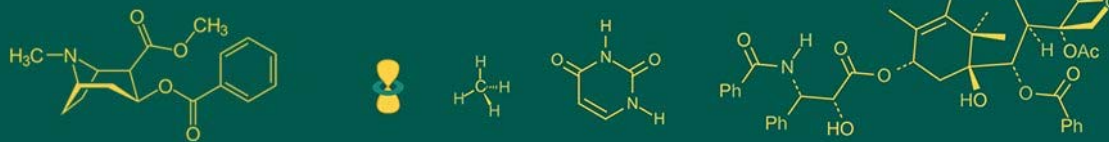


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In-vitro compatibility of *Penicillium pinophilum* with commonly used fungicides, insecticides herbicides and fertilizers in different crops

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Abstract

An *in-vitro* compatibility studies were conducted with *Penicillium pinophilum* and some commonly used, commercially available chemical pesticides and fertilizers in sugarcane. The experiment was laid out in a Completely Randomized Design (CRD) with three replications and thirteen treatments. In this study, three treatments of fungicides [Carbendazim 50% WP (Bavistin), Mancozeb 63% WP + Carbendazim 12% WP (SAAF), Thiophenate methyl 70% WP (Roko)], three treatments of insecticides [Thiamethoxam 30% FS (Slayer pro), Chlorpyrifos 50% + Cypermethrin 5% EC (Hamla), Azoxystrobin 2.5% + Thiophenate methyl 11.25% + Thiomethoxam 25% FS (Electron)], three treatments of herbicides [Atrazine 50% WP (Atrataf), Ametryne 80% WDG (Tamar), 2, 4-D sodium salt 44% + Metribuzin 35% + Pyrazosulfuron-ethyl 1.0% WG (Triskele)], three treatments of fertilizers (Urea, Di-ammonium phosphate, Muriate of potash) and *P. pinophilum* culture grown on PDA served as a control. Among fungicides, Carbendazim and Thiophenate methyl were found to be compatible, whereas Mancozeb + Carbendazim was found to be toxic. The insecticides Thiomethoxam and Azoxystrobin + Thiophenate methyl + Thiomethoxam were compatible, while Chlorpyrifos + Cypermethrin proved incompatible. The herbicides, Ametryne and 2, 4-D sodium salt + Metribuzin + Pyrazosulfuron were incompatible and Atrazine was found to be slightly toxic. Among fertilizers, Urea and Di-ammonium phosphate were found to be compatible and Muriate of potash showed additional colony growth over control.

Keywords: Compatibility, *Penicillium pinophilum*, fungicides

Introduction

Control of plant diseases and pests by chemicals can be spectacular but this is relatively short term measure and moreover, the accumulation of harmful chemical residues sometimes causes serious ecological problems. In agriculture, biocontrol agents are a safe and environmentally acceptable alternative to pesticides (Gampala and Pinnamaneni, 2010) [5]. Recommended doses of insecticides along with biocontrol agents show promising effects on the management of various plant pests than the chemicals alone (Vinit *et al.*, 2012) [17]. Combining a fungicide tolerant biocontrol agent with respective fungicides has improved the extent of disease control and reduced the quantity of fungicides required for effective management (Buck, 2004) [3]. Therefore the combine use of biocontrol agents and chemical pesticides has enticed much attention as a way to obtain synergistic or additive effects in the control of soil borne pathogens (Locke *et al.*, 1985) [8]. The herbicides, insecticides and fungicides applied as foliar spray or soil drench ultimately reach the soil and affect beneficial non-target mycoflora. Hence, knowledge of compatibility of all these biocontrol agents with important pesticides may help opt for better plant protection measures. Tolerance to commonly-used pesticides enhances the efficacy and expands the scope of application of biocontrol agent. Phosphorous and potassium is important nutrients for growth and yield of sugarcane. Fungus, *P. pinophilum* has been found to have tremendous potential for making the naturally unavailable potassium and phosphorus in the soil into easily available form for the plant (Maity *et al.* 2014) [10]. Hence, an *in vitro* study was conducted to assess the compatibility of some commonly used, commercially available fungicides, insecticides, herbicides and fertilizers with the growth of *P. pinophilum*.

