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A SYSTEMATIC REVIEW OF AN INVASIVE PLANT SPECIES: *VERBESINA ENCELIOIDES* (CAV.) BENTH. & HOOK. F. EX A. GRAY

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ABSTRACT: An introduced, non-native, exotic, or alien species are those that grow in areas outside of their natural habitats. They get introduced deliberately or accidentally into new areas by anthropogenic activities or naturally through water, wind, *etc*. In non-native areas, these species invade rapidly due to the non-availability of natural enemies (prey) in a new habitat. Therefore, these fast-spreading exotic species are called invasive alien species. *Verbesina encelioides*, an invasive species of the family Asteraceae, is introduced from the United States and started to invade many arid or semi-arid regions. It is commonly known as 'Golden crownbeard'. The weed is used as a source of traditional medicines by the indigenous people. Some researchers investigated its toxic, allelopathic, antimicrobial and nematocidal properties. Its phytochemical analysis has shown the presence of flavonoids, terpenoids, alkaloids, carbohydrates, saponins, phenolic compounds, sterol, *etc*. The pharmacological studies showed this plant's antibacterial, antitumor, antifungal, antiprotozoal and hypoglycemic potential. Attempts have been made to cover various aspects related to *V. encelioides* in this review article.

INTRODUCTION: Exotic species are also referred to as alien, non-native, non-indigenous, invasive or introduced species and are defined as species that usually occur outside their natural geographic areas [1]. These species start dominating and negatively affecting the native flora. Nowadays, the invasion of these exotic species has threatened the ecological diversity of natural ecosystems in different parts of the world.

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The invasion occurs by different means, which subsequently replace the native vegetation. Short reproductive cycle, production of a large number of seeds, tolerance to a variety of biotic and abiotic stresses encourages the invasion of these species.

These species release certain chemical compounds in their vicinity, which hindered the growth of local species. These chemicals either reduce the interaction of natives with their environment or modify the surroundings, thus making it unfit for the natives and the best fit for the exotic species. Irrespective of their centre of origin, these alien species are capable of establishing a copious population in new areas. These species used to grow with high density on barren lands, along roadsides, open fields, canal banks, *etc*.

According to Wilcove *et al.,* 1998, more than 40% of species became threatened and endangered because of the excessive growth of invasive species around them [2]. With only 2.4% land area of the World, India supports about 7-8% of the all-recorded species, including over 45,000 species of plants and 91,000 species of animals [3]. Alien plant species (173 species) negatively impacted the native biodiversity and subsequently affected the Indian floristic diversity [4]. Now, several invasive species, including *Verbesina encelioides* have been naturalized in India.

A survey was conducted from 2008 to 2017 in Punjab, India. A total of 10 weed species, including *Verbesina encelioides* were registered to be emerging in different parts of Punjab during the last decade [5]. The probable centres of origin of *Verbesina encelioides* are the United States and Mexico. This weed was found growing along canal banks, roadsides, barren lands and has also invaded residential areas. Its invasive characteristics include high seed production, seed dormancy, ability to tolerate dry conditions, and possible allelopathic effects. With relatively sandy substrate within warm, arid climate zones are vulnerable to invasion by *V. encelioides* [6].

This may be the reason behind the abundant growth of *V. encelioides* in the South-west districts of Punjab like Ferozepur, Fazilka, Bathinda, Barnala, Sangrur and arid sandy areas of district Kapurthala [5]. At the same time, it was not documented till 2017 from Pathankot, Hoshiarpur, Nawanshahar and Jalandhar district [7]. Jain *et al.,* 2008b have discussed various properties of *V. encelioides* including ethnopharmacology, allelopathy, phytochemistry, toxicity and bioefficacy [8]. The current review will provide updated and comprehensive information related to this species.

Accepted Name: *Verbesina encelioides* (Cav.) Benth. & Hook. f. ex A. Gray [9].

