Indian J. Applied & Pure Bio. Vol. 39(1), 624-631 (2024).
ISSN: 0970-2091
A Web of Science Journal

Thiram-mediated Endosulfan potentiates cytotoxicity in *Saccharomyces cerevisiae* by oxidative stress and necrotic cell death

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Abstract

In *Saccharomyces cerevisiae* (yeast cells), the cytotoxicity of two pesticides, thiram and endosulfan, was investigated. Thiram cytotoxicity was substantially lower than endosulfan cytotoxicity, with LC_{50} concentration (1 h exposure) values of 0.6 and 1.0 mM, respectively. The insecticides' lethal activity on cells was characterized by cell death, lactate dehydrogenase (LDH) leakage, glutathione depletion and the production of reactive oxygen species (ROS). The pesticide-induced cell death, LDH leakage confirmed that it was necrotic. When cells were exposed to a mixture of both compounds, thiram potentiated the cytotoxicity of endosulfan at lower concentration. Excessive glutathione depletion and induction ROS were found to be more potentiating of cytotoxicity than the cumulative effects of individual substances.

Key words : Thiram, Endosulfan, oxidative stress, cell death.

The widespread usage of pesticides has raised severe environmental concerns. Pesticides are predicted to be utilized in excess of two billion tons worldwide[18]. The majority of pesticides used end up poisoning the environment, creating a substantial health danger to humans, domestic animals, and wildlife. Although the toxicity of individual pesticides has been extensively researched[7], the toxicological impact of a mixture is comparatively unknown. As a result, investigations on a mixture of chemicals or pesticides are required in order to understand their interactions with biological systems and estimate their risk.

Thiram, a carbamate chemical, is widely used as a fungicide and for seed treatment. Thiram is also a byproduct of the fungicides ziram and ferbam's oxidation[3]. Thiram has modest acute toxicity and has been linked to chronic effects on the neurological system, reproduction, and development.

Endosulfan, an organochlorine pesticide, because of its severe toxicity and environmental persistence, is prohibited or restricted in many countries. Endosulfan is extremely poisonous to fish and animals, wreaking havoc on their neurological, immunological, and reproductive systems.

Previous cell culture experiments have demonstrated that both thiram and endosulfan produce cytotoxicity via oxidative stress[2,9,19]. Thiram cytotoxicity has been linked to glutathione depletion and changes in cellular antioxidant defense mechanisms[6]. The goal of this study was to look into the interaction of two pesticides on cytotoxicity in *Saccharomyces cerevisiae* (yeast cells) to study reactive oxygen species (ROS) and oxidative stress. Yeast cells have been found to be a useful biological model for studying xenobiotic cytotoxicity mediated by oxidative stress[5].

Chemicals :

Baker yeast from local market, technical endosulfan (98% pure) was procured from Excel Industries Ltd., Bhavnagar. Technical thiram (98% pure) was pro- cured from Dupont Co., USA. Trypan blue, 5–50 dithiobis (2-nitrobenzoic acid) (DNTB), trichloroacetic acid (TCA), nicotinamide adenine dinucleotide (reduced) disodium salt (NADH), reduced glutathione (GSH), and bovine serum albumin (BSA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Nitro blue tetrazolium (NBT), nicotine adenine dinucleotide (NAD), dimethylsulphoxide (DMSO), were obtained from Sisco Research laboratory, India.

Cell culture :

Saccharomyces cerevisiae (Baker yeast) was cultured in YPD broth. Two to three granules of yeast granule was inoculated to 1000 ml of autoclaved YPD broth, further incubated in orbital shaker at 3000 rpm at 37ºC for 24 hr.

Cytotoxicity :

To find the least cytotoxic concentration, *Saccharomyces cerevisiae* (10×10^6) suspended in 1 ml of YPD broth were treated with varied doses of thiram for 1 hour at 37ºC on a shaker water bath to determine least lethal concentration (LC). Cells were similarly treated with different doses of endosulfan to determine the LC_{50} concentration. The pesticides were dissolved in DMSO, and the final solvent content did not exceed 20 µl/ml, which had no influence on cell survival. In another experiment, Yeast cells were exposed for 1 hour at 37ºC to a combination of sublethal thiram doses and the LC_{50} concentration of endosulfan. Parallel controls using just the solvent were also used. The trypan blue exclusion method was used to determine cell viability[15].