Materials and Methods

The present study of testing the compatibility of *P. pinophilum* with pesticides was conducted at the Microbiology Laboratory at Bharti green tech. Dahiwadi, during the year 2023, using the poisoned food technique (Nene and Thapliyal, 1993) [13], Potato Dextrose Agar medium (PDA) and three replications in CRD. All nine chemical pesticides were used according to the recommended application rate for field crops and fertilizers were used at 1 percent.

Estimation of fungal colony growth

Observations of the mycelial growth of biocontrol agents were recorded by measuring the diameter (mm) of radial growth by using the measuring scale. Observations of four replications are recorded every 72 hours, till 144 hours are completed. The growth inhibition of biocontrol agents was estimated by using the following formula given by Vincent

(1947) [16] and percent inhibition of mycelial growth was obtained.

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Percent growth inhibition.

C = Colony diameter in control (mm).

T = Colony diameter in treatment (mm).

On the basis of the results at 144 hrs, the pesticides were classified into 4 categories based on percent growth inhibition (Ambethgar, 2009) [1] as below.

| Category | Percent growth inhibition | Toxicity level |
|----------|---------------------------|------------------|
| 1 | >50 | Toxic |
| 2 | 35 – 49.9 | Moderately Toxic |
| 3 | 25 – 34.9 | Slightly Toxic |
| 4 | < 25 | Compatible |

Table 1: Chemical pesticides and their concentrations

| Treatment No. | Trade name | Content | Dose per 100 ml media |
|---------------------|-----------------------|--|-----------------------|
| Fungicides | | | |
| T ₁ | Bavistin | Carbendazim 50% WP | 0.2 gm |
| T ₂ | SAAF | Mancozeb 63% WP + Carbendazim 12% WP | 0.2 gm |
| T ₃ | Roko | Thiophenate methyl 70% WP | 0.05 gm |
| Insecticides | | | |
| T ₄ | Slayer pro | Thiamethoxam 30% FS | 0.05 gm |
| T ₅ | Hamla | Chlorpyrifos 50% + Cypermethrin 5% EC | 0.2 ml |
| T ₆ | Electron | Azoxystrobin 2.5% + Thiophenate methyl 11.25% + Thiomethoxam 25% FS | |
| Herbicides | | | |
| T ₇ | Atrataf | Atrazine 50% WP | 0.2 gm |
| T ₈ | Tamar | Ametryne 80% WDG | 0.5 gm |
| T ₉ | Treskele | 2, 4-D sodium salt 44% + Metribuzin 35% + Pyrazosulfuron-ethyl 1.0% WG | 0.6 gm |
| Fertilizers | | | |
| T ₁₀ | Urea | 46:00:00 | 1 gm |
| T ₁₁ | Di-ammonium phosphate | 18:46:00 | 1 gm |
| T ₁₂ | Muriate of potash | 00:00:60 | 1 gm |
| T ₁₃ | Control | - | - |

Results and Discussion

1. Effect of fungicides on *P. pinophilum*.

Among the fungicides (Table 2), Carbendazim and Thiophenate methyl inhibited growth by 6 and 9 percent at 144 hrs, respectively, and were found to be compatible. Hence, it gives chances to use where this *P. pinophilum* is applied. However, *P. pinophilum* was completely incompatible with the fungicide Mancozeb + Carbendazim, which showed 100 percent colony growth inhibition.

Khalil *et al.* (1985) [7] discovered that the fungicides Thiophenate methyl and Mancozeb were partially incompatible with the fungus *Verticillium lecanii*. Mohamad and Radwan (2017) [12] discovered that Mancozeb was incompatible with *T. harzianum*. Maheshwary *et al.* (2020) [9] discovered that the fungicide Mancozeb + Carbendazim was incompatible with the fungus *Trichoderma asperellum*.

