Study on the interaction between melatonin and basagran herbicide on faba bean plant and its associatedweeds

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Abstract: A pot experiments was conducted to study the possible role of melatonin (50 µM or 100 µM) alone or in combination with Basagran herbicide at recommended dose or ½ recommended dose in reducing the deleterious effect of weed associated with *Vicia faba* plants. Results show that all applied treatments significantly reduced the weed dry weight at 45 DAS and at harvest accompanied by significant increase in growth parameters of bean plants (at 45 DAS), all components of photosynthetic pigments, seed yield/plant and its components relative to unweeded treatment. It is obvious that herbicide at recommended dose was more effective than herbicide at ½ recommended dose. Similarly, melatonin at 50 µM was more effective than melatonin at 100 µM. It is worthy to mention that interaction between melatonin at 50 µM and herbicide at recommended dose was the most pronounced treatments, since it significantly decreased weed dry weight by 97.49 % at 45 DAS and at harvest by 94.11% and significantly increased dry weight of plant by 171%, total photosynthetic pigments by 31.75%, faba bean seed yield/plant by 152%, and total carbohydrate of the yielded seeds by 17.77% relative to unweeded treatment. It could be concluded the availability of using the antioxidant as melatonin with basagran herbicide to decrease the deleterious basagran herbicide effect on growth and yield of *Vicia faba* plants without affecting the efficacy of the herbicide on weeds and decreased weed propagation with significant degree.

Keywords: weed control, herbicides, *Vicia faba*, melatonin, antioxidant, tolerance

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1 Introduction

Faba bean (*Vicia faba* L.) is the most important food legume and staple foods in Egypt due to its high nutritional value as its high content of carbohydrate, protein, vitamins, minerals, and fibre (Saleh et al., 2019).So, one of the most important targets of agricultural policy is to increase faba bean production.

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Weeds are considered as a major problem in bean crops causing great losses in seed yield due to direct weed-plant competition for light, moisture and soil nutrients. The reduction of faba bean yield due to weed infestation may be reached to83% and can interfere with harvest efficiency and may cause reduce seed quality(Gomaa et al., 2022).Weeds not only compete with crop for nutrients, soil moisture, space, and light but also serve as alternative hosts for several insect pests and disease (Yadav et al., 2015).Legume species generally have an open growth habit and a slow growth rate in the early stages of the crop cycle, characteristics that favor the emergence and growth of weeds (Aboali and Saeedipour, 2015;Daba and Sharma, 2018).Weed control plays an important role in raising the productivity of crops. Nowadays, the chemical control of weeds became widespread in the world because it brings rapid and desirable control of weeds. The advantages of herbicide application are characterized by high efficiency in weed control and high selectivity. Soliman et al. (2015) indicated that weed control treatments reduced dry weight of broadleaved, grassy and total weeds compared with unweeded treatments. One of the herbicides which can be applied in beans cultivation is Basagran. Basagran is a unique soluble herbicide formulation of Bentazon 480gL^{-1}. It is a selective and contact post-emergence herbicide indicated for the control of weeds associated with beans, soyabean, sorghum, corn, peanut, peas etc.Basagran is a member of the benzothiadiazole group of herbicides which is absorbed mainly by the foliage, with little translocation. It inhibits photosynthesis at photosynthesis II and interferes with photosynthesis of susceptible plants interrupting the use of carbon dioxide in the plant and therefore prohibiting the production of carbohydrates. Thus, the weed ultimately starves to death due to carbohydrate depletion.Bentazon is a benzothiadiazole herbicide that can selectively control broad leaved weeds such as *Sinapis arvensi s*L. (wild mustard),*Ambrosia artemisiifolia* L. (common ragweed), *Ambrosia trifida* L. (giant ragweed),*Polygonumper sicaria* L. (ladysthumb), *Chenopodium album* L. (*Common lambsquarter*L.),*Amaranthus retroflexus* L. (redroot pigweed), *Abutilon theophrasti* Medic. (velvetleaf),*Portulaca oleracea* L. (purslane), *Raphanusraphanistrum*L. (wild radish), *Galinsog aciliate*(hairy galinsoga), *Senecio vulgaris* (common groundsel), *Daturastr ammonium* L. (jimsonweed),*Xanthium strumarium* L. (cocklebur), *Capsella bursa-pastoris*(L.) Medic. (shepherdspurse) and *Stellaria media* (L.)Vill. (common chickweed) (OMAF, 2004; Vencill, 2002).The excessive and non-judicious use of herbicide may lead to crop injury, human and animal health concerns, soil, and water pollution as well as herbicide resistant in weeds (Jabran et al., 2008; Farooq et al., 2011).So, a new approach must be applied to decrease or tolerate the harmful effect of herbicide on growing plants via increasing plant tolerant to unfavorable conditions through applying antioxidant as melatonin.