Lactate dehydrogenase (LDH) leakage :

Following incubation, cells were centrifuged at 2500 rpm for 5 minutes, and the supernatant was tested for LDH using lactate as the substrate by detecting the change in absorbance at 340 nm [1].

Reactive oxygen species (superoxide anion):

At 37ºC on a shaker water bath, *Saccharomyces cerevisiae* (10×10^6) suspended in YPD were treated with NBT (0.2 mM) and

individual pesticides or the mixture in a final volume of 1.0 ml. Cells were treated with NBT (0.2 mM) and solvent (DMSO) in parallel control incubations. After incubation, cells were placed in a boiling water bath for 30 minutes and 1 ml dioxan was added. The colored formazan generated in cells as a result of NBT reduction by ROS was measured using a spectrophotometer at 500 nm [18].

Glutathione assay :

After incubation, the cells were rinsed with physiological saline, homogenized in 1.0 ml tris buffer (0.1 M, pH 7.4), centrifuged at 5000 rpm for 5 minutes at 4ºC, and glutathione levels in the deproteinized supernatant were determined using Ellman's reagent[4].

Cell viability :

Thiram was not cytotoxic up to 0.2 mM, and the LC_{50} for 1 h exposure was 0.6mM. Endosulfan produced dose-dependent cell killing, with an LC_{50} of 1 mM (Figs. 1 and 2). Cell mortality was greater in cells subjected to a mixture of sublethal concentrations of thiram (0.2 mM) and endosulfan (LC_{50}) than in cells exposed to individual chemicals (Figs. 3 and 4). Cell mortality was increased in cells exposed to sublethal doses of thiram (0.2 mM) and endosulfan (LC_{50}) 1mM.

LDH leakage :

There was no substantial LDH leakage in cells exposed to sublethal quantities of thiram (0.2mM), whereas endosulfan (LC_{50}) induced a threefold increase in enzyme leakage. LDH leakage was increased 4-fold higher when cells were treated with a mixture of sublethal concentrations of thiram (0.2 mM) and LC_{50} concentrations of endosulfan (Fig. 5).

ROS :

In contrast to endosulfan, which increased ROS generation by 4.6-fold at LC_{50} concentration, thiram did not cause ROS production in cells exposed to sublethal concentrations. ROS induction was 7.26 times higher in cells treated with both chemicals (thiram, 0.2 mM + endosulfan, 1.0mM) than it was with either component alone (Fig. 6). ROS production in pesticide-treated cells is associated with cell death.

GSH :

In cells treated with thiram at sublethal concentrations, glutathione cellular levels were decreased. Glutathione was reduced by 37% in cells treated with thiram alone, 56.92% in cells treated with endosulfan, and 80.76% in cells treated with both chemicals.

Several *in vivo* experiments have revealed that the toxicity of a xenobiotic is modulated by another chemical exposure[10]. For example, atrazine, a non-toxic herbicide, increased the toxicity of organophosphorous insecticides[17]. Phosphorothioate compounds increased the acute toxicity of carbamate insecticides[20]. In vitro tests on the human leukemia cell line HL-60 revealed that the insecticide pirimiphos methyl inhibited the cytoxicity of the fungicide benomyl[12]. Although thiram has been shown to reduce pesticide toxicity[21], the mechanisms involved remain unknown.

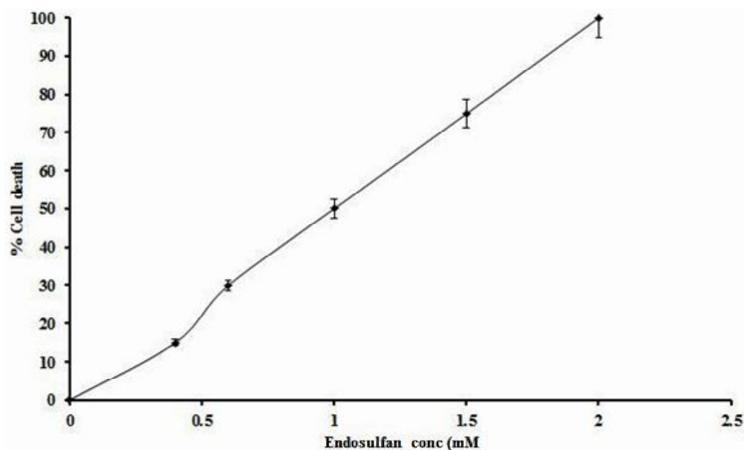

Fig. 1: Cytotoxicity of endosulfan on *S. cerevisiae* cell viability at 1 h exposur

