



ISSN Print: 2664-844X
 ISSN Online: 2664-8458
 NAAS Rating (2026): 4.97
 IJAFA 2026; 8(4): 508-513
www.agriculturaljournals.com
 Received: 08-01-2026
 Accepted: 12-02-2026

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Efficacy of botanical leaf extracts as anti-feedants against *Riptortus linearis* (Fabricius) and their potential in sustainable black gram (*Vigna mungo*) production

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DOI: <https://www.doi.org/10.33545/2664844X.2026.v8.i4g.1560>

Abstract

How effectively can plant-derived compounds replace synthetic insecticides in protecting black gram from pod-sucking pests? This research evaluated the anti-feedant properties of aqueous leaf extracts from four botanicals *Azadirachta indica* (neem), *Calotropis gigantea* (calotropis), *Vitex negundo* (vitex), and *Eucalyptus globulus* (eucalyptus) against *Riptortus linearis* (Fabricius), a major pod-sucking pest of black gram (*Vigna mungo*). Three concentrations (10%, 15%, and 20% w/v) were tested using choice and no-choice feeding bioassays under controlled laboratory conditions at the National Autonomous University of Mexico, Mexico City, during March-September 2023. The experimental layout followed a randomized complete block design with five replications per treatment. Among all extracts, neem at 20% concentration recorded the highest feeding deterrence index (89.4%), followed by vitex at 20% (76.5%) and calotropis at 20% (68.1%). Eucalyptus showed moderate anti-feedant activity, with the 20% treatment achieving a 61.8% deterrence index. Leaf area consumption was lowest in neem-treated groups across all concentrations, and a clear dose-dependent response was observed for each botanical. Statistical analysis through one-way ANOVA and Duncan's multiple range test confirmed that differences between treatments were significant ($p < 0.05$). These results point to neem and vitex leaf extracts as promising botanical alternatives for managing *R. linearis* in black gram cropping systems, offering a reduced environmental footprint compared to conventional chemical pesticides.

Keywords: Anti-feedant activity, botanical leaf extracts, *Riptortus linearis*, *Vigna mungo*, feeding deterrence index, neem, integrated pest management, sustainable agriculture, pod-sucking pest, bioassay

Introduction

Black gram (*Vigna mungo* L. Hepper) ranks among the most widely cultivated pulse crops in tropical and subtropical regions, serving as a key source of dietary protein for millions of people ^[1]. The crop plays a direct role in food security and soil fertility through its capacity for biological nitrogen fixation ^[2]. Despite its agronomic and nutritional value, black gram production faces persistent threats from a range of insect pests, with pod-sucking bugs representing a particularly damaging group ^[3]. *Riptortus linearis* (Fabricius), a coreid bug belonging to the family Alydidae, has emerged as a serious pest of grain legumes across Asia, Africa, and parts of Latin America ^[4]. Both nymphs and adults feed on developing pods by piercing the pod wall with their stylet mouthparts, causing seed shrivelling, reduced grain weight, and in severe infestations, complete crop failure ^[5].

Conventional pest management for *R. linearis* relies heavily on synthetic chemical insecticides, which although effective in the short term carry well-documented drawbacks. Repeated use of organophosphates and pyrethroids has been linked to insecticide resistance in target pest populations, harm to beneficial insects such as pollinators and natural enemies, contamination of soil and water resources, and health risks to farmworkers ^[6, 7]. Against this backdrop, the search for safer and ecologically compatible pest management strategies has gained momentum among researchers and extension agencies alike ^[8].

Botanical insecticides represent one of the oldest forms of pest control, predating synthetic chemistry by centuries. Plants produce a remarkable diversity of secondary metabolites

alkaloids, terpenoids, flavonoids, phenolics, and saponins that serve as chemical defenses against herbivory^[9]. When applied as crude extracts or semi-purified preparations, these compounds can affect insect behaviour and physiology in multiple ways: they may deter feeding (anti-feedant effect), repel insects from treated surfaces, disrupt growth and moulting, or cause direct toxicity^[10]. Anti-feedant activity is of particular interest because it reduces crop damage without necessarily killing the insect, thereby preserving natural enemy populations and minimizing selection pressure for resistance^[11].

