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Eco-friendly synthesis of Mgo nanoparticles from *Azadirachta Indica* and their XRD analysis with antibacterial and antifungal studies

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Abstract: *Azadirachta Indica* is a medicinal plant used in traditional medicine due to bioactive constituents for a variety of human ailments as domestic treatment, including its parts, such as stem, leaves, roots, bark, flowers, and fruit. Antimicrobial and insecticide agents were observed in the derived extract. Over microbes, fewer biohazards, and safe and easily available plant extracts are favored for the production of nanomaterials. Unique properties of metal/metal oxide nanoparticles have widespread applications in multiple domains of technology and science. The aim of our study is to synthesize MgO NPs from Azadirachta indica by using Magnesium Acetate, its XRD analysis, and antimicrobial studies. In NPs synthesis, Azadirachta indica acts as a stabilizing and reducing agent. And characterization of NPs is done through XRD analysis to confirm its semi-crystalline structure embedded in amorphous background. Antibacterial bioassays show that Neem-mediated MgO NPs have enhanced antimicrobial activity than Neem extract against *Ecoli*, *P. aeruginosa and S.aureus* due to increased surface area, oxidative stress induction, and phytochemical effects. In antifungal assays Neem-mediated MgO NPs showed enhanced antifungal activity through various mechanisms i.e. spore germination destruction, ergosterol synthesis in fungal cell membrane, and ROS-mediated cell death. These findings suggest the NPs as a promising candidate for biomedical applications. The incorporation of nanotechnology with plant extract is an ecofriendly, effective and sustainable approach to develop a best biomedical solution. By utilizing both the wisdom of nature and nanoscience we can address the health challenges like antibiotic resistance, chronic wound infections, site-specific delivery, and open way for safe therapeutics in the future.

Keywords: *Azadirachta Indica* (Neem), MgO Nanoparticles (NPs), Antimicrobial Activity, Biomedical Applications, Sustainable and Ecofriendly

1. Introduction

Neem has been utilized in various ways since the time of Vedic civilization in India. In ancient times,

as a domestic treatment for a variety of human ailments, all components of the tree, i.e., stem, leaves, bark, gum, roots, flowers, fruits, and seeds, were utilized as traditional medicines. The evergreen plant Azadirachta Indica, also known as Neem, has multiple chemical components, such as nimbinine and nimbendiol, and it grows quickly in tropical and subtropical regions. Due to medicinal advantages, the earliest plant species, Azadirachta indica (Neem), is renowned. The Meliaceae family to which it belongs. It has a long history of being known as the "wonder tree" in India(Devi & Sharma, 2023).

In multiple diseases and crop pests control demonstration of numerous bioactive components of Neem as beneficial for mankind.(Adusei & Azupio, 2022)..Antipyretic, antibacterial, antacid, antiviral, antiparasitic, antidiabetic, anti-inflammatory, contraceptive, anticancer, antidermatitic, healing, dental, antifungal and protective properties are the declare properties due to which, in traditional Indian medicines for millennia, Neem tree parts have been utilized. For human diseases, household remedies have been employed by using the indica parts (leaves, roots, etc.) (Wylie & Merrell, 2022).

Particles having size ranges from 1-100nm are called nanoparticles. And for certain nanoparticle materials its catalytic and adsorptive properties are good (Joseph et al., 2023).

Widespread attention captivated by nanotechnology is an innovative concept. With other methods, risk of problems, application and nanomaterial manufacturing cutback by exemplary effort of green nanotechnology.

Nanoparticle production by biological process presented by fig1.

Constructure characteristics i.e. shape and size is altered by reaction situations such as pH, temperature and chemical conditions. In comparison to massive form nanoparticle much large surface area and very small size is employed by nanotechnology. Thermal potential, chemical and optical are collection of assets that nanomaterial holds. At nanoscale many bulks material have different properties. In environmental, biological and medical applications includes anti-inflammatory, antimicrobial, bioactivity, biocompatibility, bioavailability, biological absorption, tumor targeting and effective drug delivery nanoparticles are used. For nanoparticles synthesis by green synthesis methods from biological agents i.e. viruses, plants, algae, fungi and bacteria should not hinder synthesized nanoparticles application due to nonpathogenic in nature (Pandit et al., 2022).