Melatonin (N-acetyl-5-methoxytryptamine) is a naturally occurring, low-molecular weight, and multi-regulatory molecule that exists in all living organisms, including plants and animals (Wang et al., 2017; Arnao and Hernández-Ruiz, 2018). Since its detection in plants, scientists curiosity regarding melatonin has increased, because of its diversified biological role as a plant master regulator and defensive roles in capricious environmental conditions, such as extreme temperatures, salinity, drought, heavy metals, UV radiation, and oxidative stress (Wang et al., 2017; Arnao and Hernández-Ruiz, 2018; Li et al., 2018; Wei et al., 2018). It can be speculated that melatonin is inexpensive and safe for animals and humans, its application as a biostimulator could be a good, feasible, and effective method used in agriculture to decrease environmental stress (Bonnefont-Rousselot and Collin, 2010) as well as increase food quality.

Melatonin at the low level (1 lM) caused auxinic response concerning the number and length of roots, but at the higher level (10 lM); it inhibited the root growth as in sweet cherry rootstocks (Sarropoulou et al. 2012).Moreover, Hernandez-Ruiz et al. (2004) reported that melatonin at high concentrations, acts as an inhibitor (probably reaching the toxic level in tissues) while at lower concentration, it induced the growth of *Lupinus albus* hypocotyl segments. Moreover, melatonin inhibited root elongation in some monocots even at very low concentrations.Tousi et al. (2020) reported that lower concentrations of melatonin at 15 and 50 µM, significantly improved growth and chlorophyll content in mallow plants exposed to Cd. Likewise, Esmaeili et al. (2023) showed that melatonin at low levels did not show any toxicity by unchanged of hydrogen peroxide, while at high melatonin dosages exhibit toxicity effects (increases hydrogen peroxide). They added that melatonin treatments increased level of phenolic hormone salicylic acid thus it indicates that melatonin affects the defensive responses in *L. album* cells through a SA-dependent pathway.

Melatonin has a range of possible cellular and physiological effects. Since, melatonin regulated the changes in intracellular Ca^{2+}, the permeability of membranes mediated by ion transporters (Li et al., 2016), changes in the opening and/or closing of stomata, regulated carbohydrate, lipid, and nitrogen metabolisms, and also osmoprotector metabolites (Shi et al., 2015; Wei et al., 2015) as well as optimizing efficiency of leaf water/CO_2 exchange (Li et al., 2017) and regulated other processes, such as ripening and/or senescence, flowering, and the internal biological clock (Liang et al., 2018; Liu et al., 2018; Arnao and Hernández-Ruiz, 2018). Melatonin acts as an effective free radical scavenger against hazardous reactive molecules, both reactive oxygen and reactive nitrogen species (ROS/RNS), among others. The excellent properties of melatonin as a natural antioxidant against ROS/RNS was mentioned by Tan et al. (2000), Teixeira et al. (2003), Reiter et al. (2014), and Arnao and Hernandez-Ruiz (2015). Under environmental stresses, the mitochondria can be damaged due to the over-production of ROS (Gupta et al., 2018). However, melatonin was reported to recover the damaged mitochondria (Franco et al., 2018). Evidence also indicated that melatonin has an ability to increase the production of crops.

This work aimed to study the possible role of melatonin alone or in combination with basagran herbicidein reducing the deleterious effect of weed associated with faba bean plants.