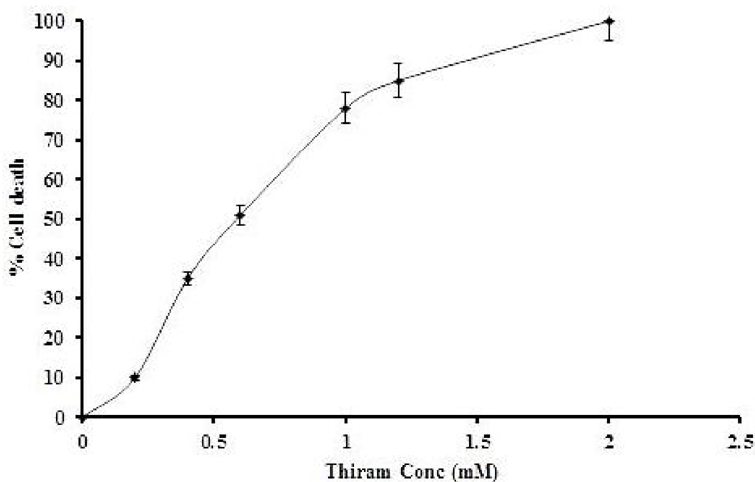

Fig. 2. Cytotoxicity of thiram on *S. cerevisiae* cell viability at 1 h exposure.

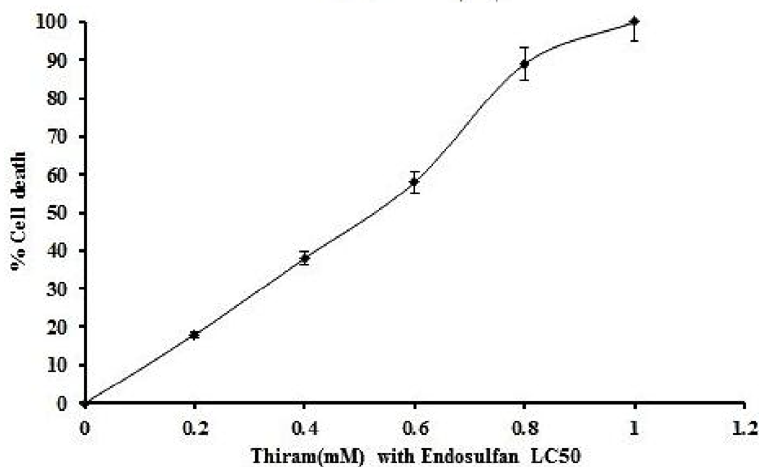

Fig. 3. Effect of sublethal concentrations of thiram (0.2mM) + LC_{50} concentration of endosulfan (1mM) on *S. cerevisiae*: cell viability at 1 h exposure.

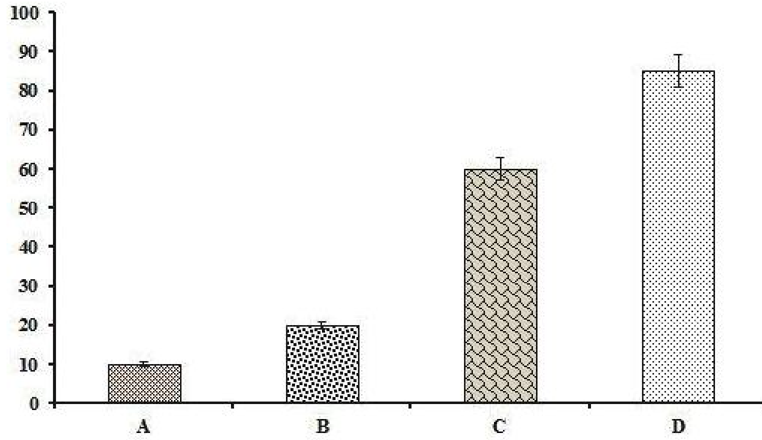

Fig. 4. Potentiation of endosulfan cytotoxicity by thiram in *S. cerevisiae*: cells were exposed to sublethal concentration (0.2 mM) of thiram + LC_{50} concentration (1mM) of endosulfan for 1 h. A: solvent control; B: thiram (0.2 mM); C: endosulfan (1 mM); D: thiram (0.2 mM) + endosulfan (1mM).