2. Effect of insecticides on *P. pinophilum*.

In the case of insecticides (Table 2), only Chlorpyrifos + Cypermethrin was found to be incompatible, which showed 56 percent inhibition, while Azoxystrobin + Thiophenate methyl + Thiomethoxam was found to be slightly toxic. Thiomethoxam showed 1 percent growth inhibition and found to be totally compatible.

Similar results were found by Oliveira *et al.* (2003) [14] insecticide, Thiomethoxam was found to be compatible and Chlorpyrifos was found totally incompatible with the fungus *B. bassiana*. Martinez Toledo *et al.* (1998) [11] and Desai and Kulkarni (2004) [4] also reported that strong inhibition of *Trichoderma harzianum* by Chlorpyrifos in addition to methyl Pymifos.

3. Effect of herbicides on *P. pinophilum*.

Except Atrazine (Table 2), the remaining two herbicides were incompatible with *P. pinophilum*. Herbicides Ametryne and 2, 4-D sodium salt + Metribuzin + Pyrazosulfuron-ethyl showed 88 and 76 percent inhibition and were found toxic to *P. pinophilum*. Atrazine showed 29 percent inhibition and was found to be slightly toxic. Desai and Kulkarni (2004) [4] observed similar results, reporting that Atrazine was a comparably safer pesticide when used with *Trichoderma harzianum*. Gardner and Storey (1985) [6] found that the herbicide 2, 4-D substantially reduced both germination and development of *B. bassiana* at 6 mg (AI)/ml.

4. Effect of fertilizers on *P. pinophilum*: Among the fertilizers (Table 2), Urea, Di-ammonium phosphate and

Muriate of potash proved totally compatible with *P. pinophilum*, which showed 20, 6 and -5 percent colony growth inhibition, respectively. Muriate of potash showed more colony growth of *P. pinophilum* than control. Similar results were observed by Bhai and Thomas (2010)^[2] Fertilizers showed compatible with *T. harzianum*. In

addition they were found to be favourable in increasing the *T. harzianum* population. Gampala and Pinnamaneni (2010)^[5] stated that Muriate of Potash and Super Phosphate showed greater compatibility than Urea.

Table 2: Colony growth inhibition

| Treatments | Average colony diameter of <i>P. pinophilum</i> (mm) | | | | Toxicity level |
|--|--|--------------|---------|--------------|----------------|
| | 72 hrs | % inhibition | 144 hrs | % inhibition | |
| Fungicides | | | | | |
| T ₁ Carbendazim 50% WP (Bavistin) | 2.88 | 33 | 26.22 | 6 | Compatible |
| T ₂ Mancozeb 63% WP + Carbendazim 12% WP (SAAF) | 0.00 | 100 | 0.00 | 100 | Toxic |
| T ₃ Thiophenate methyl 70% WP (Roko) | 3.78 | 13 | 25.33 | 9 | Compatible |
| Insecticides | | | | | |
| T ₄ Thiamethoxam 30% FS (Slayer pro) | 3.11 | 28 | 27.52 | 1 | Compatible |
| T ₅ Chlorpyriphos 50% + Cypermethrin 5% EC (Hamla) | 0.11 | 97 | 12.22 | 56 | Toxic |
| T ₆ Azoxystrobin 2.5% + Thiophenate methyl 11.25% + Thiomethoxam 25% FS (Electron) | 3.66 | 15 | 19.12 | 31 | Slightly toxic |
| Herbicides | | | | | |
| T ₇ Atrazine 50% WP (Atrataf) | 2.66 | 39 | 19.77 | 29 | Slightly toxic |
| T ₈ Ametryne 80% WDG (Tamar) | 0.00 | 100 | 3.44 | 88 | Toxic |
| T ₉ 2, 4-D sodium salt 44% + Metribuzin 35% + Pyrazosulfuron-ethyl 1.0% WG (Triskele) | 0.00 | 100 | 6.77 | 76 | Toxic |
| Fertilizers | | | | | |
| T ₁₀ Urea | 2.43 | 43.87 | 22.20 | 20 | Compatible |
| T ₁₁ Di-ammonium phosphate | 2.55 | 41 | 26.11 | 6 | Compatible |
| T ₁₂ Muriate of potash | 3.66 | 15 | 29.22 | -5 | Compatible |
| T ₁₃ Control | 4.33 | - | 27.89 | | |
| SE (m) | 0.53 | | 0.96 | | |
| CD at 1% | 1.55 | | 2.86 | | |

