In *Vitro* Synergistic Fungicidal Activity of Tricyclazole (TCZ) and Tropolone (TRO) in Combination with Voriconazole (VCZ) against VCZ-Resistant (R) *Aspergillus flavus.*

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Background: TCZ and TRO are known melanin inhibitors (MIs) that inhibit melanin via di-hydroxynaphthalene melanin (DHN-M) and tyrosine pathway respectively. **AIM:** (i) To investigate the involvement of these pathways in melanin pigmentation in *A. flavus* and (ii) to evaluate the in *vitro* fungicidal activity of the combination of melanin inhibitors and VCZ against VCZ-R *A. flavus*. **Methods:** Fresh conidial suspensions were prepared from 6-day old *A. flavus* cultures (n=10; 1 wild type and 9 VCZ-R *A. flavus* with MIC > 4 mcg/ml) grown on SD agar +/-TCZ and TRO (at 8 mcg/ml) to evaluate for melanin inhibition. MIC, FICI (fractional inhibitory concentration index), MTT and time kill assays (at 0, 8, 24 and 48 hrs) were performed using MIC and sub-MIC of TCZ, TRO and VCZ in RPMI using CLSI M-38A2 protocol. The fungicidal activities of MIs alone and in combination with VCZ were determined and confirmed using MTT assay. **Results:** *A. flavus* lost its pigmentation in the presence of 8 mcg/ml of TCZ or TRO; TCZ (256 mcg/ml) and TRO (32 mcg/ml) demonstrated intrinsic antifungal activity against WT and VCZ-R *A. flavus*. Sub-MICs of MIs plus VCZ demonstrated synergism against wild type (WT) and VCZ-R *A. flavus* at 24h and 48h with FICI < 0.5. Subsequent MTT-based spectrophotometry and time kill assays showed >99.9% killing of *Aspergillus* spores at 48 hrs. Neither VCZ nor MIs alone showed fungicidal activity at sub-MIC against VCZ-R *A. flavus*. **Conclusions:** Both TCZ and TRO (MIs) have intrinsic antifungal activity against *A. flavus* and demonstrate good synergistic antifungal activity when used in combination with VCZ against VCZ-R *A. flavus* and need further investigation.