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The use of Cannabis sativa L. for pest control: From the ethnobotanical knowledge to a systematic review of experimental studies

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Abstract:	Background. Despite the benefits that synthetic pesticides have provided in terms of pest and disease control, they cause serious long-term consequences for both the environment and living organisms. Interest in eco-friendly products has subsequently increased in recent years. Methods. This article briefly analyzes the available ethnobotanical evidence regarding the use of Cannabis sativa as a pesticide and offers a systematic review of experimental studies. Results: Our findings indicate that both ethnobotanical and experimental procedures support the use of C. sativa as a pesticide, as remarkable toxicity has been observed against pest organisms. The results included in the systematic review of experimental studies (n = 23) show a high degree of heterogeneity, but certain conclusions can be extracted to guide further research. For instance, promising pesticide properties were reported for most of the groups of species tested, especially Arachnida and Insecta; the efficacy of C. sativa as a pesticide can be derived from a wide variety of compounds that it contains; it is crucial that future studies use certain cultivars/chemovars that are being developed for industrial purposes; appropriate extraction methods should be explored; and upper inflorescences of the plant may be preferred for the production of the essential oil, but further studies should explore better other parts of the plant. Conclusion. In the coming years, as new findings are produced, the promising potential of C. sativa as a pesticide will be elucidated, and reviews such as the present one constitute useful basic tools to make these processes easier.

The use of *Cannabis sativa* L. for pest control: From the ethnobotanical knowledge to a systematic review of experimental studies

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ABSTRACT

Background. Despite the benefits that synthetic pesticides have provided in terms of pest and disease control, they cause serious long-term consequences for both the environment and living organisms. Interest in eco-friendly products has subsequently increased in recent years. Methods. This article briefly analyzes the available ethnobotanical evidence regarding the use of *Cannabis sativa* as a pesticide and offers a systematic review of experimental studies. Results: Our findings indicate that both ethnobotanical and experimental procedures support the use of *C. sativa* as a pesticide, as remarkable toxicity has been observed against pest organisms. The results included in the systematic review of experimental studies (n = 23) show a high degree of heterogeneity, but certain conclusions can be extracted to guide further research. For instance, promising pesticide properties were reported for most of the groups of species tested, especially Arachnida and Insecta; the efficacy of *C. sativa* as a pesticide can be derived from a wide variety of compounds that it contains; it is crucial that future studies use certain cultivars/chemovars that are being developed for industrial purposes; appropriate extraction methods should be explored; and upper inflorescences of the plant may be preferred for the production of the essential oil, but further studies should explore better other parts of the plant. Conclusion. In the coming years, as new findings are produced, the promising potential of *C. sativa* as a pesticide will be elucidated, and reviews such as the present one constitute useful basic tools to make these processes easier.

Keywords: biopesticides, pest control, *Cannabis*, hemp, ethnobotany, traditional uses

Introduction

Synthetic pesticides have been largely used for crop protection for decades. Although the global diversity outlook published by the Convention on Biological Diversity in 2020 stated that the use of chemical fertilizers and pesticides has stabilized globally,[1] the presence of pesticides in agriculture has increased by almost 1% per year over the last decade,[2] so their use is still remarkably high. Chemical pesticides tend to have concerning negative impacts on the environment at large and local biodiversity. For instance, a major study conducted in eight European countries reported that the use of pesticides was responsible for reduced diversity in terms of plants, insects and birds.[3] Later studies systematically confirmed this association.[4,5] In addition to harms to the environment, human health is also affected by the widespread use of pesticides.[6] Apart from safety concerns related to both the environment and human life, there are limitations in terms of efficacy, since it has been observed that many pathogens develop resistance to chemical pesticides over the long term.[7-9]