2 Materials and methods

A pot experiments was conducted at the green-house of the National Research Centre, Dokki, Cairo, Egypt on the middle of November during two winter seasons (2019/2020) and (2020/2021). Seeds of faba bean (*Vicia faba* L. cv. Giza 461) were obtained from the Legumes Crops Research Department, Ministry of Agriculture and Land Reclamation, Egypt. Six faba bean seeds and *Anagalisar vensis* weed seeds (0.01 g) were sown in each pot (30 cm diameter) at a depth of 30 mm, in approx. Seven kilogram of clay: sand (3:1 v/v) soil. Granular ammonium sulphate (20.5 (w/w) % N) was applied at a rate of 40 kg N ha^{-1}, and single superphosphate (15% P_2O_5) was added at a rate of 60 kg $P_2O_5ha^{-1}$to each pot. These doses of nitrogen and phosphorous were added and mixed thoroughly into the soil of each pot immediately before sowing. Ten days after sowing (DAS), faba bean seedlings were thinned leaving four uniform seedlings per pot. At 15 days old plants, faba bean seedling and its associated weeds were sprayed by basagran herbicides at recommended dose (1 L fed^{-1}) and 1/2 recommended dose as well as by melatonin at 50 µM and 100 µM. Additionally, two control treatments weed free and unweeded were applied for comparison. Treatments were arranged in a complete randomized block design with six replicates for each treatment.

2.1 Data recorded

2.1.1 Weeds

Three replicates were collected from each treatment at 45 DAS and at harvest. Weeds were dried in an oven at 40 ℃ for 48 h to record dry biomass (g pot^{-1}).

2.1.2 Vegetative growth parameters of faba bean

Plants were collected from each treatment during vegetative stage (45 days after sowing) for measurement of some growth parameters (plant height (cm), number of branches and leaves/plant, fresh and dry weights of plant (g)).Moreover, fresh leaves were used for determination of photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) according to the method of Moran (1982).

2.1.3 Yield and yield attributes

At harvest, three replicates of faba bean plants were taken from each treatment to determine weight of pods/plant, number of seeds/pod, weight of 100 seeds and seeds weight/plant.

2.2 Chemical analysis

A total carbohydrates and soluble carbohydrates were determined according to DuBois et al. (1956). Polysaccharides were calculated by the difference between total carbohydrates and soluble carbohydrates. Total phenolic content was extracted and measured as the method described by Tavarini et al. (2008).

2.3 Statistical analysis

The experiments were carried out in complete randomized design. All obtained data were subjected to proper statistical of variance according to Snedecor and Cochran (1980) at 0.05 probability level.

3 Results

Table 1 shows that the applied treatments (herbicide at ½ recommended dose; melatonin at 50 μM and 100 μM as well as their interaction) caused significant decreases in weed fresh and dry weight at 45 days old plants and at harvest. While, herbicide at recommended dose completely eradiated weeds. Herbicide at ½ recommended dose reduced weed dry weight at 45 days old plants by 83.78% and at harvest by 62.54% relative to unweeded treatment. Regarding melatonin effect, it was noted that the inhibitory effect melatonin at low level was more pronounced than inhibitory effect of melatonin at higher level. Since, lower level of melatonin reduced weed dry weight at 45 days old plants by 52.88% and at harvest by 44.97% relative to unweeded treatment. Likewise, interaction between herbicide at recommended dose and melatonin at two levels caused highly reduction of weed dry weight than interaction between herbicide at ½ recommended dose and melatonin at two levels. It is worthy to mention that the interaction between herbicide at recommended dose and melatonin at 50 μM was the most optimum treatments, since; it reduced weed dry weight at 45 days old plants by 97.49% and at harvest by 94.11% relative to unweeded treatment.

Table 1 Effect of the melatoninin combination with herbicide basagran on weed growth at 45 days old and at harvest

Treatments	Concentrations	45 DAS		At harvest
		Weed fresh weight/pot (g)	Weed dry weight/pot (g)	weed dry weight/pot (g)
Weed free plants	0	0.00	0.000	0.00
Unweeded plants	0	49.13	5.976	33.80
Rec	1L/fed.	0.00	0.000	0.00
½ Rec	½ L fed.	8.78	0.969	12.66
M1	50 μM	17.45	2.816	18.60
M2	100 μM	21.48	3.533	25.70
Rec+M1	1L/fed.+50 μM	0.78	0.150	1.99
Rec+M2	1L/fed.+100 μM	2.33	0.441	3.21
½ Rec+M1	½ L/fed.+50 μM	2.44	0.533	3.50
½ Rec+M2	½ L/fed.+100 μM	5.33	0.818	10.40
LSD at 5%		0.63	0.085	1.31

