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Review Article

New and emerging trends in phytopathology of medicinally bioactive geographical indicator of Kashmir: Saffron (*Crocus sativus* L.)

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Abstract

Saffron (*Crocus sativus* L.) is an important geographical indicator (GI) tagged cash crop from the Kashmir valley of India, making it a global brand. This spice crop, valued for its dried stigma with a characteristic odor and coloration, is most sought after for its diverse pharmacological activities (anti-tumor, anti-cancer, anti-aging, and their use in cosmeceuticals and pharmaceuticals). Saffron is used against diseases of the central nervous system, age-related diseases, ocular diseases and neuro-degenerative diseases. India contributes 5% of global saffron production and 90% of this produce comes from Jammu and Kashmir. However, the yield of 2.0 to 2.5 kg/ha is very low compared to the global average of 3.4 kg/ha. This has been attributed mainly to the loss of genetic yield potential due to vegetative propagation, primitive cultivation practices, dependence on natural climatic conditions, and stress caused by abiotic and biotic factors. Among the biotic factors, corm rot is the most severe disease, causing a drastic reduction in yields due to the compounding effect of the causal pathogens over the years. The current review has highlighted some of the biological control measures that have been adopted in recent years for the management of this disease. Many new diseases reported in the last 20 years have also been highlighted. The paper presents some of the futuristic trends in saffron research.

Keywords: Saffron, Phytopathology, Biocontrol, Futuristic trends, Bioactivity.

Introduction

Saffron (*Crocus sativus* L.), the golden spice or yellow spice, is a spice of choice in the cuisine of many cultures, particularly exotic cuisine. The dried stigma of the flowers has been traditionally used as a spice in food, a natural dye for silk and fabric, in painting color, in perfumes and cosmetics, and last but not least, in the preparation of medicines. Some of the famous European bakeries like pastries, pies, casseroles, cakes, and dishes like *Arroz con pollo* (Chicken and rice) and *Bacalao viz Caino* (codfish a la Biscay) depend on saffron for their color and characteristic flavor. In Kashmir, saffron is an essential ingredient for the traditional cuisine viz. *Wazwan* as well as special tea *Kehwa* that is served to special guests. Saffron ink finds use in writing amulets and charms and is applied to the forehead in "tilak" in many rituals (Dhar, 2000). After a brief lull in its use, recently, there has been a renewed interest in this mythical drug and a number of cosmetological and pharmacological uses have been highlighted. The Global Industry Analysis Forecast has valued the Saffron Market at US$ 1.07 Bn in 2022, reaching US $ 1.77 Bn in 2029 at an expected growth rate of 7.3%. India ranks second in production of saffron after Iran. It is listed as the most expensive medicinal cash crop in the world with a global production of 418 t y^{-1}, with 90% of global production coming from Iran. Besides it is also grown in Iran, India, Afghanistan, Greece, Morocco, Italy, and Spain (Cardone *et al*., 2020). The higher concentrations of glycosidic carotenoids are reported from India, Greece, New Zealand, and Spain (Caballero-Ortega *et al*., 2007). Kashmir in India is world famous for its saffron with the Pampore region known as the saffron bowl of Kashmir. The Pampore Saffron Heritage of Kashmir has been recognized as a Globally Important Agricultural Heritage System (GIAHS) in India and recently, it has been provided with GI-tagged status (Figure 1). The National Mission on Saffron was introduced in Kashmir in 2010 as part of the Rashtriya Krishi Vikas Yojana (RKVY) and in 2020, the mission was extended to commence saffron cultivation in the northeastern regions of India. Efforts

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Table 1: Health benefits of saffron documented in the recent past

