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# Effect of adjuvants on herbicidal activity of phytotoxins produced by *Aspergillus* isolate#11

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***Abstract:*** Weeds are responsible for significant losses in agriculture. Synthetic herbicides have adverse environmental effects. The utilization of fungal phytotoxins as an alternative to weed control is still limited. This study evaluated the herbicidal potential of CFCF and culture extracts of *Aspergillus* isolate against *Parthenium*. Formulation of culture extracts with adjuvants was also assessed for herbicidal potential by bioassays. CFCF of Czapek-Dox medium supplemented with casein was observed to be more phytotoxic in comparison to CFCF of Czapek-Dox medium. Formulation of culture extract of Cz with adjuvant tween 80 exhibited maximum phytotoxicity, while maximum phytotoxicity of culture extract of Cz+C was observed when it was formulated with mustard oil.
*Index Terms*- Adjuvant, formulation, phytotoxicity, culture extract.

**Introduction**

One of the major challenges facing agriculture for many decades around the world is control of weeds in agriculture areas (Daniel Jr. *et al*., 2020). The application of synthetic herbicides has helped humanity to increase crop productivity for many years, but intensive use of synthetic herbicides has caused adverse environmental effects, contamination of soil and water and herbicide resistance in weeds (Westwood *et al*., 2018). Introduction of other methods are needed to regulate weed infestation for activities aimed at sustainable development (Kołodziejczyk, 2015). Therefore, the search for new herbicides with safer toxicological and environmental profiles and with new modes of action has been increased (Dayan and Duke, 2014).

Several biomolecules with different structural and biological characteristics are produced by microorganisms. In some cases, these biomolecules can cause chlorotic and necrotic lesions in host plants. These biomolecules are secondary metabolites and they can be a source for production of new natural herbicides (Duke *et al*., 2000, Hoagland *et al*., 2007). Substances produced by secondary metabolism of fungi can be an alternative to the intensive use of chemical herbicide (Confortin *et al*., 2018).

Some studies were done using fermented broth containing the secondary metabolites produced by fungi via submerged fermentation. These studies have shown promising results in weed control (Brun *et al*., 2016, Souza *et al*., 2017). Secondary metabolites produced by fungi can damage weeds by penetrating the plant followed by the destruction of the cell wall and induction of necrotic lesions (Kaur and Aggarwal, 2015).

These biomolecules are present in very low concentration in fermentation media, so they exhibit low efficiency (Varejão *et al*., 2013). To increase herbicidal activity of these biomolecules some strategy is needed to concentrate these molecules as well as to use adequate combination of adjuvants in formulation (Daniel Jr. *et al*., 2020).

Most natural phytotoxins seem to be unable to penetrate the plant cuticle. In the case of chemical herbicides, the problem of their effective absorption into plant tissues is often solved by supplementation with the appropriate adjuvants (surfactants, penetrants, etc.) (Dubovik *et al*., 2020).

The lifestyles of fungi associated with plants belonging to Aspergillus species can range from saprophytic and symptomless endophytes to weak and opportunistic phytopathogens (Pfliegler *et al*., 2020). *Aspergillus* species have been shown to produce phytotoxins (Wu *et al*.,2023). Aflatoxins hinder chlorophyll and carotenoid synthesis thus inhibiting plant photosynthesis (Anjorin and Inje,2014).

Necrotic lesions are induced by ochratoxin A on *Arabidopsis thaliana* leaves (Peng *et al*., 2010). Cichorine a novel phytotoxin produces foliar blight in Russian knapweed. It has been discovered from several fungi including *A. nidulans* (Liao *et al*., 2019).

The objective of this study was to evaluate phytotoxic potential of *Aspergillus* isolate to weed *Parthenium* using fermented broth produced by this fungus and to screen the most compatible adjuvant for foliar application of culture extract obtained from fermented broth of this *Aspergillus* isolate. Adjuvants having different hydrophilic and lipophilic properties were used for screening.

**Materials and Methods-**

Fungal material-

*Aspergillus* isolates #11 was isolated from soil samples of Jabalpur.

Growth medium- PDA of Hi Media was used for culturing *Aspergillus*# 11 isolate.

Production of secondary metabolites-

Two media Czapek-Dox medium and Czapek-Dox medium supplemented with 1% casein were used for *Aspergillus*#11 isolate for secondary metabolite production.

Composition of Czapek-Dox medium (Cz) is as follows- Sodium nitrate- 0.3%, Sucrose- 3%, $K_2HPO_4.3H_2O$- 0.13%, $MgSO_4.7H_2O$- 0.05%, KCl- 0.05%, $FeSO_4.7H_2O$- 0.001%, $CuSO_4.5H_2O$- 0.0005% and $ZnSO_4.7H_2O$- 0.001%.