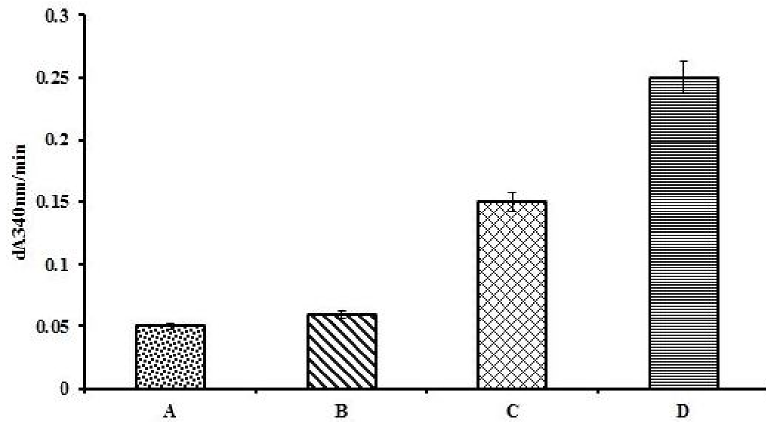

Fig. 5. Potentiation of endosulfan cytotoxicity by sublethal concentration of thiram In *S. cerevisiae* : LDH leakage. A: Solvent control; B: thiram (0.2 mM); C: endosulfan (1mM); D: thiram (0.2 mM) + endosulfan (1mM).

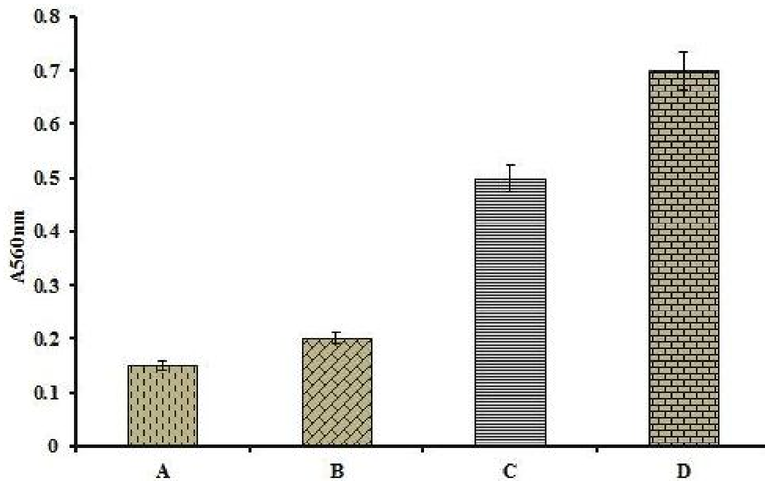

Fig. 6. Effect of combined exposure of sublethal concentration of thiram and endosulfan (LC50 concentration) on ROS induction in *S. cerevisiae*. A: Solvent control; B: thiram (0.2 mM); C: endosulfan (1 mM); D: thiram (0.2 mM) + endosulfan1mM