Family: Asteraceae (Compositae)

Synonyms: *Encelia albescens A. gray, Verbesina australis Baker; Verbesina encelioides subsp. encelioides; Verbesina microptera* (DC.) Herter; *Verbesina scabra* Benth.; *Ximenesia australis* Hook. & Arn. ex DC.; *Ximenesia encelioides* Cav.; *Ximenesia encelioides* var. *encelioides* [9].

Common Name: Crownbeard; Wild sunflower; Cowpen daisy, Golden crownbeard, Butter daisy, Yellow top, American dog weed, South African daisy. Gold weed, Golden crown and Skunk daisy [6].

Indian Vernacular Name: Jangli Suraj Mukhi, Bansla Genda, Nakli Suraj Mukhi, Mirasol [10, 11, 12, 13, 14].

Native Status: Native to Southeastern North America and parts of Central and South America, specifically Mexico and the southwestern United States of Texas, Arizona [15, 16].

Status in India: Introduced, invasive species [17].

Growth Form: Annual Herb/forb

IUCN Status: Not evaluated[14].

Chromosomes Number: x= n = 17; 2n=34 [18, 19].

Habitat / Ecology: Disturbed areas, along roadsides, field sides, railway tracts, barren land, wastelands, canal banks and uncultivated areas [5].

Morphology:

Habit: Annual herb, odor unpleasant. Root: Taproot system. Stem: Erect, woody, densely short, hairy, branched. Leaf: Simple, mostly alternate, petiolate, petiole dilated at the base to form a pair of stipules like auricles, blade lanceolate to triangular-ovate, coarsely dentate margin, apex acute, reticulate venation. Inflorescence: Heterogamous capitulum or racemose head, solitary at the end of long peduncle or in clusters of 2-3. Flower: Ray Florets: At the periphery of floral head, bright yellow, incomplete, unisexual, pistillate, zygomorphic, epigynous, corolla ligulate, 3 seriate with outer series considerably larger than inner. Disk Florets: At the centre of the floral head, light yellow, corolla five, toothed, tubular, hermaphrodite, Androecium: Stamens 5, epipetalous, syngenesious, bithecous, anthers basifixed, yellow to light brown, Gynoecium: bicarpellary, syncarpous, inferior, unilocular, basal placentation; style simple, long, stigma bifid. Seeds: grayish brown, flattened and broadly winged along margins.

Global Distribution: It is drought tolerant, thus well adapted invasive species and has successfully naturalized in many warm regions of the world.

The occurrence of golden crownbeard was reported at five locations in South Texas from 1992- 1995. Pearsall locations were infested moderately (4-6 plants/m^2) while the Charlotte sites were infested heavy with golden crownbeard populations (>8 plants/m^2) [20]. The plant was also reported in Western Arid Chaco of Cordoba, Argentina, a few semi-arid regions in the Center-East of Tunisia, the mid-rift valley of Ethiopia and some parts of Libya [12, 21, 22, 23]. Presently it is distributed in 5 continents of the world namely America (Argentina, Arizona, Hawaii, Mexico), Africa (Algeria, Egypt, Morocco, Tunisia), Asia (India, Saudi Arabia, Yemen), Europe (Belgium, France, Spain) and Oceania (Australia, Victoria) [23].

Distribution in India: Since, the species is drought tolerant and require less water for its survival, thus invaded arid and semi-arid regions of India including some parts of Punjab, Haryana, Rajasthan, Delhi [5, 24], Eastern dry zones of Karnataka [25], Uttar Pradesh [26], Madhya Pradesh, Bihar and Tamil Nadu [27].

Dakshini and Singh, 1970 reported *V. encelioides* from Delhi [28]. It was found to grow abundantly in the Sariska and Siliserh regions of Alwar district, Rajasthan [11]. In Haryana, *V. encelioides* was reported from two sites namely Nigana Khurad and Khanak [13]. It was a major part of floristics of the Thar desert of western Rajasthan [14] and predominant in the Tal Chhapar Wildlife Sanctuary of Rajasthan with importance value index (IVI) 1.74, Simpson's Dominance Index (SDI) 0.00092 and Whitford's index (A/F ratio) 0.337 [29]. It was also prevailing in the area of Eastern Ghat of Tamil Nadu [30]. A study was conducted to understand the potential invasion of this taxon in India. A total of 361.88km^2 area in the semi-arid region of India, i.e., some parts of Rajasthan, Haryana and Delhi were found to be the most suitable habitat for *V. encelioides.* Precipitation seasonality, annual precipitation, mean temperature of the coldest quarter and maximum temperature of the warmest month were the important factors that affect the distribution of this species. The probability of occurrence of *V. encelioides* increased with the increase in precipitation seasonality and annual precipitation [24]. Recently, a cluster of *V. encelioides* was recorded growing wild in a village (Laurvara) of the Banaskantha district of Gujarat [31].