Composition of Czapek-Dox medium supplemented with casein (Cz+C) is as follows- Sodium nitrate- 0.3%, Sucrose- 3%, $K_2HPO_4.3H_2O$- 0.13%, $MgSO_4.7H_2O$- 0.05%, KCl- 0.05%, $FeSO_4.7H_2O$- 0.001%, $CuSO_4.5H_2O$- 0.0005%, $ZnSO_4.7H_2O$- 0.001% and Casein- 1.0%.

250mL of medium was taken in 500mL conical flasks. Autoclaved media was inoculated with spore suspension of *Aspergillus* isolate #11 and incubated for 15 days at 25°±2°C temperature.

Preparation of cell free culture filtrates (CFCF)- Filtrates of cultures were obtained by filtration with Whatman filter paper.

**Phytotoxicity test** – Phytotoxicity test of CFCF obtained from fermented broth of *Aspergillus* isolate was done by cut shoot bioassay. Shoots of *Parthenium* was taken and placed in CFCF obtained from isolate. Observations were taken after 24 and 48 hours.

Phytotoxicity rating (0-10) scale- Phytotoxicity scale of 0-10 was adopted, in which 0 as 0%, 1 as 10%, 2 as 20% injury and so on was considered (Method of Gupta *et al*., 2019).
Preparation of culture extracts- Filtrates of cultures were extracted two times with chloroform. The solvent fractions were dried and then dissolved in 2.5mL of ethanol.

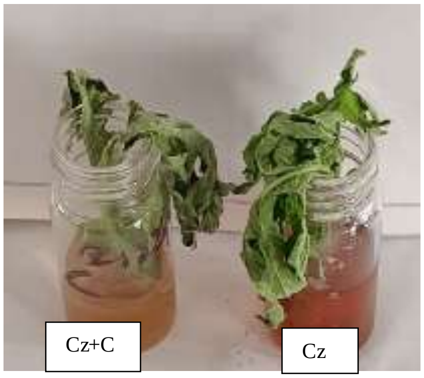
**Detection of toxins-** Detection of toxins was done by thin layer chromatography. TLC was carried out in subdued light on silica gel G plates, which were activated at 110°C for 2 hours (Scott *et al*., 1970). 10 µL of culture extracts were applied on the plate. The plate was developed in Toluene: Ethyl acetate: Formic acid :: 6:3:1solvent system. Phytotoxins were observed by exposing the plate to iodine vapors.

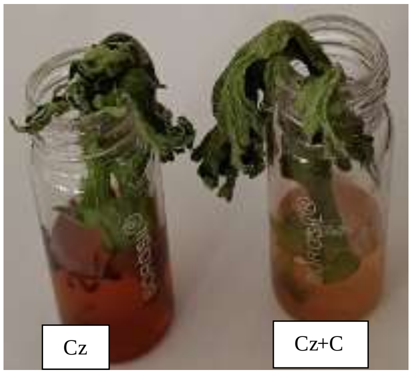
**Formulation of adjuvant with culture extract**- Formulations were prepared by adding different adjuvant at the rate of 0.5% to solution containing 100µL of culture extract in 9.9 mL of distilled water. All the components in formulation were thoroughly mixed by stirring. A total of 10 formulating agents were used namely –tween 80, triton x 100, sucrose, glycerol, coconut oil, groundnut oil, soybean oil, mustard oil, gelatin and toxin were tried. Phytotoxicity test was done by cut shoot bioassay. Shoots of *Parthenium* was taken and placed in solution containing culture extract and adjuvant in distilled water. Observations were taken after 24- and 48- hours post treatment.

**Result and Discussion**

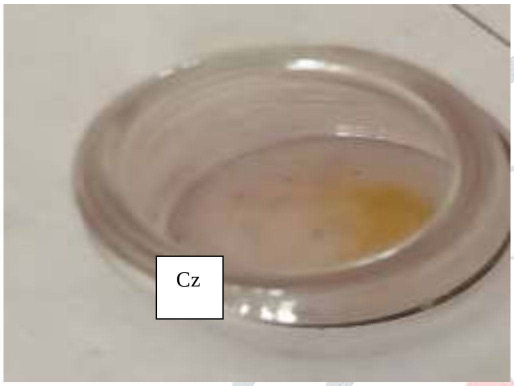
Phytotoxicity test of CFCF

Filtrates from two-week old cultures of *Aspergillus* isolate #11 exhibited phytotoxicity against *Parthenium*. Culture filtrate obtained from Czapek-Dox media supplemented with casein was observed to be more phytotoxic in comparison to culture filtrate obtained from Czapek-Dox medium. This may be due to presence of casein in media which might be providing amino acids for phytotoxin synthesis. More phytotoxic effects are observed on leaves of *Parthenium* after 48 hours of treatment with both culture filtrates.