In light of the stated above, and especially considering the concept of "one health," which emphasizes the interconnection between people, animals, plants, and their environment,[10] interest in eco-friendly or green alternatives for pest control has increased exponentially during recent years.[11] Specifically, products derived from plants and other natural products, commonly termed "biopesticides," have received special attention. Nicotinoids from *Nicotiana tabacum* L., pyrethrins from *Tanacetum cinerariifolium* (Trevir.) Sch.Bip., and secondary metabolites of *Azadirachta indica* A.Juss. are among the products that have generated significant interest.[12] Besides being barely toxic to humans and vertebrates in general, Benelli and their colleagues highlighted four main benefits associated with botanical pesticides: 1) they are relatively inexpensive and easy to employ; 2) some of them are effective at very low doses; 3) aqueous botanical extracts can be easily employed to treat mosquito breeding sites, without the further addition of other synthetic surfactants; and 4) these products exert their action through multiple mechanisms, which prevents the development of resistance by pathogens.[13] However, there are also some drawbacks and limitations that restrict their research and use. First, most of these products consist of the essential oil (EO) of the plant of interest. The composition of the EOs is commonly subjected to variations, and the vegetal material needed for large-scale production could be enormous if the plant does not produce high amounts of EO. Furthermore, certain EOs have shown toxicity for non-target organisms, such as the EO of *Melaleuca alternifolia* (Maiden & Betche) Cheel.[14] Thus, despite green alternatives seeming preferable to chemical pesticides, they require technological innovations in order for appropriate formulations and delivery methods to be developed, as well as the cautious selection of the best botanical candidates, and subsequent safety studies regarding non-target organisms, including humans.

In recent years, *Cannabis sativa* L. has been proposed as an interesting candidate for use as a pesticide.[15] It should be noted that while there is only one recognised species—*C. sativa*—different chemovars and cultivars exist.[16] Plants that contain less than 0.3% of Δ^9-tetrahydrocannabinol (THC) and which are grown for fiber and seed production are normally called hemp, while marijuana/cannabis refers to plants that contain higher THC, which are used primarily for the production of medicinal cannabis.[17] Apart from the two well-known cannabinoids, THC and cannabidiol (CBD), the plant produces more than 500 compounds, including other cannabinoids (cannabichromene, cannabigerol), terpenoids (myrcene, limonene, trans-caryophyllene), and flavonoids (apigenin, luteolin, quercetin), among others.[18] The compounds and mechanisms through which *C. sativa* exerts its pesticide effects are not currently elucidated. However, the pharmacological action of terpenes and cannabinoids, as well as interactions between certain compounds, could be involved.[19] *Cannabis sativa* has a high content of EO; therefore, its cultivation and extraction is economically feasible. The EO is mainly produced in the glandular trichomes of the aerial parts, as part of the viscous and sticky mixture that traps and/or repels insects. As a consequence, *C. sativa* shows a particular resistance of pests, which allows for eco-friendly cultivation.[15] Additionally, certain chemovars and cultivars have been developed for industrial use (e.g., 'Futura 75'), suggesting control of the growing environment and thus the standardization of the compounds and their proportion, avoiding the variability concern.[20]

It should be noted that the use of *C. sativa* as a pesticide is quite old, as a 1950 report[21] from the United Nations cited a reference from *Chemiker Zeitung* (a German scientific journal) published in 1922, reporting the use of preparations with a basis of *C. sativa* leaves as insecticides. According to that reference, the leaves and stalks were dried at a low temperature and the desiccated product reduced to a fine powder. The powder obtained, when spread on pieces of material, woolen cloths, or sprayed over plants, was said to protect them from insects.[21] From historical, non-Western records, we can mention the Arabic *Kitāb al-Ḥāwī fī l-ṭibb* (*The Comprehensive Book of Medicine*, 10th century), in which it is recommended to place branches of hemp on the bed in order to avoid bedbugs and mosquitoes.[22] Similarly, in the Byzantine Empire, Casiano Baso, in his *Geoponica*, stated that sleeping next to a flexible branch of hemp would repel mosquitoes.[22]

The crucial role that the traditional knowledge plays in the discovery of natural products and their uses is well known. In the specific case of *C. sativa*, the Botanical Institute of Barcelona (IBB; https://www.ibb.csic.es/en) and the University of Barcelona (UB; https://www.ub.edu/portal/web/dp-bsma/botanica) have recently developed a database (CANNUSE; https://cannusedb.csic.es) collecting the global ethnobotanical uses of *C. sativa*, among which pesticide uses are also reported.[23] Given the evidence coming from traditional knowledge, and the recent interest by many researchers in the potential use of *C. sativa* as an eco-friendly alternative to current pesticides, further research in this field is considered valuable and necessary.