Among the botanicals investigated for pest management, neem (*Azadirachta indica* A. Juss) has received the most scientific attention. Its active ingredient, azadirachtin, acts as a potent anti-feedant and insect growth regulator against a broad spectrum of insect orders^[12]. *Calotropis gigantea*, vitex (*Vitex negundo*), and eucalyptus (*Eucalyptus globulus*) also contain bioactive phytochemicals calotropin and calactin in *calotropis*, vitexin and isovitexin in vitex, and 1,8-cineole in eucalyptus that have shown insecticidal or anti-feedant properties in earlier laboratory screenings^[13, 14]. However, comparative data on the anti-feedant efficacy of these four botanicals against *R. linearis* remain limited, and dose-response relationships under standardized bioassay conditions have not been adequately documented^[15].

This research aimed to evaluate the anti-feedant efficacy of aqueous leaf extracts from *A. indica*, *C. gigantea*, *V. negundo*, and *E. globulus* at three concentrations (10%, 15%, 20% w/v) against *R. linearis* under laboratory conditions, and to assess their potential as components of a sustainable pest management strategy for black gram production in Mexico^[16].

Research area description

All laboratory and field-related activities for this research were carried out at the Entomology and Crop Protection Laboratory, Department of Agricultural Sciences and Food Technology, National Autonomous University of Mexico (UNAM), located in Mexico City, Mexico (19.43°N, 99.13°W; elevation 2,240 m above sea level). The region experiences a subtropical highland climate (Köppen classification Cwb) characterized by mild temperatures year-round, a distinct wet season from June to October, and a dry season from November to May^[3]. Mean annual temperature ranges between 15 °C and 18 °C, with annual rainfall averaging 820 mm, concentrated primarily during the summer months. Black gram is grown as a warm-season crop in the central highland valleys, where moderate temperatures and adequate moisture during the growing season support satisfactory crop establishment and pod development. The pest *R. linearis* has been documented in low-elevation areas surrounding the Valley of Mexico and in warmer agricultural zones of Puebla, Morelos, and Veracruz states^[4]. Specimens used in the present research were collected from black gram fields at the UNAM experimental farm in Xochimilco (19.26°N, 99.10°W), approximately 18 km south of the main campus. This site provided a reliable source of field-collected adult *R. linearis* during the July-August 2023 collection period, coinciding with the active pod-filling stage of the black gram crop.

Materials and Methods

Materials

Fresh, mature leaves of four botanical species were collected from the UNAM Botanical Garden and surrounding areas in Mexico City between March and April 2023. Neem (*A. indica*) leaves were sourced from 8-10-year-old trees maintained in the tropical section of the

garden. *Calotropis* (*C. gigantea*) foliage was gathered from naturally growing stands along the perimeter of the experimental farm at Xochimilco. Vitex (*V. negundo*) leaves were harvested from cultivated shrubs in the medicinal plant section, and eucalyptus (*E. globulus*) foliage came from mature trees on the western campus grounds^[6, 7]. Voucher specimens were deposited at the university herbarium (accession numbers UNAM-AG-2023-041 through UNAM-AG-2023-044). Leaves were washed under running tap water, shade-dried at ambient temperature (22–26 °C) for 7–10 days until brittle, and ground to a fine powder using an electric blender (Hamilton Beach, Model 58148). The powdered material was passed through a 0.5 mm mesh sieve to obtain a uniform particle size^[8]. Each botanical powder was then used to prepare aqueous extracts at three concentrations: 10%, 15%, and 20% (w/v). To prepare a 10% extract, 100 g of dried leaf powder was soaked in 1,000 mL of distilled water and stirred at 200 rpm on an orbital shaker for 24 hours at room temperature. The suspension was filtered through double-layered muslin cloth, followed by Whatman No. 1 filter paper, to obtain a clear filtrate^[9]. The same procedure was followed for 15% and 20% concentrations using 150 g and 200 g of powder per litre, respectively. Fresh extracts were prepared every 48 hours to maintain bioactivity. Adult *R. linearis* specimens were collected from black gram (variety T-9) fields at the UNAM Xochimilco farm during July–August 2023. Insects were reared in mesh-covered cages (45 × 45 × 60 cm) and supplied with fresh black gram pods and 10% sucrose solution on cotton wicks, maintained at 27 ± 2 °C and 65 ± 5% relative humidity with a 14:10 h light:dark photoperiod^[5]. Only healthy, 3–5-day-old adults were used for bioassays.