S. No.	*Compound*	*Activity*	*Method*	*Health benefits*	*References*
1.	Polyphenols	Antioxidant activity	Spectrophotometry, histopathological studies, assay kits	Protection of kidney, liver, lungs, and heart from free radicals	Makhlouf *et al.*, 2011
2.	Polysaccharides, crocin contents, and antioxidants	Scavenging, antioxidant and reducing activity	Fourier-transform infrared spectra (FTIR), HPLC, antioxidant assays	Spice, food colorant, and medicine	Zhang *et al.*, 2019
3.	AgNPs using an aqueous extract of saffron	Antibacterial effects	FTIR, X-ray diffraction analysis, transmission electron microscope	Antibacterial effect against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Shigella flexneri* and *Bacillus subtilis*	Bagherzade *et al.*, 2017
4.	Saffron juices	Antioxidant and cytotoxic activities	FRAP and DPPH assays, MTT assay	Preventing cholesterol degradation and protection against Cu^{2+}-mediated degradation of the liposomal unsaturated fatty acids	Tuberoso *et al.*, 2016
5.	Flavonoids and their derivatives	Antioxidant activity	LC-MS, DPPH, NBT and FRAP	Alleviating oxidative stress in plants, yeast, and bacteria	Baba *et al.*, 2015
6.	Crocin-1, crocin-2, crocin-3, picrocroein, acid form of picrocrocin, HTCC-diglycosil-kaempferol trans-crocin-4, trans-crocin-2, trans-crocin-3, safranal, crocetin and cis-crocin-3	Chemo-protective effects	HPLC, colony formation assay	Cytotoxic, mutagenic and antimutagenic activities, potential cancer chemo-preventive agent	Abdullaev and Espinosa, 2004
7.	Crocetin and safranal	Memory enhancing effects	MTT assay, DCF assay	Improvement in learning and memory	Papandreou *et al.*, 2011
8.	Crocin, crocetin, picrocrocin, safranal, kaempferol-3-*O*-sophoroside, kaempferol-3-*O*-glucoside, and quercetin-3-*O*-sophoroside	Antidepressant activity	Analytical method	Sources of flavonol glucosides, treatment of neurodegenerative damage,	Mottaghipisheh *et al.*, 2020
9.	Crocetin di-(β-d-glucosyl)-ester [dicrocin], crocetin-(β-d-gentiobiosyl)-(β-d-glucosyl)-ester [tricrocin] and crocetin-di-(β-d-gentiobiosyl)-ester [crocin]	Free radical scavenging activity	Measurements of cell viability, peroxidized membrane lipids and caspase-3 activity	Neuroprotective effects	Ochiai *et al.*, 2007
10.	Saffron	Protective activity on ischemic hearts	Basal and final serum levels of heart troponin I, heart tissue antioxidants and histopathological indices	Myocardial infarction	Joukar *et al.*, 2010
11.	Saffron supplementation	Antidepressant activity	Random effects modeling procedures	Depression in adults with major depressive disorder	Hausenblas *et al.*, 2013
12.	Carotenoids	Chemoprotective activities	Saffron pre-treatment	anticancer and antitumor	Premkumar *et al.* 2006

are being made to expand the areas to Himachal Pradesh, Arunachal Pradesh, Sikkim, Uttarakhand, Manipur, and Tamil Nadu to meet the domestic as well as global demand (Kumar *et al.*, 2022).

Saffron is acclaimed worldwide for its pharmacological, cosmetological and aphrodisiac properties. From the Persian physician Avicenna of 10^{th} century to the ancient Greeks and Romans, from Cleopatra of Egypt to the

Figure 1: A field view of Saffron (*Crocus sativus* L.) cutivation at SKUAST, Kashmir, India

Maharanis of India, the benefits of saffron were known to all. Now we have well-documented evidence of the impact of saffron on diseases of the central nervous system, *viz*. depression, anxiety, schizophrenia, Parkinson's; age-related diseases like atherosclerosis, hypertension, dyslipidemia, type-2 diabetes; ocular diseases like age-related macular degeneration, diabetic retinopathy, glaucoma, retinitis pigmentosa; neurodegenerative diseases like Alzheimer's disease, Parkinson's disease and muscle weakness in the elderly. Table 1 provides some of the activities and compounds of saffron and their beneficial effects documented in the last 20 years.