The present work aims, first, to summarize the traditional ecological knowledge regarding the use of *C. sativa* as a pesticide, from the references found in the CANNUSE database, and, second, to perform a systematic review of experimental studies assessing the pesticide potential of this plant.

Methods

Search in CANNUSE database and systematic review of experimental studies

Records related to the use of *C. sativa* as a pesticide were hand-searched in the CANNUSE database, and the whole document was stored for data extraction. The review of experimental studies was carried out in accordance with the preferred reporting items for systematic reviews and meta-analysis guidelines (PRISMA).[24] We attempted to identify all experimental studies available to review in which *C. sativa* or isolated cannabinoids were assessed against pests, from 1970 to March 2021. Electronic searches were performed using PubMed, Web of Science, and Google Scholar databases. The following keywords were used: (cannabis OR hemp) AND (pest* OR insect* OR plague). References were retrieved through searching electronic databases and manual searches through the reference lists of identified literature.

Eligibility Criteria

The following inclusion and exclusion criteria were established prior to the literature search: i) Article type. All studies published in peer-reviewed journals involving the use of any chemovar or cultivar of *C. sativa* or isolated cannabinoids against pests were included. Pests are understood in a broad sense. Mosquitoes, flies, ticks, mites, worms, aphids, bugs, thrips, snails, or beetles, among others, that are considered pests for crops were included. Reviews, abstracts, comments, and editorials were excluded. ii) Study design. The review included bioassays using *C. sativa* extracts or isolated cannabinoids against pests. iii) Experiments. All designs evaluating the potential pesticide use of *C. sativa* or cannabinoids were included. iv) Outcomes. We included all reports that assessed the biological effects of *C. sativa* extracts or cannabinoids systematically (with standardized biological measures).

Data Extraction

One author (GO) screened all studies, and doubts were resolved by a team of botanical experts. From the articles included, we recorded the names of authors, year of publication, study design, type of experiment, chemovar of *C. sativa* used, parts of the plant used, method of extraction, pest organisms used, results of phytochemical analysis (if any), and main findings observed. Pest organisms were further classified in taxonomic groups.

Results and discussion

This investigation comprised an initial selection of references included in the CANNUSE database reporting on traditional ecological knowledge regarding the use of *C. sativa* as a pesticide. Then, a systematic review of available experimental evidence possibly supporting such use was performed. Studies assessing the potential activity of *C. sativa* extracts were collected and summarized. They mostly consisted of bioassays using various chemovars or cultivars of *C. sativa* against distinct species. While synthesizing the extant literature, we have bridged traditional and experimental knowledge, providing a fruitful basis that may help to develop a more sustainable and eco-friendly agriculture practice.

First, it should be noted that, from a broader perspective, the problem of pests in large-scale agriculture is primarily caused by unsustainable models prioritizing monocultures. This kind of agricultural practice is associated with biodiversity loss and, therefore, enhanced vulnerability to outbreaks of virulent pest insects.[25,26] Thus, the use of *C. sativa* or other products for pest control should be conceived of as partial solution that must be accompanied by other strategies capable of addressing the underlying causes of the problem. In that regard, however, insect pests have always existed, and the use of EOs or crushed plant parts against insects has been extensively reported on as being successful in traditional medicines.[27,28] Nevertheless, the use of *C. sativa* for that indication has not received much attention, possibly because of its several other uses (medicinal, as a textile or alternative material, among others) have received more interest.