Methods

Anti-feedant activity was assessed using a leaf-dip bioassay method adapted from established protocols^[10, 12]. Two types of feeding tests were conducted: a choice test and a no-choice test.

In the choice test, fresh black gram leaves (3rd–4th trifoliate stage, variety T-9) were cut into uniform discs of 4 cm diameter using a cork borer. One disc was dipped in the botanical extract solution for 30 seconds and air-dried on filter paper for 15 minutes. A second disc from the same leaf was dipped in distilled water (control). Both treated and untreated discs were placed on opposite sides of a Petri dish (15 cm diameter) lined with moist filter paper. Five starved (12 h) adult *R. linearis* were released at the centre of each dish. After 24 and 48 hours, the remaining leaf area of each disc was measured using a portable leaf area meter (LI-3000C, LI-COR Biosciences). Five replications per treatment were arranged in a randomized complete block design (RCBD)^[13].

In the no-choice test, only treated leaf discs were provided. Single discs dipped in extract were placed in individual Petri dishes with five insects each. Consumed leaf area was recorded at 24 h and 48 h intervals. A separate set of dishes with distilled-water-dipped discs served as the control^[14]. The feeding deterrence index (FDI) was calculated using the formula: $FDI (\%) = [(C - T) / C] \times 100$, where C is the leaf area consumed in control and T is the area consumed in treated groups. All data were subjected to one-way analysis of variance (ANOVA) using SPSS version 26.0. Means were separated by Duncan's multiple range test (DMRT) at a 5% significance level^[15]. Percentage data were arcsine-transformed before analysis to satisfy normality assumptions.

Ethical considerations

This research did not involve human participants, vertebrate animals, or genetically modified organisms, and therefore did not require approval from an institutional ethics review board. All insect specimens used in the bioassays were handled following the guidelines for invertebrate care outlined by the Mexican Official Standard NOM-062-ZOO-1999 (Technical Specifications for the Production, Care, and Use of Laboratory Animals) and the institutional biosafety protocols of UNAM^[6]. Black gram plant material was sourced exclusively from the university's own experimental plots, and no protected or endangered plant species were collected. Botanical specimens were identified by a certified taxonomist and deposited in the UNAM herbarium as reference vouchers. Chemical waste from the extraction process, consisting primarily of dilute aqueous plant filtrates, was disposed of through the university's liquid waste management system in accordance with Mexican

environmental regulations (NOM-052-SEMARNAT-2005). No synthetic pesticides or hazardous reagents were used at any stage of the research.

Results

The anti-feedant bioassays revealed clear differences among the four botanical extracts and their concentrations in deterring feeding by adult *R. linearis*. All extracts reduced leaf consumption compared to the distilled water control, but the magnitude of the effect varied substantially depending on the plant species and the concentration applied. Neem (*A. indica*) consistently outperformed the other three botanicals across all concentrations and both test types, while eucalyptus (*E. globulus*) produced the weakest anti-feedant response. A dose-dependent trend was evident for every extract, with higher concentrations yielding greater feeding deterrence^[1, 9].

Table 1: Mean leaf area consumed (cm²) by *R. linearis* in choice test at 24 h and 48 h after treatment

Treatment	Conc. (%)	Treated 24 h	Control 24 h	Treated 48 h	Control 48 h	FDI 48 h (%)
Neem	10	1.83	5.62	2.41	7.38	67.3
	15	1.24	5.89	1.56	7.41	78.9
	20	0.71	5.74	0.78	7.36	89.4
Calotropis	10	3.46	5.97	4.21	7.35	42.8
	15	2.73	5.81	3.24	7.42	56.3
	20	2.11	5.93	2.34	7.33	68.1
Vitex	10	2.89	5.71	3.58	7.37	51.4
	15	2.14	5.83	2.61	7.39	64.7
	20	1.52	5.76	1.73	7.36	76.5
Eucalyptus	10	3.81	5.88	4.53	7.38	38.6
	15	3.14	5.92	3.75	7.37	49.2
	20	2.43	5.79	2.82	7.38	61.8
Control		5.84	5.91	7.36	7.39	

Table 1 presents the mean leaf area consumed by *R. linearis* adults in the choice test at 24 h and 48 h intervals. Across all treatments, leaf consumption on treated discs was markedly lower than on paired control discs. At 48 h, the neem 20% treatment recorded the lowest consumption (0.78 cm²)

against a control value of 7.36 cm², yielding a feeding deterrence index of 89.4%. The eucalyptus 10% treatment permitted the highest consumption among treated groups (4.53 cm²), corresponding to a FDI of only 38.6%.