In India, the demand for saffron is about 100 tonnes, whereas only 6 to 7 tonnes are produced here. The yield and production of saffron are severely limited by genetic erosion due to vegetative propagation, traditional agricultural practices, lack of awareness about modern technological interventions, and mechanized farming. The cultivation of saffron is highly labor intensive and there is little scope for intercropping or crop rotation as it is a perennial crop lasting for about 7 to 15 years. In addition, it is highly dependent on climatic conditions leading to scarce production in some years and bountiful production in others. However, novel techniques in saffron cultivation are coming up like soilless production, hormone therapy, and omics approach, that have revolutionized the cultivation of saffron.

The major bottleneck in realizing the full yield potential of saffron is diseases besides pests and weeds. Over the years, the major diseases causing significant crop losses year after year are corm rot, root rot, bulb rot, bacterial rot, leaf spot, and blight. However, corm rot remains the major disease-causing significant yield reductions year after year. The disease is a complex one reported to be caused by several pathogens, particularly *Fusarium* sp., *Rhizoctonia* sp. and *Sclerotinia*. The disease incidence ranges from 30 to 80% in different regions and years, depending on the conditions favorable for the disease development and in some years, there is a complete loss of the crop (Gupta, *et al.*, 2021). The present review is an attempt to consolidate the current knowledge on diseases of saffron, particularly corm rot of saffron and its biological control and highlight some of the emerging diseases of saffron under the changing climatic scenario.

Corm rot

Corm rot is an important disease of saffron reported to be caused by many pathogens. In a study from Jammu and Kashmir, India, five fungi, *viz., Fusarium solani, Aspergillus flavus, Trichoderma harzianum, Fusarium neocosmosporiellum,* and *Mucor circinelloides* were found to be pathogenic to saffron causing corm rot, but all the fungi required injury before causing infection (Mansotra *et al.*, 2023) indicating a significant deviation from earlier studies. *F. oxysporum, F. o.* f. sp. *gladioli, F. monoliforme* var. *intermedium* are also reported as pathogenic to saffron, causing corm rot (Sameer *et al.*, 2012). Recently, morphological and multilocus phylogenetic studies revealed that highly virulent *F. nirenbergiae* caused corm rot of saffron in China (Mirghasempour *et al*., 2022). *Penicillium* sp. has also been shown to be pathogenic, causing corm rot of saffron in India and China (Zhang *et al*., 2020). *Sclerotium rolfsii* Sacc. was reported as a causal agent of saffron corm rot from Jammu, India (Kalha *et al.*, 2007). Other fungi associated with corm rot of saffron are *Penicillium* spp, *Stromatinia gladioli,* and bacteria like *Burkholderia* sp. and *Pseudomonas* sp. (Fiori *et al*., 2007).

Traditionally, the disease has been managed by the application of fungicides. Carbendazim and hexaconazole have been efficiently used for the management of disease (Shahnaz *et al*., 2013; Shah *et al*., 2012). Myclobutanil has also been found effective in reducing the disease severity (Ahmad *et al*., 2012). However, due to the adverse effects of chemicals on health and the environment, a search is on for alternatives for chemicals in general and fungicides in particular.

Biological control is amassing a great interest in the management of plant diseases and saffron is no exception. A number of studies have reported the efficacy of bio-control agents in the management of corm rot of saffron. Some of these have been enlisted in Table 2. *Trichoderma asperellum* was found to reduce the mycelial growth of the pathogen, *F. oxysporum,* under *in-vitro* conditions, reduce the population density under field conditions and simultaneously reduce the average number of days to sprouting and flower emergence after sowing (Gupta *et al.,* 2020). The *P. aeruginosa* YY322 encodes genes for phenazine, surdactin, salicylate, hydrogen cyanide, colonization, and biofilm formation, indicating its role in biocontrol and plant growth. Scanning electron microscope (SEM) and transmission electron microscope (TEM) showed the effect of *P. aeruginosa* on the hyphae and conidia of *F. oxysporum* and *F. solani* (Hu *et al*., 2021). Real-time imaging of *Bacillus* sp. strain D5 and *F. oxysporum* in saffron corm revealed that the bacterial species reduced the infection load of infected corms by 50%, whereas there was upregulation and downregulation of various Fox R1

