, *F. verticillioides* has been in culture, at 48 hours of incubation, *P. fluorescens*' effect on the pathogen was significantly different and not significantly different from *T. pseudokoningii, B. subtilis* and *P. fluorescens* at 48 hours. There were no significant differences at 72 and 96 hours of incubation but, *P. fluorescens* at 96 hours was significantly different. The second trial, 48 hours of incubation gave significant effect of the antagonists on the pathogen. *P. fluorescens* gave a significant inhibitory effect on the pathogen followed by *T. harzianum* and *T. pseudokoningii*. At all the times of observation, *P. fluorescens*' effect on the pathogen was distinct. Next to its effectiveness was *T. harzianum*.

*Table 3.* *Growth response of F. verticillioides when paired with the each antagonist after 24 hours of the pathogen's inoculation.*

*1st trial Time of incubation (hrs) of F. verticillioides*

| Antagonists | 48 | 72 | 96 | 120 | 144 | 168 |
|---|---|---|---|---|---|---|
| *T. harzianum* | 1.40b | 1.80b | 2.30b | 3.10c | 3.50c | 3.90c |
| *T. pseudokoningii* | 1.10c | 2.03b | 2.50b | 3.70b | 4.13b | 4.53b |
| *B. subtilis* | 1.20bc | 1.90b | 2.46b | 3.23c | 3.66bc | 4.06bc |
| *P. fluorescens* | 1.00c | 1.40b | 1.90c | 2.40d | 3.13c | 3.53c |
| Control | 2.30a | 4.30a | 6.30a | 8.70a | 8.90a | 9.00a |
| LSD | 0.269 | 0.674 | 0.393 | 0.357 | 0.539 | 0.533 |
| 2nd Trial | | | | | | |
| *T. harzianum* | 1.00c | 1.60bc | 2.06bc | 2.80c | 3.20c | 3.60c |
| *T. pseudokoningii* | 1.00c | 1.80b | 2.30b | 3.60b | 4.00b | 4.40b |
| *B. subtilis* | 1.30b | 1.50bc | 2.23bc | 3.00c | 3.40c | 3.80c |
| *P. fluorescens* | 0.80d | 1.33c | 1.76c | 2.10d | 2.50d | 2.90d |
| Control | 2.10a | 3.90a | 6.00a | 8.20a | 8.60a | 8.8ba |
| LSD | 0.181 | 0.393 | 0.467 | 0.514 | 0.514 | 0.44 |

Means of 3 replicates (cm). Means in the same column followed by the same letter are not significantly different at 0.05 level (LSD).

### 3.2. Effect of the Three Pairing Methods of Antagonists on the Pathogen

The pairing method of introducing the antagonists 24 hours before the pathogen showed no significant difference at 48, 72 and 96 hours (Fig. 1). At 120 hours, the treatments were significantly different from each other at p = 0.05. The effect of *T. harzianum* on the pathogen was significantly different from the other antagonists. It gave the highest inhibitory effect on the pathogen; *F. verticillioides* growth was 1.40 cm. This effectiveness as an antagonist was followed by *P. fluorescens*

which limited the growth of the pathogen to 1.86 cm. There were significant differences in the growth of *F. verticillioides* due to the effect of *B. subtilis* and *T. pseudokoningii* to 3.00 cm and 2.33 cm respectively. At 144 hours the action of *T. harzianum* was significantly different from all the antagonists tested against the pathogen's growth was 1.73 cm on the average. This action was followed by the *P. fluorescens* with *F. verticillioides*' growth as 2.16 cm. Similar trend was observed at 168 hours after pairing. *T. harzianum* was the most effective antagonist among all the antagonists tested against *F. verticillioides* when the antagonists were introduced 24 hours before the pathogen.