Table 2 Effect of melatonin in combination with the herbicide basagranon faba bean growth at 45 days old

Treatments	Concentrations	Plant height cm)	Number of leaves/plant	Number ofbranches/plant	Fresh weight /plant (g)	Dry weight/plant (g)
Weed free plants	0	56.53	13.66	2.33	23.94	2.486
Unweeded plants	0	41.50	8.66	1.00	14.82	0.920
Rec	1L/fed.	54.50	12.00	2.00	22.72	2.283
½ Rec	½ L fed.	49.66	9.83	1.83	16.19	1.526
M1	50 μM	56.00	10.5	1.66	19.20	2.120
M2	100 μM	51.33	9.83	1.50	17.77	1.316
Rec+M1	1 L/fed.+50 μM	58.00	14.33	2.33	23.59	2.493
Rec+M2	1 L/fed.+100 μM	56.00	11.66	2.00	22.12	2.186
½ Rec+M1	½ L/fed.+50 μM	51.66	12.00	1.33	19.31	2.166
½ Rec+M2	½ L/fed.+100 μM	45.00	9.83	1.16	19.21	1.580
LSD at 5%		2.73	0.79	0.33	2.21	0.168

Table 2 shows that all applied treatments significantly increased growth parameters of faba bean plants as plant height, number of leaves, fresh and dry weight of plant at 45 DAS relative to unweeded plant treatments. Regarding herbicide effect, it was noted that recommended dose of herbicide was more pronounced than ½ recommended dose of herbicide. Similarly, melatonine at 50 µM was more effective than melatonine at 100 µM. It was noted that the interaction between herbicide at recommended dose and melatonin at 50 µM was the most optimum treatments in increasing values of different growth parameters under investigation followed by herbicide at recommended dose lonely. Since, the interaction between herbicide at recommended dose and melatonin at 50 µM significantly increased dry weight of bean plant at 45 DAS by 171%. Recommended dose of herbicide or melatonin at 50 µM increased dry weight of faba bean plant at 45 DAS by 148% and 130% respectively.

Table 3 Effect of melatonine in combination with the herbicide basagran on chlorophyll a, chlorophyll b, carotenoids (mg/g fresh leaf tissues) in faba bean at 45 days old

Treatments	Concentrations	Chlorophyll a	Chlorophyll b	Carotenoids	Total Photosynthetic pigments
Weed free plants	0	2.336	0.687	0.371	3.394
Unweeded plants	0	1.753	0.531	0.254	2.538
Rec	1L/fed.	2.227	0.614	0.321	3.162
½ Rec	½ L fed.	1.905	0.594	0.305	2.804
M1	50 µM	1.946	0.588	0.329	2.863
M2	100 µM	1.926	0.561	0.254	2.741
Rec+M1	1 L/fed.+50 µM	2.303	0.672	0.369	3.344
Rec+M2	1 L/fed.+100 µM	2.186	0.635	0.357	3.178
½ Rec+M1	½ L/fed.+50 µM	2.153	0.561	0.352	3.066
½ Rec+M2	½ L/fed.+100 µM	2.022	0.548	0.329	2.899
LSD at 5%		0.104	0.032	0.028	0.145

Table 3 shows that all applied treatments significantly increased all components of photosynthetic pigments relative to unweeded treatments. Recommended dose of herbicide was more effective in increasing photosynthetic pigments than ½ recommended dose. Likewise, melatonin at 50 µM was more pronounced than melatonin at 100 µM. Regarding the interactions, it was noted that the most optimum results were recorded due to interaction between herbicide at recommended dose, and melatonin at two levels followed by herbicide at recommended dose lonely. The interaction between herbicide at recommended dose and melatonin at 50 µM increased total photosynthetic pigments by 31.75% meanwhile, herbicide at recommended dose lonely increased total photosynthetic pigments by 24.58%.