Table 2: Biocontrol strategies against some major pathogens of saffron in the recent past

S. No.	*Pathogen*	*Bioagent*	*Method/Technique*	*References*
1.	*Fusarium oxysporum*	*Trichoderma asperellum*	Dual culture and poison food techniques	Gupta *et al.*, 2020
2.	*F. oxysporum, F. solani, Penicillium citreosulfuratum, P. citrinum* and *Stromatinia gladioli*	*Pseudomonas aeruginosa* strain YY322	Plate confrontation, scanning electron microscopy, transmission electron microscopy	Hu *et al.*, 2021
3.	*Fusarium oxysporum, F. solani* and *Penicillium* sp.	*Bacillus amyloliquefaciens* strain W2	Well diffusion and dual culture assay, pot trials	Gupta and Vakhlu, 2015
4.	*Sclerotium rolfsii* and *F. oxysporum*	*Brevibacterium frigoritolerans (AIS-3), Alcaligenes faecalis* subsp. *Phenolicus (AIS-8) and Bacillus aryabhattai (AIS-10)*	Dual culture	Rasool *et al.*, 2021
5.	Corm rot pathogens in soil	*T. viride-2* and *T. viride-1*	*In-vivo* studies	Ahmad *et al.*, 2012
6.	*F. oxysporum, F. solani, P. citreosulfuratum,* and *P. citrinum*	*Streptomyces yangpuensis*	Dual culture, SEM, TEM	Tian *et al.*, 2022
7.	*F. oxysporum, F. solani,*	*T. viride-2, T. viride-1 and T. harzianum-2*	Dual culture	Shah *et al.*, 2012
8.	*F. monliforme* var. *intermedium, F. oxysporum* and *F. gladioliis*	*T. viride, T. harzanium and P. florescence*	Seed treatment	Sameer *et al.*, 2012
9.	*F. oxysporum*	*T. asperellum*	Dual culture and poison food techniques	Gupta *et al.*, 2020

genes in the presence of Bar D5 (Nancy and Vakhlu, 2024) Gupta and Vakhlu (2015) used native *Bacillus amyloliquefaciens* strain W2 against *F. oxysporum,* reducing the disease incidence by 40%. The antifungal isolates of the bacteria *viz., Brevibacterium frigoritolerans* (AIS-3), *Alcaligenes faecalis* subsp. *Phenolicus* (AIS-8) and *B. aryabhattai* (AIS-10) controlled the growth of *Sclerotium rolfsii* and *F. oxysporum* and had excellent plant growth-promoting properties (Rasool *et al.,* 2021). Similarly, different isolates of *T. viride* reduced the severity of corm rot when applied as corm treatment or incorporated with FYM (Ahmad *et al*., 2012). Application of *T. harzianum* and *Azospirillum* reduced the disease and increased yield (Sameer *et al*., 2012). A similar study using microbial rhizospheric isolates identified bacterial strains *viz. B. pumilus* and WG6 inhibited the pathogenic *Fusarium* by more than 50% (del Gallo *et al.,* 2023).

The endophyte BG-E39, produces chitinases and β-1,3 glucanase, which may cause cell wall degradation of *F. oxysporum* and induce host resistance (Ahmad *et al.,* 2022). S*treptomyces yangpuensis* inhibited the growth and development of pathogenic fungi of saffron, probably due to the production of enzymes and secondary metabolites (Tian *et al.,* 2022). Du *et al.,* (2023) reported that the endophytes *Talaromyces* sp. and *Penicillium* sp. increased the stress-related enzymatic activity, enhanced the biomass of saffron, improved production and accumulation of specific metabolites and prevented corm rot disease.