A survey was conducted in Punjab during 2008-2017 to study the appearance of new weed species [5]. Golden crown beard was probably introduced in Punjab from Rajasthan using land-fill and sand meant for construction purposes. Now it is naturalized, found growing along roadsides, canal banks, and vacant places and gradually replacing the native plant species of Punjab state. This weed was found profusely growing in South-west parts of Punjab, including Ferozepur, Fazilka, Bathinda, Barnala, and Sangrur districts. The plant was also reported in some semi-arid areas of district Kapurthala [7].

Ecology and Phenology: *Verbesina encelioides* produce rapid vegetative and reproductive growth seedlings [32]. Al-Farraj *et al.,* 1988 demonstrated the control of germination in *Verbesina* seeds by the osmotic inhibitors in clay soil. Their experiment showed no promising results in the case of Petri-dishes and more pronounced results in the case of soil with osmotic inhibition, which may be advantageous in checking the establishing of seedlings in adverse conditions [33].

During summer, the foliar ozone injury on leaves was recorded in Great Smoky Mountains National Park, and severe injuries were notified on lower leaves [34]. Seeds of this weed can germinate in a wide range of temperatures (15/5-35/25°C), with optimum germination at 25/15°C. Germination of seeds was minimum at pH <5 or >8. Germination was 95% when seeds were placed on the soil surface. No emergence was observed when seeds were buried to 6 cm or more depth in the soil. Results indicated that this weed can emerge in multiple flushes of germination. Seeds can invade drought-affected areas and can grow in moderately saline, slightly acidic, or alkaline soils [35]. Cold stratification can reduce seed dormancy, which requires a low (15/5–20/10°C) temperature for germination [36]. Taylor *et al.,* 2020 studied the reproductive phenology of *V. encelioides* at Midway Atoll National Wildlife Refuge (NWR) and suggested that the plant took an average of 76 days from the leaves to seed drop; thus, invasion control can be scheduled accordingly [37]. A new variant of croton yellow vein mosaic virus naturally infecting wild sunflower was reported in Noida (Uttar Pradesh), India [26]. *Verbesina encelioides* were designated as an alternative and new host of

Spongospora subterranean (fungus), a causal organism of Powdery Scab disease in Israel [38].

Toxicology: Earlier, the toxicity of *Verbesina encelioides* for livestock was believed to be because of the high level of nitrates [39, 40], but later on Oelrichs *et al.*, 1981 isolated a toxic compound (galegine) from this plant [41]. The plant is widely distributed in the U.S. and thus, represents a potential hazard for grazing livestock. The concentration of galegine in *V. encelioides* and its toxicity and pathologic effects on sheep was also worked out [42]. Affected animals had shown dullness, lack of appetite, and severe lesions in different organs, which finally led to death. A toxic compound (galegine) was said to be responsible for the death of sheep [43]. Therefore, *Verbesina* was classified as a poisonous plant in the United States. Jain *et al.,* 2008a also categorized galegine as a toxic compound present in this plant that causes poisoning in livestock [44].

