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Research Article

EFFECTS OF MICROWAVE, ULTRAVIOLET LIGHT AND LOW TEMPERATURE ON KHAPRA BEETLE MORTALITY AND QUALITY OF SAFFRON

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ABSTRACT. Microbial contamination and the presence of physical and biological agents are the main limitations for exportation of saffron in Afghanistan. Recently, the presence of khapra beetle (*Trogoderma granarium*) in saffron has been reported. In this research, an attempt was made to study the effects of the microwave, ultraviolet light and low temperature treatments on control of khapra beetle (larvae and adult) and their impact on quality of saffron (crocin, picrocrocin and saffronal). The results indicated that application of microwave treatment @ 2450 MHz and 1850 W power for 60 seconds was effective in controlling khapra beetle with 100 percent mortality of both larvae and adults after 6 days of incubation. It was observed that the ultraviolet, microwave and low temperature treatments had increased corcin and picrocrocin contents decreased saffranal content slightly. The findings suggested that the use microwave irradiation could be an effective method to control of khapra beetle infestation (adult and larvae) in saffron with 100 percent of mortality.

Keywords*: saffron, khapra beetle, microwave, low temperature, ultraviolet.*

INTRODUCTION

Saffron stigma is a spice obtained from the dehydrated stigmas of *Crocus sativus* L. which belongs to Iridaceae family [1]. Saffron is one of the most important cash crops that are commonly used in nutrition, cosmetics, medicine, edible pigment production, aromatization, and art purposes [2]. Saffron stigma is widely used in traditional healthcare as antimicrobial agent to control variety of diseases, as spice in several recipes, as therapeutic agent in medicines and as coloring agent around the world [3]. Iran, Afghanistan, Greece, Morocco, India, Spain, Turkey and Italy are the main producers of saffron [4]. A variety of biological and phytochemicals properties, including antioxidation, anti-inflammation, antidepressant, and hypolipidemic actions have been associated with saffron. According to the latest figures from the Ministry of Agriculture, Irrigation, and Livestock, in 2019, saffron production in Afghanistan has reached to19.5 ton with cultivation area of about 7557 hectares, Herat province account as the main producer of saffron [5]. Around 150 volatile and non-volatile compounds have been reported from saffron stigma, in which about 50 constituents have been, identified [6]. The major bioactive compounds of saffron are crocin, picrocrocin and safranal, wherein picrocrocin and crocin are water-soluble compounds derived from the xanthophyll zeaxanthin [7]. Crocin is glycosides of crocetin, contains water-soluble carotenoids that impart red color, it is the main biologically active metabolites of saffron [8], whereas, Picrocrocin and saffranal are present in lower quantities. Picrocrocin is a colorless glycoside of the aglycone 4-hydroxy-2, 6, 6-trimethyl1-carboxaldehyde-1-cyclohexene (HTCC), which is the main substance responsible for the bitter taste of saffron. Safranal is the main component of the essential oil and is responsible for the characteristic saffron aroma, which is obtained from picrocrocin and HTCC during the saffron drying process [9].

Trogoderma granarium, known as khapra beetle is the world's most destructive stored grain pest, it is ranked as one of the 100 worst invasive pests worldwide [10]. Khapra beetle is distributed in more than 35 countries of the world [11]. The larval stage of this pest is considered as the most destructive stage that causes heavy economic losses to stored grains and other food commodities [12]. Khapra beetle is a serious pest of grain products under hot dry conditions [13]. The dry hot condition of cargo containers is a potential intimidation to the worldwide food security. Severe infestations by khapra beetle have caused the grains unfit for consumption or marketable [14]. Khapra beetle has been designed as a technical barrier to trade due to quality deteriorating characteristics [15]. Recently contamination of saffron stigma with khapra beetle has been reported in Herat saffron [16], therefore, control of khapra beetle infestation and spread is very essential to safeguard the quality and marketability of saffron. Several treatments and control methods may be employed to control khapra beetle in saffron, such as chemical, biological, physical heat, controlled atmosphere, and irradiation [17]. The use of chemicals and botanicals may control the khapra beetle to some extent, however the residues remain in the material and also, application of chemical in food stuffs has been restricted in most of the countries [18], therefore, physical treatments such as microwave, ultraviolet light and low temperature could be better strategies to control khapra pest infectation in saffron.