Simultaneous pairing of the pathogen with the antagonists showed that *T. harzianum* was still the most effective followed by *P. fluorescens* then *T. pseudokoningii* and the least effective was *B. subtilis*. The 24 hours pairing after the pathogen has been in the growth media showed that *T. harzianum* was still the most effective at all times of incubation which make the antagonist a promising biocontrol agent against *F. verticillioides*. The fungus was most effective in the experiment against the pathogen at all the three pairing times. The introduction of the antagonist before the pathogen *in vitro* was observed to be the best followed by the simultaneous pairing and the least effective (though effective too it was better than the control) was the introduction of the antagonist 24 hours after the pathogen. It was observed that all the antagonists tested had good inhibitory potentials on the pathogen, *F. verticillioides*.
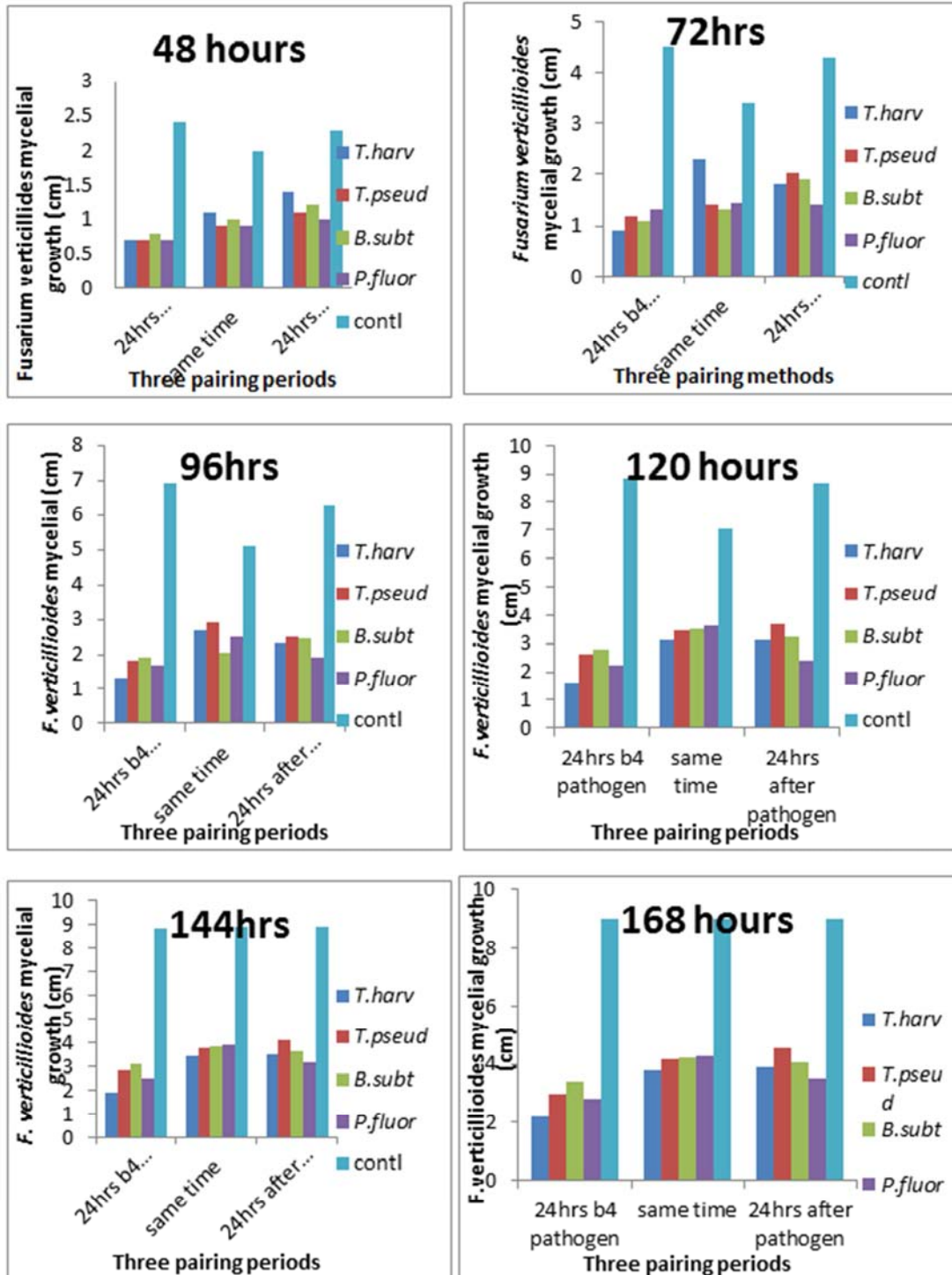