Allelopathy: *Verbesina encelioides* had shown an allelopathic effect on some native taxa including *Tephrosia purpurea, Cassia occidentalis, Crotalaria medicagenia* and *Indigofera linnaei.* Extracts prepared from different parts of *Verbesina* inhibited the seed germination of native plants. But Leaf and floral extracts were comparatively more toxic [45]. The allelopathic potential of this plant was the major cause behind its dominance in any habitat and was explained using plant extracts on radish seedlings [46]. Field experiments were performed to measure the effects of seven crownbeard densities (0, 0.2, 0.4, 0.8, 1.6, 2.4, and 3.2 weeds/m) on the yield of groundnut (*Arachis hypogaea*), and it was observed that at 3.2 weeds/m density of *Verbesina*, the groundnut yield decreased by about 50% [47]. Different concentrations of the extracts of *Verbesina encelioides* had also negatively affected the growth of some members of Cyanophyceae, Chlorophyceae, and a few species of Bacillariophyceae [48].

Vegetative Propagation: Attempts were made to propagate *V. encelioides* by *in-vitro* methods. The cell cultures of *V. encelioides* were established from seeds on Murashige and Skoog's (MS) basal medium in the absence or presence of IAA, NAA, Kn, and BAP individually or in different combinations, and callus was successfully established on MS medium supplemented with 10 mg/L NAA and 0.4 mg/L Kn [49]. Later, an efficient protocol for *in vitro* micropropagation of *V. encelioides* on MS medium was developed [50]. Highly organized callus induction and proliferation were achieved on MS medium +NAA (1.00 mg/L) + BAP (3.00 mg/L). Optimum shoot multiplication (13.00±0.28) was achieved on MS medium + NAA (0.50 mg/L) + BAP (1.00 mg/L) and similarly, efficient root induction (8.30±0.40) was achieved on MS medium + indole 3-acetic acid (IAA, 1.00 mg/L). The plantlets were hardened in a controlled plant growth chamber before *ex-vitro* transfer.

The *in-vitro* plant regeneration from leaf segments of *V. encelioides* exhibited different responses when cultured on MS supplemented with 0.5 mg/l BAP. The addition of IAA/NAA to the basal medium completely inhibited the formation and growth of callus. When assayed, the endogenous content of IAA in the different segments of the leaf revealed that the middle segment had the highest level of IAA and apical and basal segments contained the lowest contents [51]. Axillary buds, hypocotyls, immature leaves of 7 days old seedlings and nodal segments were also used as explants. *In-vitro* regeneration seemed to be more successful by axillary bud regeneration than by indirect organogenesis [52].

Phytochemical Profile: Phytochemicals are secondary metabolites of plants that exert significant pharmacological and toxicological effects on humankind. They often play an important role in plant defense mechanisms against herbivory and other pathogens. The most critical phytochemicals in plants include terpenoids, phenolics, flavonoids, alkaloids and glycosides, which act as an important source of active principles in modern medicines. Secondary metabolites also have a good antioxidant property which can be used as an effective natural antioxidants source in nutraceuticals.

The phytochemical screening of different parts of *V. encelioides* showed various bioactive compounds responsible for its medicinal and allelopathic properties. The alcoholic and aqueous root extract of *V. encelioides* showed the presence of two amino acids viz. nor-lucine and ornithine, and two carbohydrates, D-ribose and sucrose.

Instead of these, flavonoids, carbohydrates, saponins, phenolic compounds, and sterol were also present in both root and shoot extracts [48, 53, 54, 55]. Roots of *V. encelioides* also contained tetracosan-1-oyl 1-tetradecanoate (lignoceryl myristate), β-amyrin palmitate, urs-12-en-3β-olyloleate (β-amyrin oleate) and β-amyrin stearate [56].

The amount of protein is comparatively much higher in leaf, stem, flower, and seeds extracts, whereas phenolic compounds are maximum in roots and starch is least in all these plant parts [57]. Various chemical compounds (taraxasterol acetate, ceryl alcohol, β-amyrin, β-sitosterol and stigmasterol mixture, p-coumaric acid, β-sitosterol and stigmasterol-3-O-β-D-glucopyranoside mixture, quercetin-3-O-β-D-galactopyranoside, and galegine) were isolated through the petroleum ether and ethyl acetate fractions of hydroalcoholic extracts of the aerial branches, roots, and flowers of this plant [58]. The structure of these compounds was analyzed using physicochemical characters and spectral methods like mass, UV, IR, and NMR. Several triterpenoid compounds *viz*. friedelin, epifriedelinol, lupeol, α- and β-amyrins, stigmasterol, betulin, and β-sitosterol, were isolated from the callus of this plant and identified based on their chromatographic behaviour, melting points and spectral analysis [49].