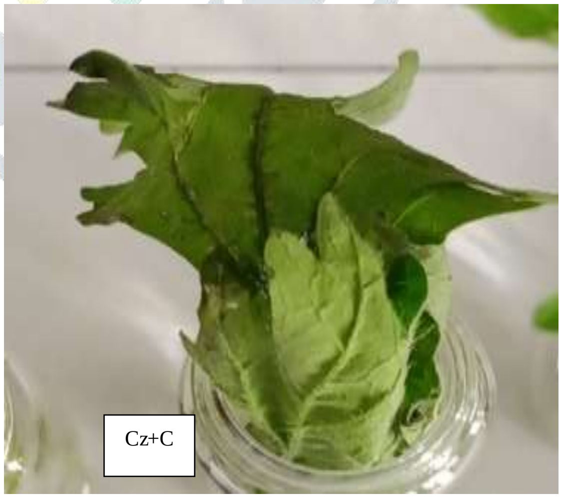



**Phytotoxicity of CFCF of *Aspergillus* isolate on *Parthenium* after 24 hours and 48 hours respectively.**

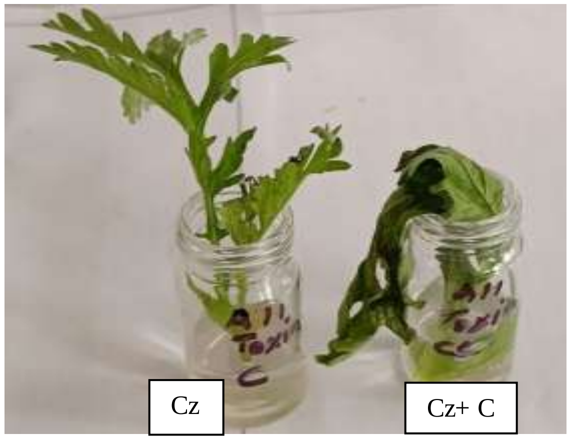



**Phytotoxins obtained from culture extract of Cz and Cz +C of *Aspergillus* isolate #11**

Different types of phytotoxins are obtained in culture extract of Czapek Dox medium (Cz) and Czapek Dox medium supplemented with casein (Cz+C) and this accounts for the difference in their phytotoxic effects. After 24 hours necrosis at margin of leaves is observed when *Parhenium* leaves are treated with aqueous formulation of phytotoxins obtained from Czapek Dox medium but in case of aqueous formulation of phytotoxins obtained from Cz+ C medium necrosis can be observed at the margins and also near the veins of *Parthenium* leaves after 24 hours.

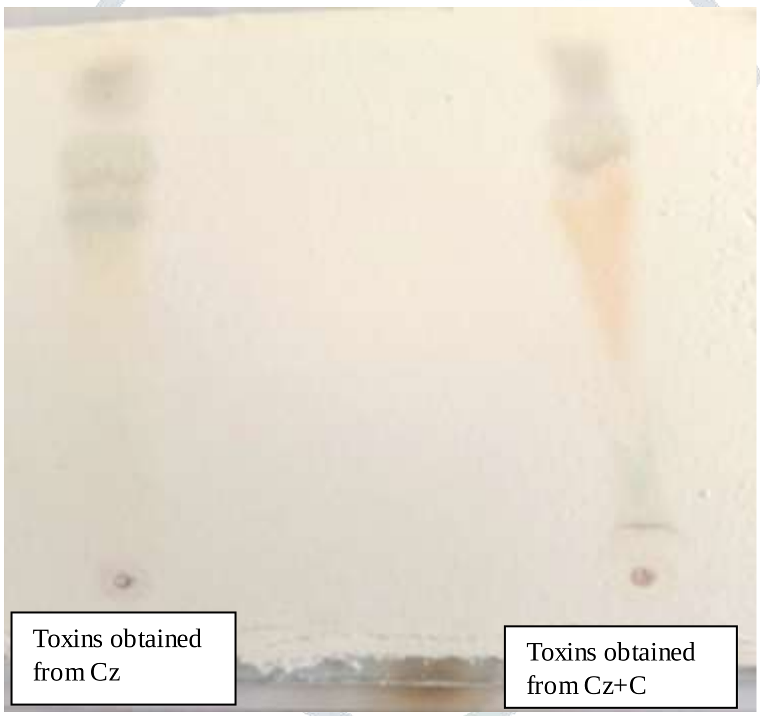



**Necrotic lesions in *Parthenium* leaves after 24 hours due to phytotoxin treatment**




**More necrotic lesions are observed after 48 hours due to phytotoxins**.

Detection of toxins obtained from Czapek Dox and Czapek Dox supplemented with casein was done by TLC. In TLC plate we can clearly see the difference in phytotoxins obtained from Cz and Cz+C.