Pathways for Production of nanoparticles

Under two definite groups i.e. top-down and bottom-up methods multiple organic and physiochemical routes lie for synthesis of metal oxide NPs (Aigbe & Osibote, 2024).

Top-Down Method

In TDM by applying lithographic method (photo-reduction, thermal evaporation, sputtering, grinding, milling, and chemical etching) a fine particle is formed from bulk materials by reducing size for NPs synthesis and all these procedures include in TDM. (Aigbe & Osibote, 2024).

Bottom -Up Method

By using different chemical and biological techniques, miniature matters (atoms and molecules) are used for NPs production and are developed further into nanoscopic particles For minimum contamination green, NPs are synthesized through this technique by using natural biomass sources i.e. biomolecules (lipids, pigments, proteins, polysaccharides etc.), plants as biologic-reducing and stabilizing agents, micro and macro-organisms (fungi, algae, bacteria and

selected viruses).Use of various biological sources is a breathtaking step, is made through NPs synthesis expansion and green methods. Nanotechnology includes wet, dry and computational engineering procedures. For NPs production various methods are involved are shown by fig. (Aigbe & Osibote, 2024). Numerous studies have been previously conducted on metal oxide NPs synthesis by using Azadirachta indica as a stabilizing and reducing agent and their properties. Various others researches investigated phytochemical analysis of A.indica and its antibacterial activity. This research aims to address the gap by evaluating MgO NPs synthesis from neem extract by using metal salt i.e. magnesium acetate and its antifungal and antibacterial properties. As we know that magnesium acetate is stable, eco-friendly, biodegradable and safer to use for green synthesis of nanoparticles. Magnesium acetate has best benefit that it is less toxic and body can metabolize it easily.

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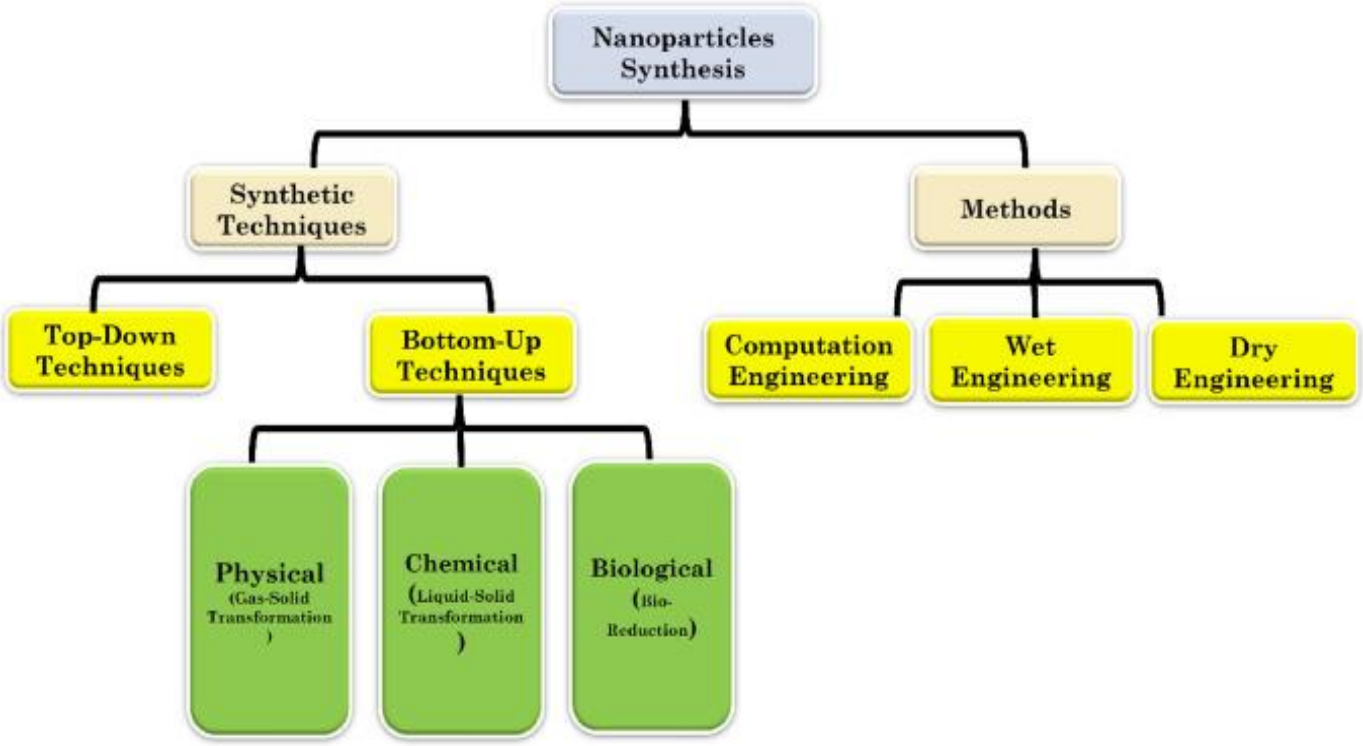