Table 4 Effect of melatonine in combination with the herbicide basagran on faba bean yield and yield components

Treatments	Concentrations	Weight of pods/plant (g)	Number ofseeds/pod	Weight of seeds/plant(g)	Weight of 100 seeds(g)
Weed free plants	0	7.85	2.66	7.83	70.81
Unweeded plants	0	4.23	1.33	3.30	54.68
Rec	1L/fed.	9.25	2.66	6.84	65.84
½ Rec	½ L fed.	8.39	2.00	5.47	58.60
M1	50 µM	7.60	2.33	6.80	64.76
M2	100 µM	6.45	2.00	4.84	60.06
Rec+M1	1 L/fed.+50 µM	10.47	3.00	8.34	76.61
Rec+M2	1 L/fed.+100 µM	9.07	2.00	6.10	64.16
½ Rec+M1	½ L/fed.+50 µM	5.44	2.33	5.28	63.29
½ Rec+M2	½ L/fed.+100 µM	5.23	1.69	4.00	62.14
LSDat 5%		0.42	0.27	0.39	2.89

All applied treatments significantly increased faba bean seed yield /plant and its components relative to unweeded treatment (Table 4). Recommended dose of herbicide was more effective in increasing seed yield and yield components than ½ recommended dose. Since, recommended dose of herbicide increased seed yield/plant by 107%, while herbicide at ½ recommended dose increased in seed yield /plant by65.75% relative to unweeded treatment. Similarly, melatonin at 50 µM was more effective than melatonin at 100 µM. The increase in seed yield due to melatonin at 50 µM was 106% and due to melatonin at 100 µM was 47%. Regarding interactions treatments, it was noted that interaction between herbicide at recommended dose and melatonin at 50 µM was the most pronounced treatments relative to all other treatments. Since it increased seed yield/plant by 152% relative to unweeded treatment.

Table 5 Effect of melatonine in combination with the herbicide basagran on some chemical constituents of the yielded faba bean seeds

Treatments	Concentration	Phenolic content %	Total soluble sugars %	Total carbohydrates %	Polysaccharides
Weed free plants	0	3.235	3.905	54.845	50.940
Unweeded plants	0	4.360	7.000	46.590	39.590
REC	1L/fed.	4.355	5.895	53.415	47.520
½ Rec	½ L fed.	3.595	5.525	49.055	43.530
M1	50mM	3.630	5.200	52.920	47.720
M2	100mM	3.280	5.825	47.080	41.255
REC+M1	1L/fed.+50mM	2.950	5.700	54.875	49.175
REC+M2	1L/fed.+100mM	3.340	6.025	51.010	44.985
½ Rec+M1	½ L/fed.+50 µM	2.925	5.945	53.005	47.060
½ Rec+M2	½ L/fed.+100 µM	2.910	5.780	50.655	44.875
LSD at 5%		0.177	0.254	0.425	0.440

All applied treatments significantly increased total carbohydrate content and polysaccharides accompanied by significant decrease in total phenolic content and total soluble sugars relative to unweeded treatment (Table 5). The herbicide treatments at both doses lonely showed the least decreases in phenolic content followed by melatonine treatments at both doses relative to unweeded treatment. Regarding interaction between herbicide treatments and melatonine treatments, it was noted that the significant decreases in total phenolic content due to Rec M1, ½ Rec M1 and ½ REC M2 were similar. On the other hand, the significant increases of total carbohydrates due to individual treatments were Rec dose > M1 > ½ Rec > M2. Whereas, the significant decrease in total soluble sugars due to M1 was > ½Rec > M2 > Rec. regarding interaction between herbicide and melatonine, it was noted that Rec M1 treatment was the most optimum treatment followed by ½Rec M1,since it increased total carbohydrate by 17.77%and 13.75% relative to unweedred treatment.