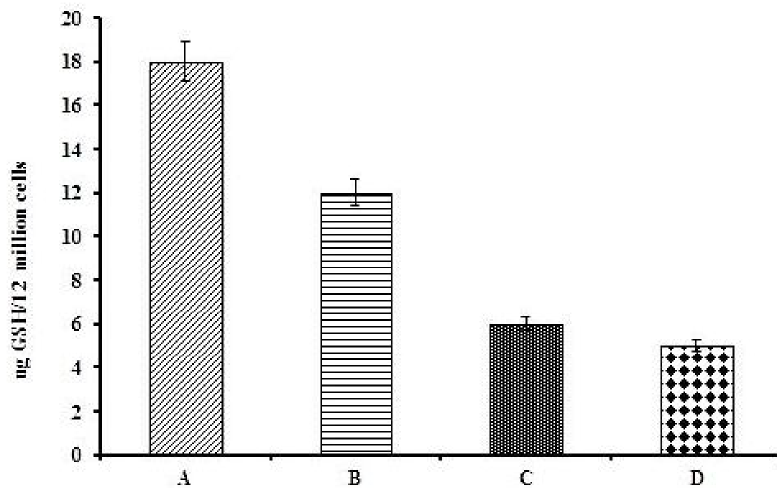

Fig. 7. Effect of thiram and endosulfan individually and in combination on glutathione levels in *S. cerevisiae*. A: solvent control; B: thiram (0.2 mM); C: endosulfan (1 mM); D: thiram (0.2 mM) + endosulfan (1 mM).

Cell culture studies provide an excellent in vitro model system for investigating the biochemical mechanisms involved in xenobiotic-induced cell death.

The cytotoxicity of thiram has been studied in human and master fibroblast cells[2,6]. Thiram produced necrotic cell death that was unrelated to gene expression and mitochondrial dysfunction, according to Cereser *et al.*,[2]. Dysfunction was a side effect of harmful action. Thiram was discovered to primarily deplete GSH, which is what causes cytotoxicity in fibroblasts[2]. By depleting GSH and altering the redox balance of the cells[6] provided strong evidence that thiram cytotoxicity involved cell death caused by free radicals.

According to our research on *Saccharomyces cerevisiae* cells, thiram had lesser cytotoxicity than endosulfan (LC_{50} = 0.6 mM) after an hour of treatment (LC_{50} = 1.0mM). Additionally, our findings clearly demonstrate that the cytotoxicity entailed necrotic cell death, as demonstrated by LDH leakage. However, greater cytotoxicity was seen when cells were exposed to a combination of sublethal concentrations of thiram and LC_{50} concentrations of endosulfan.

LDH leakage and enhanced cell death indicated necrotic type of cell death. When cells were exposed to a combination of sublethal concentrations of thiram (0.2 mM) and endosulfan (LC_{50} concentration), the cell mortality was noticeably higher than it was with either exposure alone. This clearly illustrates how endosulfan's cytotoxicity is increased by non-toxic concentrations of thiram. The ubiquitous cellular tripeptide GSH is an essential component of the body's defensive mechanisms against toxic insults and free radical oxidative damage to cells[16]. Non-enzymatically, GSH rapidly interacts with oxidizing species, while enzymatically; glutathione peroxidases and transferases detoxify harmful compounds[22].

GSH is depleted in the cells over the course of GSH-dependent detoxication

activities; it is then replenished by synthesis and glutathione reductase's reduction of the oxidized form of glutathione (GSSG)[11]. Oxidative stress develops when cells experience excessive GSH depletion as a result of chemical exposure[2]. According to Robertson and Orrenius[16] GSH-dependent processes are used to detoxify xenobiotics, including pesticides.

The depletion of cellular GSH by thiram and endosulfan has been demonstrated[2,6,14]. Our findings indicate that *Saccharomyces cerevisiae* exposed to thiram and endosulfan had reduced GSH levels. In comparison to exposure to the two compounds separately, the GSH depletion was greater in cells exposed to a mixture of thiram and endosulfan. Additionally, the mixture caused higher levels of ROS production in Yeast cells than it did in cells exposed to the individual compounds, indicating greater oxidative stress. In *Saccharomyces cerevisiae* subjected to the mixture, GSH depletion, ROS production, and cell death were greater than the sum of the effects of the separate compounds. greater cell death was directly connected with greater GSH depletion ROS generation.

Olgun *et al.*,[13] reported on the potentiation of cytotoxicity by the insecticides Malathion, lindane, and permethrin, where they demonstrated the presence of both necrotic and apoptotic types of cell death in murine thymocytes.[8] Jia and Misra recently revealed that endosulfan and zineb, a dithiocarbamate, together potentiated the cytotoxicity of neuroblastoma cells. The research suggests that the intensity and length of the toxic insult affect the kind of cell death, whether necrotic or apoptotic, in cells exposed to xenobiotics. Depending on the experimental circumstances, oxidative stress in cells brought on by chemical exposure could lead to either apoptosis or necrosis. According to Cereser *et al.*[2], thiram's cytotoxicity in human skin fibroblasts includes necrosis.