**Detection of phytotoxins by TLC**

Table 1: $R_F$ value of observed toxins obtained from culture extract of *Aspergillus* isolate#11

| Toxin spots detected | $R_F$ value of toxins obtained from culture extract of Czapek-Dox medium | $R_F$ value of toxins obtained from culture extract of Czapek-Dox + casein medium |
|---|---|---|
| Spot 1 | 0.88 | 0.89 |
| Spot 2 | 0.76 | 0.75 |
| Spot 3 | 0.67 | 0.65 |
| Spot 4 | - | 0.137 |
| Spot 5 | - | 0.09 |

**Table 2: Formulation of adjuvant with culture extract:**

Influence of various adjuvant used in formulation is presented in Table 1.

| S.No. | Adjuvant used in Formulation | Phytotoxicity rating of Formulation of phytotoxins obtained from Cz on *Parthenium* leaves | | Phytotoxicity rating of Formulation of phytotoxins obtained from Cz+ C on *Parthenium* leaves | |
|---|---|---|---|---|---|
| | | After 24 hours | After 48 hours | After 24 hours | After 48 hours |
| 1 | Sucrose | 2.6 | 3.4 | 4.7 | 5.1 |
| 2 | Glycerol | 2.6 | 3.2 | 4.3 | 4.9 |
| 3 | Gelatin | 2.5 | 3.6 | 4.6 | 5.5 |
| 4 | Triton X | 3.6 | 4.1 | 4.7 | 5.9 |
| 5 | Tween 80 | 3.8 | 4.6 | 4.6 | 5.8 |
| 6 | Mustard oil | 2.1 | 2.4 | 6.2 | 6.8 |
| 7 | Coconut oil | 1.8 | 2.0 | 5.4 | 5.9 |
| 8 | Groundnut oil | 2.8 | 3.4 | 4.6 | 5.0 |
| 9 | Soybean oil | 1.8 | 2.0 | 5.2 | 5.4 |
| 10 | Toxin (No Adjuvant) | 2.6 | 3.2 | 4.6 | 5.0 |
| 11 | Control (D.W) | 0.0 | 0.0 | 0.0 | 0.0 |

Herbicidal activity of phytotoxins against *Parthenium* leaves was evaluated using formulations containing different adjuvant. Formulations prepared with different adjuvant exhibited phytotoxicity against *Parthenium* leaves to varying degree. Maximum herbicidal activity of phytotoxins obtained from Cz culture filtrate was observed when adjuvant Tween 80 was used in the formulation. Necrosis in *Parthenium* leaves increased significantly when Tween 80 was used as adjuvant in comparison to when only toxin was present. Formulation with oil as adjuvant and phytotototoxins extracted from Cz exhibited lesser phytotoxicity as compared to Tween 80 and triton X.

**Influence of different adjuvant in formulations with phytotoxins obtained from Cz on herbicidal activity of phytotoxins against *Parthenium* leave after 24 hours.**

**Influence of different adjuvant in formulations with phytotoxins obtained from Cz on herbicidal activity of phytotoxins against *Parthenium* leave after 48 hours.**

Phytotoxins obtained from culture filtrate of Cz+ C exhibited maximum phytotoxicity when mustard oil was used as adjuvant in formulation followed by coconut and soybean oil.

**Influence of different adjuvant in formulations with phytotoxins obtained from Cz+C on herbicidal activity of phytotoxins against *Parthenium* leave after 24 hours.**

**Influence of different adjuvant in formulations with phytotoxins obtained from Cz+C on herbicidal activity of phytotoxins against *Parthenium* leave after 48 hours.**

There is an increase in herbicidal activity of phytotoxins when an adjuvant is present in the formulation. Similar results have been reported by Bastos *et al*.,2017. They have reported increase in herbicidal activity of culture filtrate from *Diaporthe* sp. using formulation containing 8.2 wt% of palm oil. Dubovik *et al*., 2020 have supported the potential of the oil-based adjuvant Hasten™ to increase the herbicidal efficacy of natural phytotoxins. The adjuvants in formulations of chemical herbicides are used as wetting agents, penetrants etc. Selection of compatible adjuvant is dependent on many factors: the nature of phytotoxin, weed and crop features and application techniques. Adjuvants help in increasing leaf penetration and herbicidal activity of natural compounds (Dubovik *et al*., 2020). Various adjuvant tested in this study varied in their hydrophilic and lipophilic segments. Herbicidal activity of phytotoxins obtained from Cz increased markedly when tween 80 was used as an adjuvant in the formulation but for phytotoxins obtained from Cz+C mustard oil was observed to cause maximum increase in herbicidal activity of phytotoxins, this might be due to difference in hydrophilic and lipophilic segments of toxins.

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