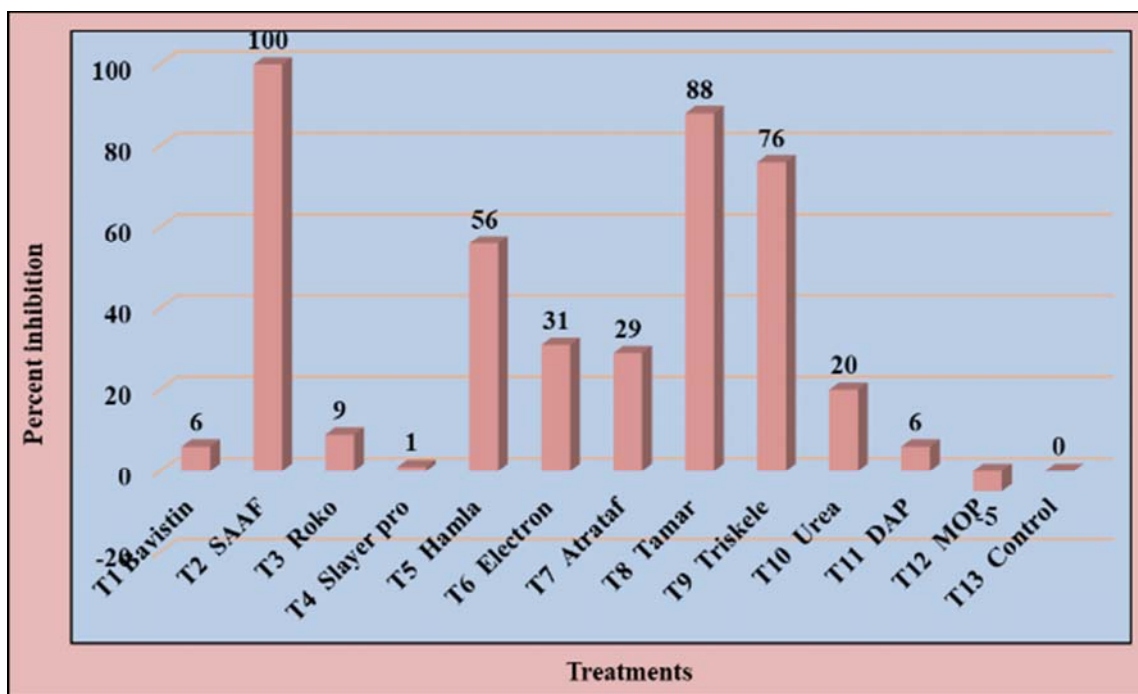


Fig 1: Inhibition percentage of colony growth of *P. pinophilum* by different pesticides and fertilizers.