The mechanism of thiram's potentiation of endosulfan's cytotoxicity included excessive GSH depletion, which increases oxidative stress, as shown by ROS production. Our research on a basic *in vitro* system shows that the exposure of cells to relatively non-toxic chemicals can significantly alter the harmful response.

Oxidative damage induced by thiram was low compared to endouslfan whereas with sublethal concentration of thiram with LC_{50} concentration of endosulfan induces high oxidative stress with necrotic cell death in *Saccharomyces cerevisiae* Therefore, it is crucial that the potential interactions of a variety of xenobiotics on living systems be considered in the toxicity risk evaluation of pesticides.

References :

1. Bergmeyer, H.U., (1974). *Toxicology 162:* 89–101.
2. Cereser, C., S. Boget, P. Parvaz, and A. Revol, (2001b). *Toxicology 163:* 153–162.
3. Edwards, I.R., D.H. Ferry, and W.A. Temple, (1991). Fungicide and related compounds. In: Hayes, W.J. *et al.* (Eds.), Handbook of Pesticides Toxicology. Academic Press, New York, pp. 1436–1469.
4. Ellman, G.L., (1959). *Arch. Biochem.*

Biophys. 82: 70–77.

5. Fernando. J.S., Jahn Luciane, Paula Ana L. Delamare, Sergio Echeverrigaray (2018). *Pest. Biochem.Physiol. 149:* 137-142.
6. Grosicka-Maciag, E., D. Kurpios, H. Czeczot, M. Szumilo, M. Skryzycki, P. Suchocki, and P. Rahden-Staron, (2008). *Toxicol. In Vitro 22:* 28–35.
7. Groten, J.P., F.R. Cassee, P.J. Van Bladeren, C. DeRosa and V.J. Feron (1999). Mixtures. In: Marquardt, H., Schafer, S.C., McClellan, R., Welsch, F. (Eds.), Toxicology. Academic Press, New York, pp. 257–270.
8. Jia, Z., and H.P. Misra, (2007). *J. Appl. Toxicol. 27:* 434–446.
9. Kannan, K., R.F. Holcombe, S.K. Jain, X. Alvarez-Hernandez, R. Chervenak, R.E. Wolf, and J. Glass, (2000). *Mol. Cell Biochem. 205:* 53–66.
10. Liang, T.T., and E.P. Lichtenstein, (1974). *Science (New York, NY) 186:* 1128–1130.
11. Lu, S.C., (2000). *Curr. Top. Cell Regul. 36:* 95–116.
12. Marinovich, M., M. Guizzetti, and C.L. Galli, (1994). *Toxicology 94:* 173–185.
13. Olgun, S., R.M. Gogal, F. Adeshina, H. Choudhury, and H.P. Misra, (2004). *Toxicology 196:* 181–195.
14. Pandey, S., I. Ahmad, S. Parvez, B. Bin-Hafeez, R. Haque, and S. Raisuddin, (2001). *Arch Environ. Contam. Toxicol. 41:* 345–352.
15. Pompeia, C., M.F. Cury-Boaventura, and R. Curi (2003). *Brazil. J. Med. Biol. Res. 36:* 1549–1560.
16. Robertson, J.D., and S. Orrenius, (2000). *Crit. Rev. Toxicol. 30:* 609–627.
17. Schuler, L.J., A.J. Trimble, J. B. Belden, and M.J. Lydy, (2005). *Arch. Environ. Contam. Toxicol. 49:* 173–177.
18. Smith, C. (2001). *Int. J. Occup. Environ. Health 7:* 266–274.
19. Sohn, H.Y., C.S. Kwon, G.S. Kwon, J.B. Lee, and E. Kim, (2004). *Toxicol. Lett. 151:* 357–365.
20. Takahashi, H., A. Kato, E. Yamashita, Y. Naito, S. Tsuda, and Y. Shirasu, (1987). *Fundam. Appl. Toxicol. 8:* 139–146.
21. Wysocka-Paruszewska, B., A. Osicka, J. Brzezinski, and I. Gradowska, (1980). *Arch. Toxicol. Suppl. 4:* 449–451.
22. Yang, M.S., H.W. Chan, and L.C. Yu, (2006). *Toxicology 226:* 126–130.