Figure 1: Approaches devoted to the NPs production

2. Material and Method

Green Synthesis of *A. indica* Mediated MgO Nanoparticles In this work, analytical-grade materials and reagents were utilized. Methanol HPLC grade 99.9% purity, magnesium acetate tetrahydrate, were purchased from Sigma. Additionally, the Neem leaves were purchased from the local market of Abbottabad, Pakistan. Distilled water is used as a solvent obtained from the scientific market of Abbottabad. NaOH was purchased from Sigma. The generated nanoparticles' antimicrobial effectiveness was tested against various types of multidrug-resistant human pathogens, including Escherichia coli and Staphylococcus aureus. The biomedical research center of Abbottabad University of Science and Technology (AUST) provided these human pathogens under study. Luria–Bertani agar was prepared by dissolving 1.25 g yeast extract, 3.5 g sodium chloride (NaCl), 2.5 g tryptone, and 3.75 g bacteriological agar in 250 mL of distilled water. The mixture was thoroughly stirred on a magnetic stirrer until all components were completely dissolved. The prepared medium was sterilized by autoclaving at 121 °C for 15 minutes under 15 psi pressure. After autoclaving, the molten LB agar was cooled to approximately 45–50 °C before being poured into sterile Petri dishes under aseptic conditions in a biosafety cabinet. The plates were allowed to solidify at room temperature and were stored at 4 °C until use for antibacterial assays.

Extraction

In teaching laboratory of AUST we took A.indica leaves from local shop. Then washed it with deionized water gently to remove all the dust and impurities. Repeat this washing three times spread washed leaves on cotton cloth for 2-8 hours at room temperature. Then grind the dried leaves in the machine into fine powder of leaves are formed. After this measure the 50grams of *Azadirachta indica* leave powder. Take 200ml of deionized water and pour it into a beaker and add 50 grams of leave powder in it and boil it on low flame at 60°C for 15 minutes. Then place it for cooling after cooling filter the extract by using a Whatman filter paper no 1.

Preparation of Magnesium Acetate Solution

Take a magnesium acetate salt(sigma) then by using weight balance measure 10.7milligrams of magnesium acetate and then put deionized water to make 50 ml solution and stir it by using a magnetic stirrer for few minutes make sure that it is completely dissolve in DI water.

Preparation of Nanoparticles

Firstly, sample of MgO NPs are prepared biologically through co precipitation technique by using magnesium acetate as a precursor of magnesium. Azadirachta indica act as reducing agent for magnesium. In

50ml of reducing extract 1mM magnesium acetate solution are added drop wise stir at 600 rpm for 30min.By adding 1% NaOH to this solution to adjust pH at 12. Precipitation of MgO synthesis is maximized. For following up an aging procedure at room temperature for 2h and is stirred. At the room temperature precipitation period of 24h is followed. At 7000rpm centrifugation is applied for recovery of nanoparticles that are generated. Unreacted ions are removed by using three rounds of washing. By using oven at 100°C sample are dried for 12h and at 450°C for 2 hours it is calcinated to remove the volatile components.