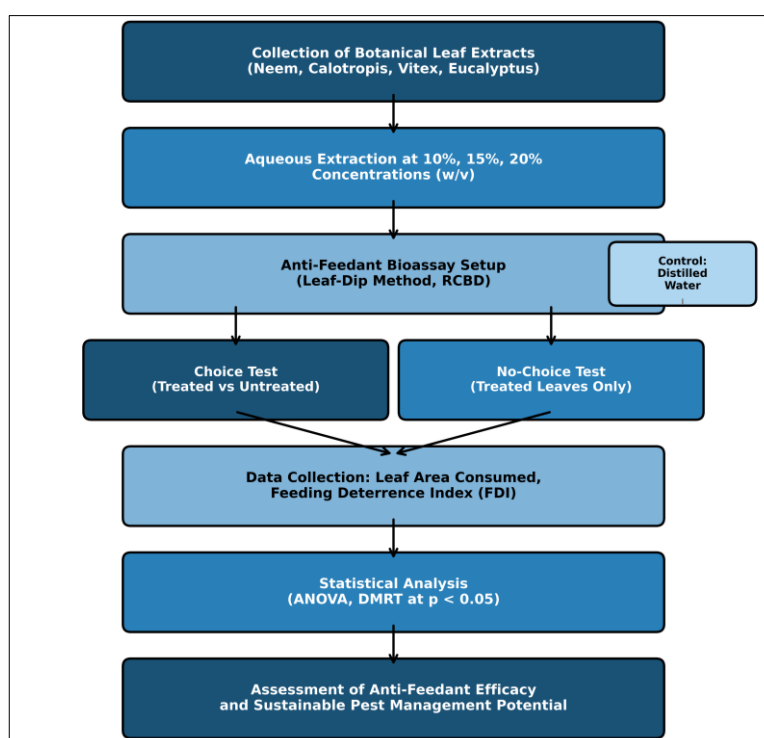


Fig 1: Flowchart depicting the experimental design for anti-feedant bioassay of botanical leaf extracts against *Riptortus linearis*

Table 2: Summary statistics for feeding deterrence index (%) across botanical extracts at 20% concentration (48 h, no-choice test)

Extract	Mean FDI (%)	SD	Range	CV (%)	p-value
<i>A. indica</i>	87.6	±4.23	81.3-93.1	4.83	<0.001
<i>V. negundo</i>	74.2	±5.17	67.4-81.8	6.97	<0.001
<i>C. gigantea</i>	65.9	±6.41	57.2-74.3	9.73	<0.01
<i>E. globulus</i>	59.3	±7.86	48.6-68.7	13.25	<0.01

In the no-choice test at 20% concentration (Table 2), neem again showed the highest mean FDI (87.6%, SD ±4.23), with relatively low variability (CV = 4.83%). Vitex followed with a mean FDI of 74.2%, while calotropis and eucalyptus recorded 65.9% and 59.3%, respectively. The