Traditional Medicinal Uses: *Verbesina encelioides* is used in traditional medicines in different parts of the world. It has been found effective in the treatment of cancer, gastrointestinal disturbance, skin ailments, snakebite, warts, haemorrhoids**,** diaphoretic, piles, dropsy, conjunctivitis, ophthalmic, anti-inflammatory and for the treatment of gum sores [8, 11, 12]. Other medicinal properties include analgesic, emetic, febrifuge, insecticide, *etc*. North Dakota Hopi Indian tribe use *V. encelioides* for the treatment of spider bite. Verbesina tea is a mild laxative, that induces sweating and lowers fever [52].

Bio-activities of *V. encelioides:* *Verbesina encelioides* had shown strong activity against many bacteria and fungi but not promising activity against different cancer cell lines [8, 10, 25, 49, 59, 60, 61, 62]. Cell cultures of *V. encelioides* were established from seeds on MS basal medium [49]. The seed-callus extract inhibits the growth of *Bacillus subtilis* (10mm), *Candida albicans,* and *Trichophyton rubrum* (15 mm)**.** The plant root extracts were prepared in methanol, cold water, and hot water to examine their antimicrobial activities against selected microorganisms (Bacteria and fungi) by disc diffusion method and antioxidant potential by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydartes) method.

All the test extracts showed antimicrobial activity but hot water extract was remarkably active against *Pseudomonas aeruginosa* and *P. crysogenum.* Hot water extract also showed 20.04% inhibition of DPPH at 80ug concentration [8]. The methanol extract of *Verbesina encelioides* exhibited appreciable antioxidant activity [10]. It also showed activity against *Klebsiella pneumoniae* and *Enterobacter arerogenes* but did not prevent any cell growth [59]. Similar studies were done by Gouda *et al.,* 2014 and Albalawi *et al.,* 2015 in Saudi Arabia. The methanol extract of *Verbesina encelioides* was tested for their *in-vitro* anti-microbial activity and *in-vitro* cytotoxicity on different human cancer cell lines. The antibacterial assay was performed by disc diffusion method and the cytotoxic activity against breast (MCF7), ovarian (A2780) and cervical (HeLa) cancer cell lines.

The plant had remarkable antibacterial activity but not promising cytotoxicity against different cancer cell lines [60, 61]. Al-Oqail *et al.,* 2016 screened the extracts of *V. encelioides* for anticancer potential against human lung cancer (A-549), breast cancer (MCF-7) and liver cancer (HepG2) cell lines. After 24 h exposure of cells with 10-1000 µg/ml extracts showed that 250-1000 µg/ml concentrations were cytotoxic against MCF-7 and HepG2 cells but not against A-549 cells. Moreover, the extract displayed a higher decrease in the cell viability in the case of HepG2 cells than MCF-7 cells and therefore, HepG2 cells were selected for further studies *viz.* oxidative stress (GSH and LPO), reactive oxygen species (ROS) generation, mitochondrial membrane potential (MMP), cell cycle arrest, and DNA damage. The cell cycle analysis and comet assay showed that *V. encelioides* considerably induced G2/M arrests and DNA damage [62]. The hydroalcoholic extracts of the aerial branches, roots, and flowers of *V. encelioides* were cytotoxic against hepatocellular carcinoma

and active against Gram +ve bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), Gram -ve bacteria (*Escherichia coli*) and fungi (*Aspergillus fumigatus* and *Candida albicans*) [61]. According to Ramakrishnan *et al.,* 2017 proteins from the young leaves of *V. encelioides* exhibits moderate antimicrobial activity against various bacterial and fungal species [25].