***Fig. 1.*** *Comparison of the effect of the antagonists on the pathogen, F.verticillioides at the three different pairing periods. (24hrs b4 = 24 hours before the pathogen, 24hrs after = 24 hours after the pathogen; Contl = Control; B. subtilis = B. subtilis; T. harv = T. harzianum, P. fluor = P. fluorescens).*

# 4. Discussion

The antagonistic potentials of the two *Trichoderma* species against *F. verticillioides* were shown in all the three pairing methods. This suggests the ability of each of the *Trichoderma* species to inhibit the mycelial growth of *F. verticillioides* irrespective of time of application. This tells us that if *F. verticillioides* occurs on a maize field before the two *Trichoderma* species were introduced; either of the two can still suppress it to some extent. Strains of *T.pseudokoningii* were successful in inhibiting growth of *F.verticillioides in vitro* in a similar manner and were also able to check occurrence of the pathogen significantly within maize (*Zea mays*) stem in the field, irrespective of pairing methods [25]. The significance of mycelial suppression of *F. verticillioides* by *T. pseudokoningii* over that of *T. harzianum* suggests *T. pseudokoningii* showed better competitive ability than *T. harzianum* against *F. verticillioides*. *T. pseudokoningii* appeared to be more promising than *T. harzianum* in checking the occurrence of the pathogen on maize fields.

Inoculating antagonist before pathogen had a significant advantage and preference over the other two pairing methods in aiding effective growth inhibition of the pathogen. This means that inoculating antagonists before pathogen is better than the other two pairing methods. In inhibition of *F. verticillioides* growth, it is better for the antagonists to be already established on the field before the pathogen comes in. The zone of inhibition of growth of *F.verticillioides* caused by *T. harzianum* irrespective of the pairing methods, showed a probable antibiosis-mode of inhibition.

The highly significant interaction between the *Trichoderma* species and pairing methods showed that mycelia suppression of F. *verticillioides* by either *T. pseudokoningii* or *T. harzianum*. For a more competitive exclusion of *F. verticillioides* from maize plant, it is better for *Trichoderma* species to be on the field before the occurrence of the pathogen.

The growth of *T. pseudokoningii* on some parts of the mycelial mass of the pathogen and the hyphal distortions of the pathogen suggested hyperparasitism. Minor morphological disturbances and hyphal distortions of one fungus by another is a form of hyperparasitism. The hyperparasitic action of the antagonist against the pathogen agreed with the work of [1, 21] which concluded that hyperparasitism also encompasses overgrowth of one fungus by another. The fading of the colour of the pathogen when *T. pseudokoningii* gradually grew over its mycelial mass might mean gradual extermination of the pathogen and thus gradual death. This is an indication of hyperparasitism. It means that hyperparasitism of the pathogen, even when it was inoculated before the antagonist could mean that the pathogen will pose little or no problem even if it arrives on the phylloplane of maize plant well before the antagonists. The growth of the antagonist might mean competition for space as its mode of action against the pathogen.