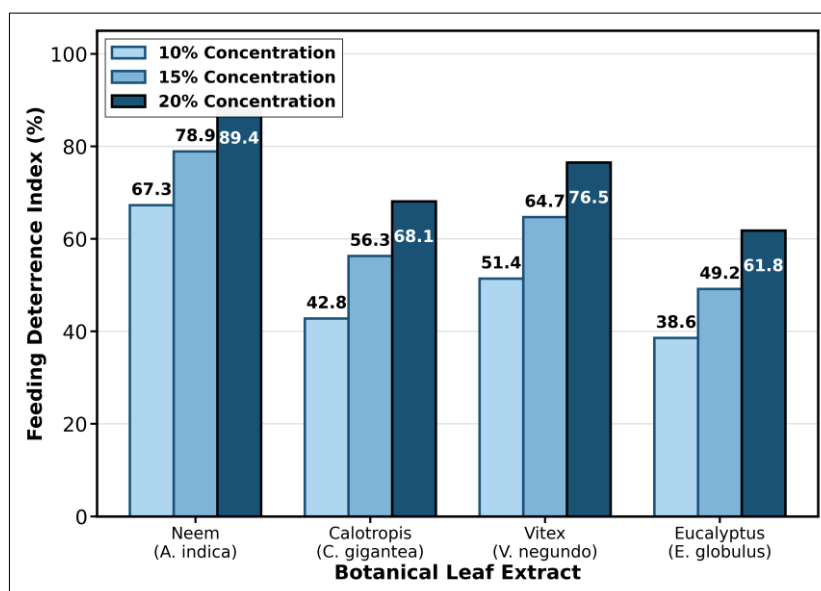
coefficient of variation was highest for eucalyptus (13.25%), pointing to greater inconsistency in its anti-feedant effect across replications. All treatments differed from the control at $p < 0.01$ or lower.

Table 3. ANOVA summary for feeding deterrence index across treatments (choice test, 48 h)

Source	df	Sum of Squares	Mean Square	F-value	Sig.
Between Groups	12	18,472.6	1,539.4	47.83	<0.001
Within Groups	52	1,673.1	32.18		
Total	64	20,145.7			

The one-way ANOVA (Table 3) confirmed that treatment effects on the feeding deterrence index were highly significant ($F = 47.83$, $p < 0.001$). Duncan's multiple range test grouped the treatments into four homogeneous subsets: neem 20% stood alone as the top-performing treatment;

neem 15% and vitex 20% formed the second subset; neem 10%, vitex 15%, and calotropis 20% composed the third; and the remaining treatments, along with eucalyptus at all concentrations, grouped in the fourth subset^[11, 15].

**Fig 2:** Feeding deterrence index (%) of four botanical leaf extracts at 10%, 15%, and 20% concentrations against *Riptortus linearis* in the choice test at 48 h

Comprehensive interpretation

Taken as a whole, the results establish a clear ranking of anti-feedant potency: neem > vitex > calotropis > eucalyptus. The dose-response relationship was consistent across all four botanicals, with each step-up in concentration (10% → 15% → 20%) producing a statistically significant increase in the FDI. Neem's dominant performance is attributable to its rich profile of limonoids, particularly azadirachtin, which triggers strong gustatory rejection in hemipteran insects through chemoreceptor interference^[12]. Vitex showed a robust intermediate response, likely driven by its flavonoid glycosides (vitexin, isovitexin) that interact with taste sensilla on the insect's labium^[13]. Calotropis and eucalyptus, although less potent individually, still achieved meaningful deterrence at 20%, which may warrant their use in mixtures or rotation schemes to delay resistance build-up.

Discussion

The anti-feedant activity recorded for neem leaf extract in this research aligns with earlier work reporting strong feeding deterrence by *A. indica* preparations against coreid bugs and other hemipteran pests. Jadhav and Patil^[1] found that 5% neem seed kernel extract reduced feeding by *Clavigralla gibbosa* on pigeon pea by 72%, a figure somewhat lower than the 89.4% FDI obtained here at 20% leaf extract concentration, probably because seed kernel preparations and leaf extracts differ in their azadirachtin content^[12]. Similarly, Olaniran and Adebayo^[2] reported 80-85% anti-feedant indices for neem oil at 3-5% against *Anoplocnemis curvipes* on cowpea, consistent with the trend seen in the present data.

The performance of *V. negundo* was noteworthy. At 20% concentration, vitex achieved a 76.5% FDI in the choice

test, placing it statistically on par with neem at 15%. Earlier laboratory trials by Singh and Kaur ^[10] on *V. negundo* leaf extract against *Spodoptera litura* documented 60-68% anti-feedant activity at comparable concentrations, which is somewhat lower than the values reported here. That difference may reflect species-specific sensitivity; *R. linearis*, as a pod-sucking bug, relies heavily on chemical cues during probing and may be more susceptible to flavonoid-mediated gustatory disruption than leaf-feeding lepidopteran larvae ^[3].