Aqueous and alcohol root extracts of *Verbesina encelioides* were allowed to dry and given to swiss albino mice to check their hypoglycemic activity [63]. The oral administration of root extracts reduces the blood glucose significantly in normal and Streptozotocin, alloxan diabetic mice. Aqueous extract of fresh flower, leaves and stem of *Verbesina encelioides* showed strong nematocidal activity against the root-knot nematode (*Meloidogyne javanica*) and the plant was recommended as green manure or its extracts as nematicides for the control of root-knot nematodes [64]. Antiprotozoal activities of major constituents (Pseudotaraxasterol-3β-acetate, benzyl 2, 6-dimethoxy benzoate, 16β-hydroxy-pseudo-taraxasterol - 3β - palmitate, pseudo-taraxasterol, β-sitosterol glucoside and β-sitosterol galactoside) of *Verbesina encelioides,* isolated by using bioactive petroleum ether was evaluated and observed that compounds like Pseudotaraxasterol-3β-acetate showed moderate to weak activity against *Leishmania infantum, Trypanosoma brucei, Plasmodium falciparum.* Compound 16β-hydroxy-pseudotaraxasterol-3β-palmitate showed moderate activity against *Leishmania infantum;* compound pseudotaraxasterol revealed weak activities against *Trypanosoma cruzi*. It was also observed that all compounds were non-cytotoxic and showed less antiprotozoal activity than the crude fraction [65].

Miscellaneous Studies: The extracts of *V. encelioides* prepared in hexane, ethanol, and water were studied for their phytopathogenic (*Alternaria solani*) activity along with commercial fungicides, 'Captan' (chemical origin) and 'Sedri 650' (biological origin). The aqueous extracts highly inhibited the development of the fungus (75 to 85%). It means the extract may be used as a viable alternative to commercial fungicides to control this pathogen [66]. The eco-friendly nanoparticles synthesized from the stem and leaf extracts of *V. encelioides* for the first time in 2012 were tested for their antimicrobial activity [67]. A powder form of the extracts of stem and leaves of *V. encelioides* was used for the biosynthesis of bio-nanoparticles characterized by UV-visible spectrophotometer, scanning electron microscopy, and X-ray diffraction FTIR analyses. The obtained particles were then subjected to an antimicrobial assay.

Comparative analyses of the antimicrobial behaviour of aqueous, ethanol and methanol extracts and bio-nano particles against 2 bacteria (*Escherichia coli* and *Vibrio cholerae*) and 2 fungi (*Aspergillus niger* and *A. flavus*) were made [68]. The gamma irradiated seeds of *V. encelioides* altered the mean total seed germination and temporal patterns of the germination when compared with control [69].

Methods of Management:

Physical/ Mechanical Control: The plant should be cut at the base and pulled by hand before the seed set. Plants with mature seeds should be burned. However, these techniques are time-consuming but still effective.

Chemical Control: Field experiments were conducted at five South Texas locations (1992-1995) for the evaluation of eight postemergence herbicides individually or in combination to control golden crownbeard and to manage peanut yield. Field studies suggested that imazapic and imazethapyr have provided sporadic control of golden crownbeard, especially under variable moist conditions and large weed size. Further, Bentazon, lactofen, pyridate, 2,4-DB and acifluorfen or pyridate plus 2,4-DB were found to be the best options for POST golden crown beard control [20]. Biological control has not yet been explored.

CONCLUSION: The biological invasion of alien species is the second worst threat after habitat destruction, which is responsible for the loss of native species. Invasive species have a wider range of ecological amplitude, thus, once invaded, they consistently reproduce and sustain populations for many generations and become naturalized in new areas. The available literature suggests that *V. encelioides* is an invasive species and has naturalized in various parts of the world and is approaching very quickly to the remaining parts. Its fast-invading properties include rapid seedling,

vegetative and reproductive growth, high seed production, toxicity to livestock and allelopathic effects. Presently in arid to semi-arid regions of India, this weed is spreading more rapidly than other weeds. Since the species contain a variety of phytoconstituents of medicinal importance (antimicrobial, antifungal, cytotoxic, antiprotozoal and nematocidal) and has also evolved in various traditional health-related formulations, it can be explored further for its use as raw material in the pharma sector. This approach may be helpful for its sustainable use and control.

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