4 Discussion

The results show that basagran herbicide and melatonin as antioxidant substance or interaction between them significantly reduced weed growth (Table 1) accompanied by significant increases in different growth parameters of faba bean plants (Table 2), photosynthetic pigments (Table 3) seed yield/plant and its components (Table 4) and carbohydrate content of the yielded seeds (Table 5). Many research workers tried to apply some growth agents with the herbicides as attempts to decrease the phytotoxic action of herbicides on main crops without affecting the herbicide efficacy on weeds (Abouziena et al., 2011; Moran et al., 2011; El-Rokiek et al., 2012). Protective agent protects main crop plants from herbicide damage without reducing activity in target weed species (Davies and Caseley, 1999, Moran et al., 2011). These results are in conformation with those of Baghestani et al. (2008) as well as Aboali and Saeedipour (2015) who reported that basagran offers sizeable increase in crop production corresponding to its weed control spectrum. Basagran inhibits photosynthesis of weeds selectively and interferes with photosynthesis of susceptible plants interrupting the use of carbon dioxide in the plant and therefore prohibiting the production of carbohydrates thus reducing weeds growth. Hence, the weed ultimately starves to death due to carbohydrate depletion. Al-Jubouri and Antar (2021) represented that all basegran herbicide doses decreased the narrow and broad leaves weeds, dose 750 mlh^{-1} superior at decrease the narrow leave weeds and their dry weight (17.89) and (53.42) gm^{-2}, the dose (375 and 750 mlh^{-1}) superior on the dose 187.5 mlh^{-1} significantly at decrease the number of broad leaves weed (4.44, 4.30 plant m^{-2}).Regarding melatonin effect, it is worthy to mention that melatonin could be used to improve the phytoremediative efficiency of plants against different pollutants. Moreover, melatonin reportedly counteracts the toxicity of various environmental chemicals, including herbicides, insecticides, fungicides, and heavy metals (Manchester et al.,2015;Asghari et al., 2017).Melatonin administration negated the effects of Paraquatin toxication (a broad spectrum agricultural pesticide, caused cellular toxicity by increasing oxidative stress levels in various biological systems), indicated by decreased levels of H_2O_2 and mitochondrial malondialdehyde, and enhanced expressions of antioxidant enzymes (Medina Leendertz et al.,2014;Pang et al.,2019). Moreover, melatonin and its metabolic derivatives possess extensive free radical scavenging abilities (Reiter et al.,2014; Zhang and Zhang, 2014).

It is well known that melatonin is growth regulator and stress conditions modulator that can improve plant performance and yield even under stress (Arnao and Hernández-Ruiz, 2014). In *Solanum lycopersicum*, melatonin modified Cd-induced toxicity by increasing the antioxidant capacity and the efficiency of photosynthesis (Hasan et al., 2015). In addition, melatonin decreased the oxidative stress by increasing superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) activities (Kaya et al., 2019). The stimulatory and inhibitory effects of melatonin on the growth appear to be dependent on its concentration. For example, under Cd stress, a moderate concentration of melatonin showed a maximum stimulatory effect on the fresh weight and root length in tomato (Hasan et al., 2015) and tobacco (Wang et al., 2019) plants. High concentrations of melatonin can act as inhibitors of plant growth, possibly due to the increased total content of endogenous melatonin and its accumulation caused toxic levels in tissues (Zhao et al., 2015). According to our results, one possible reason for the increased growth in the melatonin-pretreated plants, especially at lower concentrations, could be the stimulation and alteration of carbohydrate metabolism. Melatonin can stimulate carbohydrate metabolism and sucrose loading (Zhao et al., 2015).

It has excellent antioxidant properties and substantially scavenges reactive oxygen species by increasing the activity of enzymatic and non-enzymatic antioxidants (phenolic content) under stress conditions. Moreover, the up-regulation of stress responsive and antioxidant enzyme genes makes it an excellent stress-inducing molecule especially at harsh environments (Khan et al., 2020). Additionally, one melatonin molecule may scavenge up to 10 free radicals (Tan et al., 2007), which contrasts with the classic antioxidants that typically detoxify one radical per molecule.

5 Conclusions

It could be concluded that all applied treatments (basagran herbicide at recommended dose and at ½ recommended, melatonine at 50 µM and 100 µM, and their interactions) significantly reduced the dry weight of weeds accompanied by significant increases in faba bean plant growth and productivity. The most optimum treatment was due to interaction between melatonin at 50 mM and herbicide at recommended dose. The results suggested availability of using the antioxidant melatonin with basagran herbicide to decrease the deleterious herbicide effect on growth and yield of *Vicia faba* plants without affecting the selectivity and the efficacy of the herbicide on weeds and at the same time decreased weed propagation with significant degree.

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