Emerging diseases

Some of the new and emerging diseases reported from various saffron growing areas are:

Leaf spot and corm rot

Hossainnia and Mohammadi (2018) reported chlorosis and necrosis of leaves of saffron and corm rot caused by *Alternaria alternata* in Iran. Genetic diversity studies revealed the presence of two distinct groups with 71% genetic diversity within populations and 29% diversity located among populations (Najari and Nourollahi, 2019).

Neck and corm rot

Neck and corm rot of saffron is caused by *Stromatinia gladioli.* It causes reddish to dark brown coloration of leaf sheaths and wilting and yellowing of plants. The infected corms show rotting with a shredded appearance (Muñoz *et al.*, 2020).

Bacterial leaf and corm rot

A bacterial disease of saffron caused by *Burkholderia gladioli* pv. *gladioli* causes leaf and corm rot of saffron (Khezri *et al.,* 2023). The bacteria cause rotting of the emerging shoots and leaves, reduce flowering and cause spots on leaves and corms (Fiori *et al.,* 2011).

Black spot

A new disease of saffron caused by *Penicillium citreosulfuratum* was recently reported by Hu *et al.,* (2022). The disease caused typical black spots under the membranous scale leaves of corms and hence was named as black spot of saffron. The disease caused the darkening of the corm in the groove, with light brown coloration at the junction of lesions and healthy areas. Later, the surface of the corm became dark brown to bluish-grey. The disease was not found to be fatal but may affect the overall yield of the crop.

Future trends

The saponins and phenolics from peel and internal parts of saffron corm inhibit fungi like *Aspergillus niger*, *Bipolaris spicifera*, *F. oxysporum*, *P. raistriicki* and *Rhizopus nigricans* which cause corm rot disease of saffron, suggest that higher amounts of phytochemical compounds with antifungal properties if induced, could play a significant role in reducing yield losses (Rubio-Moraga *et al.,* 2013). The aqueous extracts of saffron stigmas acted as a stimulator, improving the growth of tomato plants, and at the same time, had an antifungal effect on *Phytophthora infestans,* causing late blight of tomatoes (Khoulati *et al.*, 2019). However, the cost considerations of using this costly spice as an alternative to chemical products must be estimated for the economical cultivation of this high-value crop.

Shuwen *et al.,* (2020) used next-generation sequencing (NGS) and ITS sequencing to detect different fungi growing on affected saffron corms. Trichocomaceae and *Talaromyces* were dominant in infected corms; Aspergillaceae dominated ungerminated corms, whereas *Penicillium* and Dothideomycetes were high in corms that germinated but could not bloom. Such type of information may prove beneficial for developing strategies to improve the germination and flowering of saffron. More than this, agro-techniques to improve the productivity of the crop must be looked into apart from generating clones with higher production capabilities and resistance to most diseases.

Saffron, the gold spice, is emerging as an important spice in today's world of consumer awareness and environmental consciousness. Although a lot of research has been done on the medicinal and therapeutic effects, there is a huge gap in the isolation of actual active ingredients responsible for such effects. Research is also hindered in human trials because of the ethical issues. The need of the hour is isolation, identification and production of safe pharma products that have high efficiency. In addition, the saffron crop needs more attention to enhance its yields and protection from damaging biotic and abiotic effects. Research should be focussed on managing the diseases in an efficient, eco-friendly way while maintaining sustainable crop yields. The latest methods and techniques, particularly the omics approach, can be a step forward in this direction.

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Author Contribution

Efath Shahnaz: Conceptualization Methodology, Investigation, Formal Analysis, Writing-original draft preparation; Saba Banday, ZA Dar, AA Lone, Mehfuza Habib, Seerat Un Nisa, Amit Kumar and Shahida Iqbal: Data acquisition and curation; Tripta Jhang: Manuscript preparation, Supervision and Editing.

Conflict of Interest

The authors declare that they have no conflict of interest.

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