X-Ray Diffraction Analysis

In the figure the X-ray diffraction (XRD) patterns of neem-derived NPs are shown. In 2θ region, at 15-30° a broad hump is observed in diffractograms showing the presence of amorphous material derive from bioactive components of neem. At 2θ = 36.9°, 42.9°, 62.3°, 74.7°, and 78.6° distinct crystalline peaks are observed. To the planes of cubic magnesium oxide (MgO, JCPDS card no. 45-0946) i.e. (111), (200), (220), (311), and (222) these reflections correspond. MgO NPs formation is successfully confirmed through these results. At 18–19° and around 29–33° minor peaks was observed that indicates the presence of residual Mg $(OH)_2$ and basic magnesium carbonates.

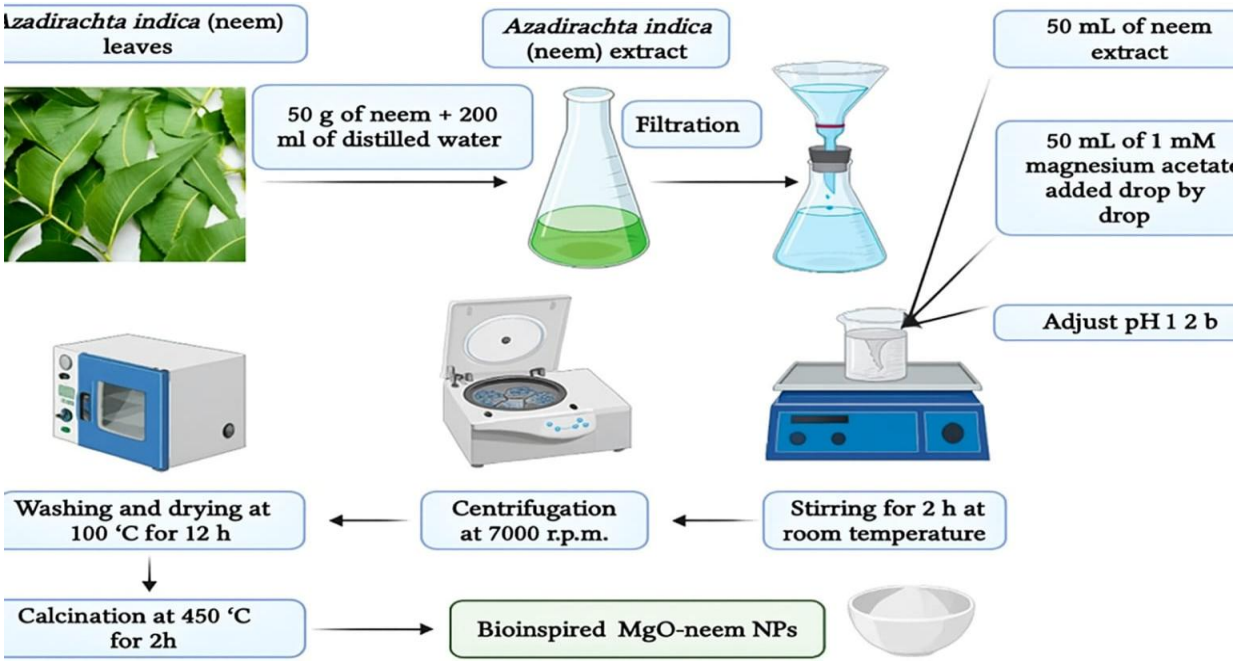

Figure 0: Schematic Illustration of the Synthesis Conditions of Bioinspired MgO NPs.