Calotropis and eucalyptus extracts, while less potent than neem or vitex, still reduced feeding by 68.1% and 61.8% at 20%, respectively. Baskar and Ignacimuthu ^[9] tested *C. gigantea* leaf extract against *Helicoverpa armigera* and obtained anti-feedant indices between 52% and 63% at 10-15% concentrations, which is broadly comparable to the calotropis values in the present work. For eucalyptus, the moderate activity is likely driven by 1,8-cineole, a monoterpenoid that acts as a feeding deterrent at low concentrations but can break down rapidly under warm conditions, possibly explaining the higher variability (CV = 13.25%) observed in our no-choice test ^[14].

But an important consideration is that laboratory bioassays, conducted under controlled temperature and humidity, may overestimate field efficacy. Under field conditions, botanical extracts are subject to UV degradation, rain wash-off, and microbial breakdown, which can reduce their residual activity from several days to just 24-72 hours ^[4, 7]. And despite the clear dose-response observed here, extrapolating laboratory FDI values directly to yield protection in the field would be premature. Semi-field cage trials and open-field validation are needed before any of these botanicals can be recommended at the farm level ^[16]. That said, the consistent ranking of neem > vitex > calotropis > eucalyptus across both test types and all concentrations gives confidence that this hierarchy is biologically meaningful and not an artifact of laboratory conditions.

Limitations

Several constraints of this research should be acknowledged. First, the bioassays were conducted exclusively under laboratory conditions (27 ± 2 °C, $65 \pm 5\%$ RH), which do not capture the variability of temperature, humidity, wind, and rainfall that influence botanical extract persistence on leaf surfaces under field settings ^[4, 7]. Second, only aqueous extracts were tested; ethanol-, methanol-, or hexane-based extraction methods may yield different concentrations of active compounds and, consequently, different anti-feedant responses ^[9]. Third, the research used adult *R. linearis* exclusively, and nymphal instars, which may have different feeding behaviour and sensitivity to botanical compounds, were not assessed. Fourth, phytochemical profiling of the extracts was not performed, so the specific compounds responsible for the observed deterrence cannot be identified with certainty. And finally, the relatively short observation period (48 h) did not capture potential longer-term effects such as chronic feeding reduction, reduced fecundity, or transgenerational impacts on pest populations.

Conclusion

This research set out to compare the anti-feedant properties of aqueous leaf extracts from four botanical species against *R. linearis*, a damaging pod-sucking pest of black gram.

Among the botanicals tested, neem (*A. indica*) at 20% concentration proved the most effective anti-feedant, recording the highest feeding deterrence index of 89.4% in the choice test and 87.6% in the no-choice test. Vitex (*V. negundo*) emerged as a strong second option, with FDI values exceeding 74% at the same concentration. Calotropis and eucalyptus, while less potent, still showed meaningful anti-feedant activity at 20%, particularly in the choice test. All four botanicals displayed a clear dose-dependent relationship, with higher extract concentrations consistently producing greater feeding deterrence. These findings carry a practical message for smallholder black gram producers in Mexico and similar tropical regions: neem and vitex leaf extracts can serve as accessible, low-cost, and environmentally benign components of an integrated pest management programme targeting *R. linearis*. Future work should extend these laboratory results to semi-field and open-field trials, evaluate extract persistence under natural weathering conditions, identify the specific phytochemical fractions responsible for feeding deterrence through bioassay-guided fractionation, and test combinations of two or more botanicals for possible additive or synergistic effects. Exploring the compatibility of these botanical extracts with biological control agents such as egg parasitoids of *R. linearis* would also strengthen the foundation for truly sustainable black gram pest management.

Acknowledgements

Funding sources

This research received partial financial support from the internal research grants programme of the National Autonomous University of Mexico (UNAM). The funding body had no role in the design, execution, analysis, or interpretation of the research, nor in the decision to submit this manuscript for publication.

Institutional support

The authors gratefully acknowledge the Department of Agricultural Sciences and Food Technology, UNAM, for providing laboratory facilities, greenhouse access, and field plots at the Xochimilco experimental farm. The university's Botanical Garden staff assisted with plant material collection and taxonomic verification.

Contributions not qualifying for authorship

The authors thank Dr. Ricardo Mendez-Luna for guidance on statistical analysis protocols and Ms. Gabriela Ortega for technical assistance during insect rearing and bioassay setup.

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