MATERIALS AND METHODS

Estimation of crocin, picrocrocin and safranal

ISO method (ISO3632-2:2010, saffron-test methods) was used to determine the main components of saffron stigma, including crocin, picrocrocin, and safranal. 0.5 g of saffron powder taken in volumetric flask and volume mad up 1000 mL with distilled water and was stirred at 1000 rpm for 1 h. Next, 20 ml of solution was added to volumetric flask and volume mad up to 200 ml. The solution was placed in a dark place for 10 min, and then filtered rapidly using filter paper to obtain a clear solution. Finally, the absorbance of this solution was measured by UV–Vis spectrophotometry. The wavelengths used were 330, 440, and 257 nm for safranal, crocin, and picrocrocine, respectively. The following equation was used to calculate the picrocrocin, safranal, and crocin values:

$$A^{1\%}_{1cm}(\lambda max) = \frac{D \times 10000}{M \times (100-W_{MV})}$$

Where D is the absorbance of the solution at the desired wavelength; M is the Mass of the test portion, WMV is the moisture and volatile matter content.

Microwave, UV light and low temperature treatments

Microwave, low temperature, and ultraviolet light treatments were applied for control of khapra beetle in saffron. One gram of fresh uninfected saffron was mixed with khapra pest (50

No.) @ 25 larvae and 25 adults each in a petri dish of 9 cm diameter. The petri-dishes containing test sample were irradiated for 60 seconds at 2450 MHz microwave oven that had the capability of producing up to 1850 W microwave power to control khapra beetle as explained by [19]. Similar set up of petri dishes containing saffron and khapra beetles were subjected for ultraviolet light treatment at 254 nm exposure for 120 minutes, where in the distance between UV source and petri dishes was 20 cm in a sterile UV cabinet. For low temperature treatment, the khapra beetles having larvae and adults was mixed with one gram of saffron in a glass vial, exposed to -10°C temperature for 2 hours by using a deep freezer. The untreated samples of saffron and khapra pests formed the controls. Later, the treated and untreated samples were kept in the controlled conditions of temperature 33±1°C and relative humidity 60±5%. At an interval of 1, 2, 3, 4, 5 and 6 days of incubation, the data were recorded in terms of the number of insects alive by observing the mobility of insect under stereo microscope. Each test was replicated five times. The results were analyzed by using Completely Randomized Design (CRD) as per the guidelines suggested by [20].

RESULTS AND DISCUSSION

Effects of Microwave, UV light and low temperature on khapra beetle mortality

The effects of physical treatments, exposing 50 khapra beetle insects (25 larvae and 25 adults) mixed with one g of saffron for microwave, UV light and low temperature treatments are shown in Fig. 1 and 2. In general, both larvae and adults of khapra were affected by treatments. Figure 1 indicates that the best method to control the larvae stage of khapra beetle was microwave which resulted in 100 percent mortality and this was followed by low temperature with a 63.2 percent mortality of khapra beetle larvae after 6 days of investigation. The use of ultraviolet light in controlling of khapra beetle was not effective; it showed minimum mortality of larvae at 41.6 percent. The most effective method to control the adult stage of khapra beetle was observed to be the application of microwave with 100 percent mortality and this was followed by low temperature (-10°C) treatment with 52.8 percent mortality of khapra beetle adults after 6 days of investigation. Ultraviolet light showed minimum mortality of khapra adults with 29.6 percent mortality (Figure 2). Similar findings were reported by [21, 22], who revealed that all stages of the pest were controlled by microwave radiation. Super cooling effect of temperature on rate and starvation Khapra beetle, chilling temperature had decreased the survival rate of khopra beetles [23].

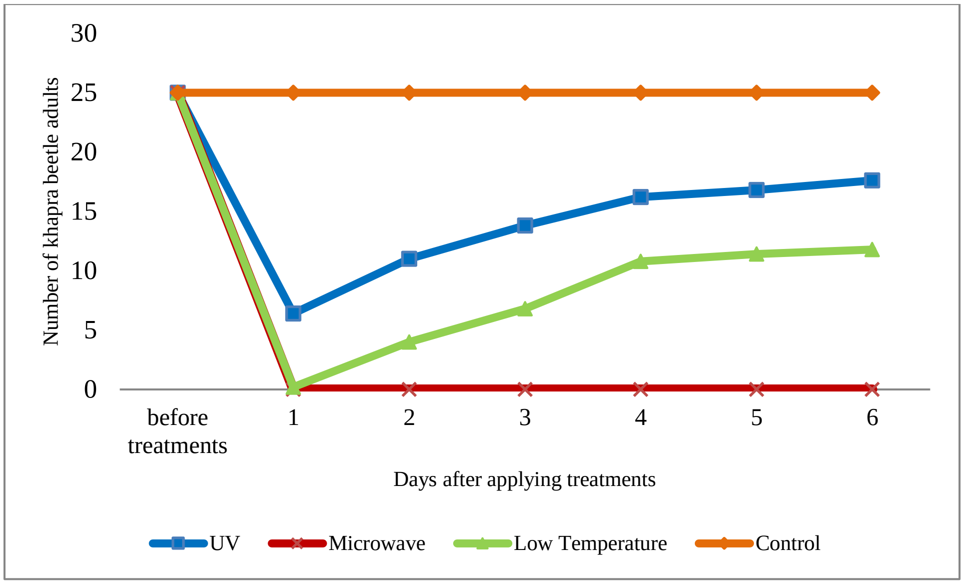

Fig. 1. *Effects of microwave, ultraviolet light and low temperature on mortality of khapra beetle adult*