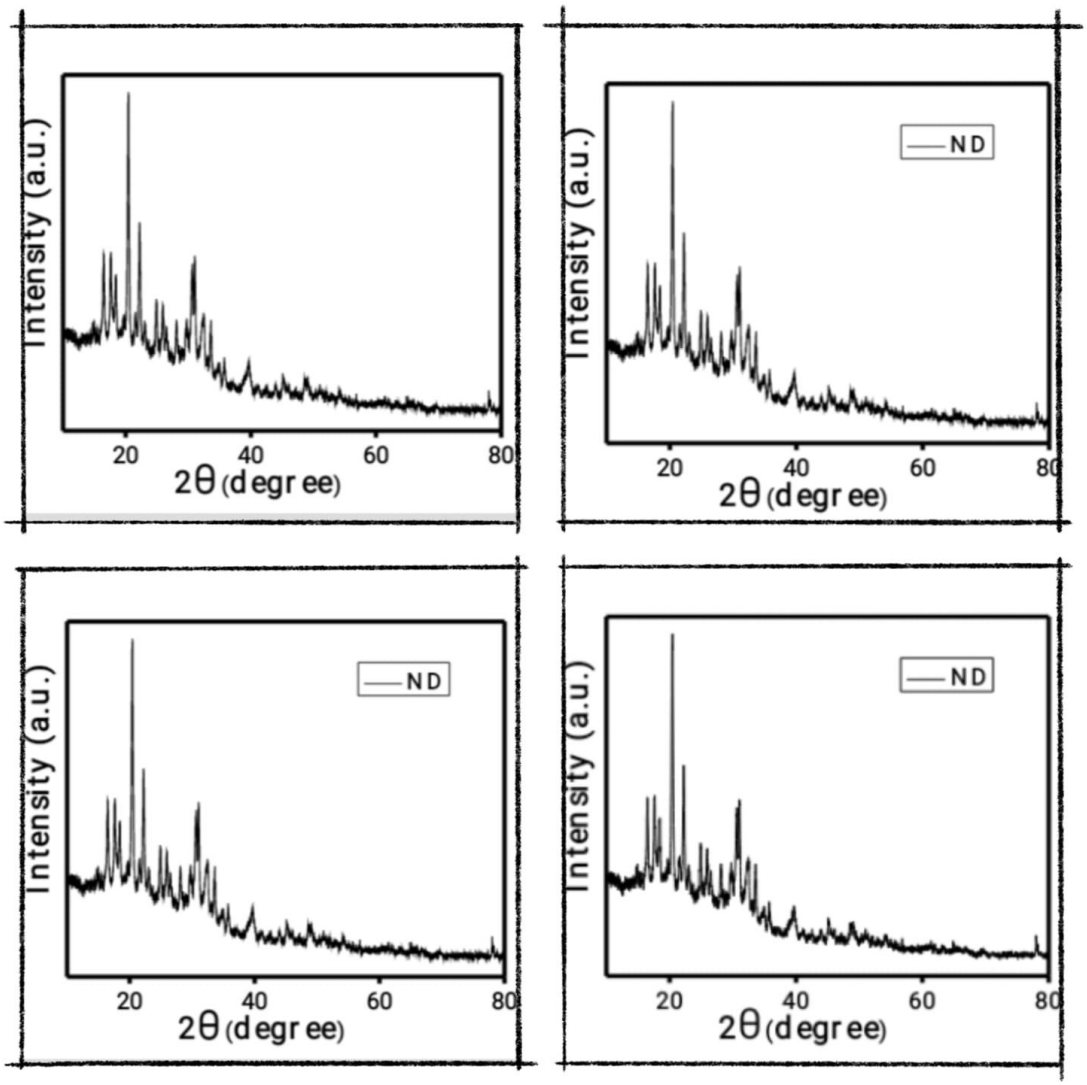

Figure 0:XRD Analysis of MgO NPs of *A. Indica*.

3. Results & Discussion

3.1 Antibacterial Activity of Nanoparticles:

Agar well diffusion method is used against three bacterial strains E. coli, S. aureus, and P. aeruginosa to analyze antimicrobial potential of synthesized NPs. Wide zone of inhibition is clearly seen in case of plate labeled with S. aureus. Against a Gram-positive bacterium i.e. *S. aureus* NPs showed a strong antibacterial activity. A smaller zone of inhibition is observed in case of *E. coli* as compared to *S. aureus*. Against a Gram-negative bacterium (*E coli*) with outer membrane that inhibits penetration of antimicrobial agents, moderate antibacterial activity is shown. Among all these three smallest zones of inhibition is detected in case of *P. aeruginosa* indicating lowest sensitivity to NPs. Strong resistance mechanisms (including efflux pumps and biofilm formation) are the property of P. aeruginosa that reduce the activity.

3.2 Antifungal Activity of Nanoparticles

Two fresh slices of bread are taken to test the antifungal property of neem-mediated NPs. Under the same environmental conditions, these bread

slices are sealed separately in polyethylene bags. Sample #1: Experimental slice (sprayed with neem NPs). Sample #2: Control slice (sprayed with deionized water)

In case of sample 1After 7 days, the bread is detected. There are no common bread molds colonization and fungal growth is observed. Spore germination inhibition and fungal cell wall synthesis are disrupted due to neem-derived compounds, i.e., limonoids, azadirachtin, etc. Additionally, antimicrobial efficacy is improved due to bioavailability and surface contact of NPs.