The inability of the pathogen to grow in certain spaces not yet colonized by *T. pseudokoningii* might be due to the yellowish-green metabolite produced by the antagonist which suggests antibiosis as a mode of action which includes mycoparasitism and competition for space and substrate. *T. pseudokoningii* antagonistic effect against *F.verticillioides* may be due to the fact that the antagonist was able to colonize as much space as possible before the arrival of the pathogen. Competition for space was involved in its mode of action against *F.verticillioides*. *T. pseudokoningii* exhibited hyperparasitism on this pathogen by growing faster and stopping growth of the pathogen by the 3rd day. This observation was also noted by [21] when *T. pseudokoningii* was used against seed-borne pathogens of cowpea.

This present study suggests the versatile and broad spectrum activity of *Trichoderma* species. Many researchers reported the potential values of *Trichoderma spp* as bioagents for the protection of various seed and soil borne diseases of crops [1, 2, 12, 7, 12, 13, and 21]. The effectiveness of the two *Trichoderma* spp used against *F. verticillioides* in this study may be due to their fast growth and high sporulating ability. Competition for space, food and hyperparasitism are their mode of actions against the pathogen.

*P. fluorescens* when paired with *F.verticillioides* achieved a remarkable and almost total inhibition of the pathogen. Though it was not a fast-growing organism, it was able to stop further growth of the pathogen at a distance by the third day after pairing. It is conjectured that its inhibitory effect may be due to a metabolite secreted by the bacterium into the medium that brought about the change in colour of the whitish fluffy mycelia of *F.verticillioides* to purple. It was the ability of *P. fluorescens* to produce the metabolite that enhanced its usefulness as a biocontrol agent since it caused inhibition of growth of the target pathogen at a distance from their site of application. This observation is in line with [22] report that a strain of *P. fluorescens* carries the gene of *Bacillus thuringiensis* toxin. It produces an intracellular protein toxin crystal (the parasporal body) during sporulation which can act as a microbial insecticide for specific insect groups. It is weakly toxic to insect pests such as the cabbage loop and the European corn borer [22]. The proven ability of *P. fluorescens* irrespective of the times of pairing with the pathogen, to provide diffusible substance toxic to the fungus supports the hypothesis and recommends *P. fluorescens* as an important bioagent that can be used against *F.verticillioides*.

*B. subtilis* grew faster than the *P. fluorescens*. The antibiotics secreted by *B. subtilis* into the growth medium stopped the hyphal growth of *F. verticillioides*. The effect of the antibiotics was not noticed as such in the *B. subtilis* used in this study, on the basis of time of pairing with the pathogen. The observation from this study could be as suggested by [20] that *B. subtilis* produces antibiotics in response to the presence of selected organisms and more so that the culture medium composition used in the study does not support the synthesis of antibiotics, as bacteria perform best on nutrient agar.

*T. pseudokoningii* is effective in the midst or in the abundance of the pathogen due to its ability to colonize the

growth media which both it and the pathogen depend on and also its ability to overgrow the pathogen thereby depriving the pathogen of oxygen and all other things essential for the organism's growth. *P. fluorescens* was observed to be as effective antagonist as it was observed in the first trial. The color change due to the effect of this antagonist on the pathogen was also observed. The second trial was a confirmation of all that were observed in the first trial.

Statistical analysis showed that any of the four antagonists is a good antagonist against the pathogen. The fact that inoculating the antagonist before the pathogen being better than the other two types of inoculation agreed with the work of [24] who believed that there were no biocontrol agents that have high enough competitive ability to displace an already established pathogen. The time lag between the inoculation of the antagonist and the inoculation of the pathogen might have contributed to the success recorded with the antagonists against the pathogen. This agreed with the emphasis on the importance of time-lapse between the arrival of the antagonist and later pathogen on the phylloplane. This allows adequate increase in sporulation and subsequent colonization of the plane by the antagonist before the arrival of the pathogen.

The overall behaviour of the antagonists against *F.verticillioides* pathogen agrees with the report of [14] on the possibility of an antagonist controlling more than one pathogen on a plant. They suggested that the mode of action of such an antagonist may be non-specific and may be better than one with a specific mode of action.

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