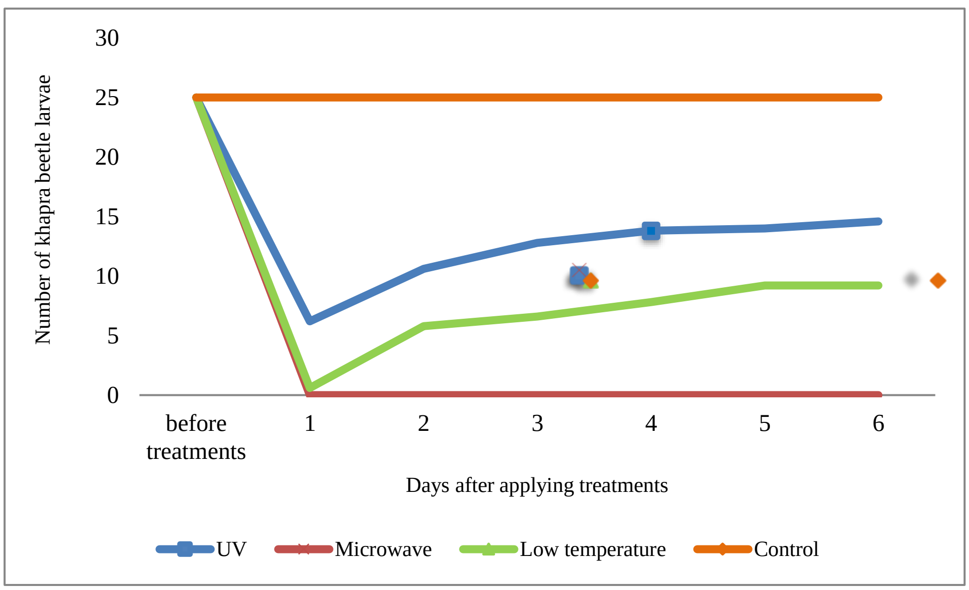

Fig. 2. *Effects of ultraviolet light, microwave and low temperature on mortality of khapra beetle larvae*

Effects of Microwave, Ultraviolet light and low temperature on saffron quality

Significant differences in quality of saffron were observed due to treatments. The tested compounds such as crocin, picrocrocin and safranol showed variations as effected by different treatments. Crocin content of saffron had decreased from 243.94 to 180.98 by applying microwave, ultraviolet light and low temperature treatments while controlling khapra beetles in saffron (Table 1). The maximum retention of crocin (241.16) was recorded in low temperature exposure treatment, which was followed by microwave (195.12). The minimum retention of crocin content (180.98) was recorded in ultraviolet light treated samples. The picrocrocin content of saffron was also found decreased significantly due to different treatments (Table 1). Maximum retention in picrocrocin content, 91.66 of saffron was recorded in low temperature treatment and it was followed by microwave treatment (79.76), while the minimum retention was recorded in ultraviolet light (51.76) besides controlling Khapra beetle insect in saffron. The results showed that the safranal content of saffron had increased from 36.91 to 39.88 by applying microwave, ultraviolet light and low temperature (Table 1). The maximum increase was recorded in treatment applying ultraviolet light which was followed by microwave to control khapra beetle in saffron and minimum increase in low temperature was recorded. Change in quality of saffron by applying microwave and ultraviolet, might be due to high temperature and oxidation bioactive compounds. Similar findings were reported by [9] in change of pigments and volatiles of saffron during processing and storage and in stability of volatile compounds of Turkish saffron (*crocus sativus*) after one-year storage [24]. Similar changes in different drying and aging treatments on saffron constituents observed [25].

***Table 1**. Effects of Microwave, Ultraviolet light and low temperature on saffron quality*

Treatments	Saffron quality parameters		
	Crocin	Picrocrocin	Safranol
UV	180.98c	51.77c	39.88a
Low Temp	241.16a	91.66a	37.10b
Microwave	195.12b	79.76b	39.10a

Note: initial crocin content: 243.94, initial picrocrocin content: 101.17, initial safranol content: 36.91

CONCLUSION

The effects of microwave, ultraviolet light, and low temperature on mortality of khapra beetle and quality of saffron showed that the use of microwave with 2450 MHz and 1850 W power for 60 seconds was most effective to control khapra beetle in saffron. The application of different treatments had changed the quality of saffron. Crocin, picrocrocin contents had decreased significantly, while safranal content of saffron had increased upon microwave, ultraviolet light, and low temperature. Saffron treatment with microwave with 2450 MHz and 1850 W power for 60 seconds is an effective method to control the khapra pest.

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