In case of sample, 2 After 7 days, bread is detected. More degradation of bread is seen due to no antimicrobial protection. Antifungal property showed by neem NP treated bread with absence of mold growth as compared to the control sample. Sustainable antifungal activity provided by neem bioactive compounds and synergic action of NPs.

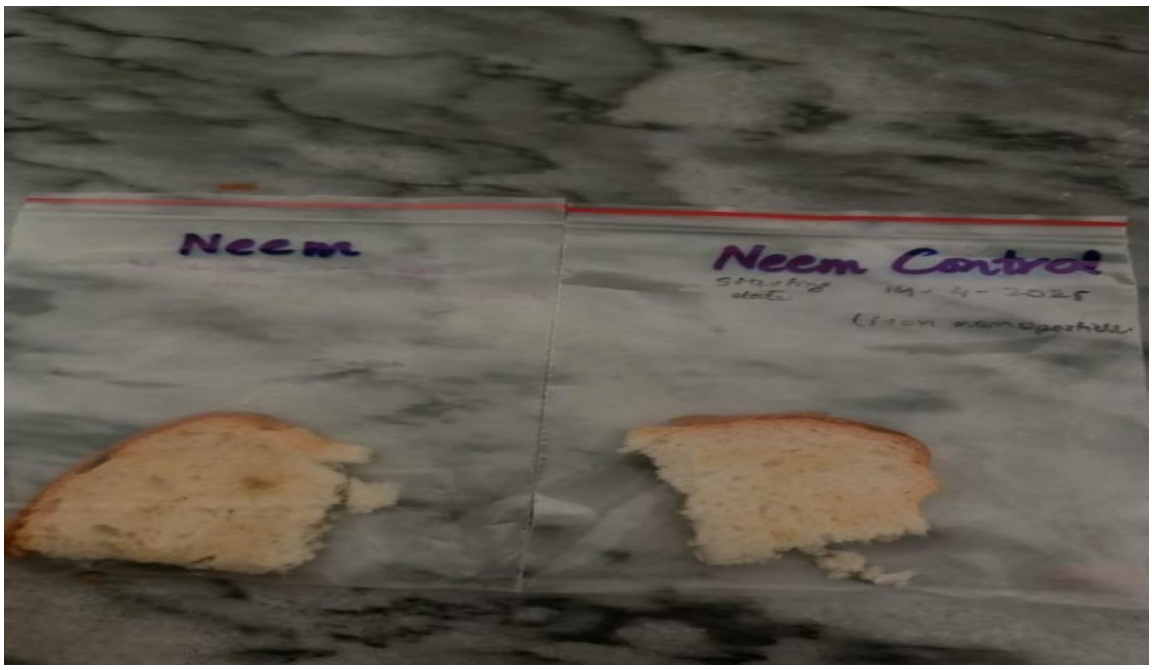

Figure 0: Antifungal assay of Neem mediated MgO NPs

4. Conclusion

Biogenic nanomaterials having prominent antibacterial and antifungal properties are derived from neem-mediated synthesis of magnesium oxide nanoparticles (MgO NPs) using magnesium acetate. Through affecting cell wall structure and intrinsic resistance mechanisms against S.aureus a highest activity moderate activity in case of *E. coli* and least inhibition of P.aeruginosa are showed by NPs. Through various mechanisms, including reactive oxygen species (ROS) generation, cell wall/membrane disruption, and bioactive neem phytochemicals, NPs perform actions that are suggested by the sensitivity of fungal pathogens. Dual antimicrobial action, i.e. Physical and biochemical, as presented by a combination of neem biomolecules and magnesium oxide, are confirmed due to the alignment of consistent inhibitory patterns with published findings on neem-derived MgO NPs. For broad-spectrum antimicrobial agents' development, a promising rout is presented by this eco-friendly and cost-effective synthesis approach, and is useful in agricultural pathogen control, biomedical applications, and surface coating. In this era of drug resistance, the potential of phyto-mediated MgO NPs as a sustainable alternative to conventional antimicrobial agents is supported by the results.

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