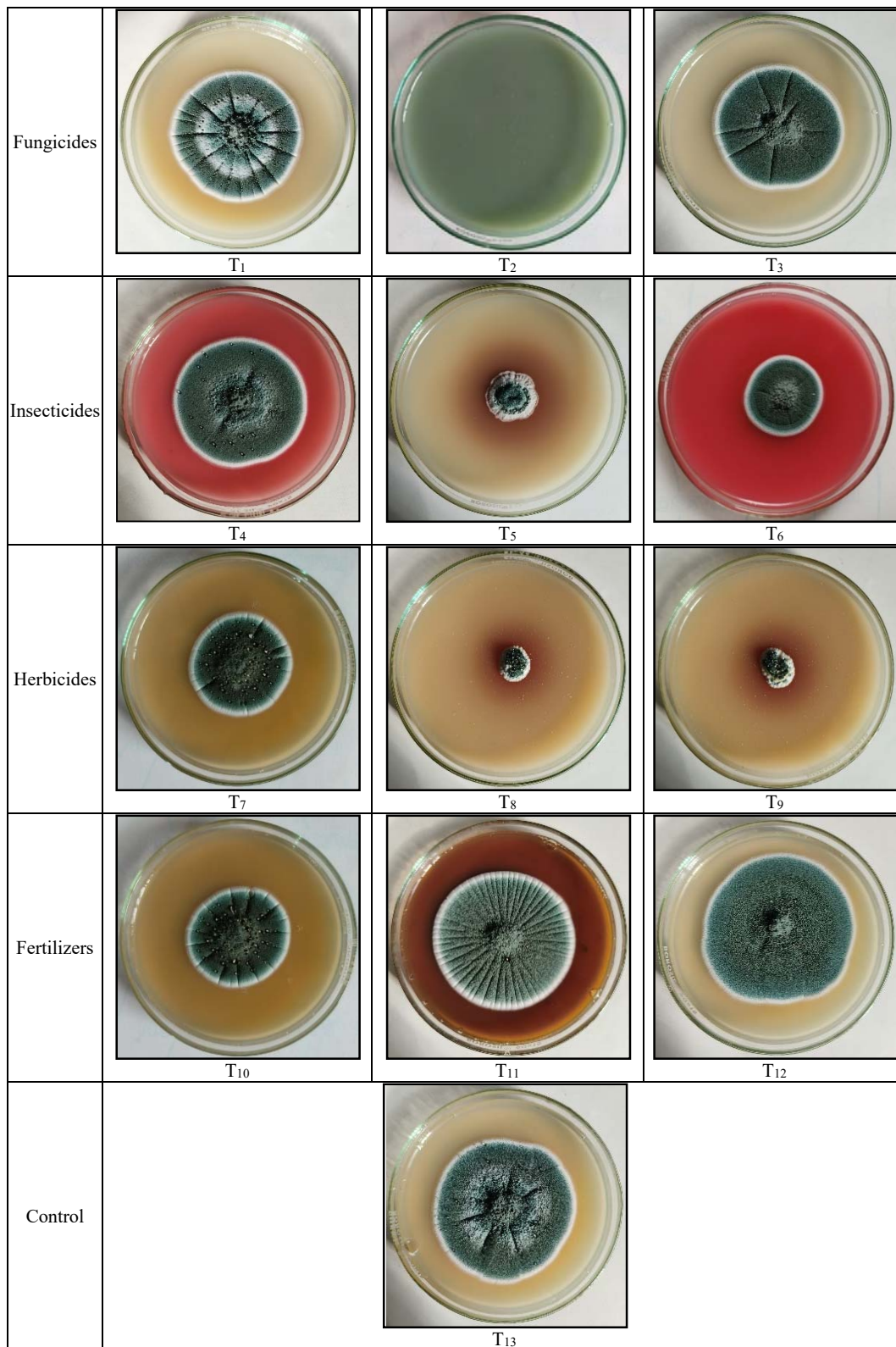


Fig 2: Colony growth of *P. pinophilum*.

Conclusion

- Carbendazim and Thiophenate methyl were found to be compatible; hence, it can be used in the sugarcane where *P. pinophilum* has already been applied for nutrient management.
- The insecticides Thiomethoxam and Azoxystrobin + Thiophenate methyl + Thiomethoxam were compatible;

hence, combined use of all these insecticides will be safer for *P. pinophilum*.

- The herbicide Atrazine was found to be slightly toxic, so it can be applied safely in the IPM system. The herbicides Ametryne and 2, 4-D sodium salt + Metribuzin + Pyrazosulfuron were incompatible, so

application of both must be avoided in *P. pinophilum*-applied soils.

- Among fertilizers, Urea and Di-ammonium phosphate were found compatible and Muriate of potash showed additional colony growth over control.
- Some chemical pesticides and fertilizers do not affect the growth and development of *P. pinophilum*. The combination of such chemical pesticides can provide an additive or synergistic effect in the nutrition and control of diseases and pests in sugarcane.

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