Traditional ecological knowledge

Nine references[29-37] were found in the CANNUSE database (see Table 1). It should be noted that other, historical references exist as well, such as the ones cited above.[21,22] This suggests a long tradition of *C. sativa* use as a pesticide. Regarding these specific references, they were mainly reported in Asian countries, such as India[30,31,33,36,37] and Pakistan.[29,35] Scarce but interesting information can be drawn from these traditional ecological reports. The leaves are the part of the plant that was mostly used (in eight out of nine references),[29-34,36,37] but the use of the whole plant is also mentioned in three references.[31,32,36] Leaves can be pressed to obtain "juice"[29] or for the preparation of extracts.[34] Among the methods used to administer *C. sativa*, smoke is mentioned in three references,[30,33,34] while fresh or dried leaves were also reportedly used in granaries or under the bed for repelling insects.[32,37]

Systematic review of experimental studies

The search of the literature yielded 496 references that were reviewed for abstract screening. Hand searching the bibliographies of the selected citations identified 10 additional citations. Following this and after the removal of duplicates, 33 potentially relevant references were identified. Full-text reports for these citations were obtained for a detailed evaluation. Ten citations were excluded for various reasons (see Figure 2). Thus, 23 citations[12,15,38-58] were included in the systematic review. The main characteristics and results of each citation are presented in Table 2.

The various organisms used in the experimental studies were classified into phylum, class, and order. Most experimental studies (16 out of 23) focused on the effect of *C. sativa* on arthropod pests. These results are in accordance with the data obtained from traditional ecological knowledge, where six out of nine studies specifically mentioned the use of *C. sativa* as an insect repellent. The studies included in the systematic review found remarkable toxicity of *C. sativa* extracts against all the Arachnida using mostly aerial parts and flowers of both 'Felina 32' and wild hemp.[12,42,51,55] In the Insecta group, *C. sativa* toxicity was reported against *Oryzaephilus surinamensis,*[47] *Tribolium confusum*,[47] *Aedes aegypti*,[56] *Aedes albopictus*,[39] *Anopheles gambiae*,[53] *Anopheles stephensi*,[53] *Musca domestica*,[15,40] *Aulacorthum solani*,[42] *Brevicoryne brassicae*,[38] *Myzus persicae*,[40] *Schizaphis graminum*,[12] *Manduca sexta*,[52] *Plodia interpunctella*,[47] *Spodoptera littoralis*,[15,40] and *Culex quinquefasciatus*,[15,49] although one study found the *C. sativa* extract to be ineffective against *C. quinquefasciatus*.[40] Other species in which *C. sativa* extracts did not show relevant toxicities included *Reticulitermes virginicus*,[54] *Brassicogethes aeneus*,[58] *Trogoderma granarium*,[43] *Chaoborus plumicornis*,[54] *Drosophila melanogaster*,[54] and *Frankliniella occidentalis*.[12] It is not possible to elucidate the reasons for this differing efficacy, since wild hemp and other chemovars/cultivars, different parts of the plant, and different extraction methods were found to be efficacious in some cases and inefficacious in others.

The results for the Chromadorea species were also mixed. Different cultivars and chemovars ('Tiborszallasi,' 'Futura 75,' MX-CBD-11, MX-CBD-707) showed toxicity against *Heterorhabditis bacteriophora, Steinernema carpocapsae*, and *Steinernema feltiae*.[46] *Meloidogyne incognita* also was affected by three different *C. sativa* plants, one being wild hemp and the others non-specified chemovars.[44,48,50] However, no toxicity was observed in three species (*Caenorhabditis elegans*, *Strongyloides papillosus*, *Haemonchus contortus*).[41,54] These three Nematoda species were tested in studies using wild hemp. The only study performed on species from Gastropoda (*Physella acuta*) reported toxicity of the EO extracted from aerial parts.[39] *C. sativa* EOs were effective against the two species of fungi tested (*Sclerotium rolfsii* and *Cryptococcus neoformans*).[45,57]

Almost half of the studies (11 out of 23) reviewed reported wild hemp uses. Wild hemp is certainly the most suitable plant-type to corroborate the ethnobotanical evidence, as no cultivar or chemovar is usually reported in traditional ecological reports. However, in terms of the reliability and replicability of the results, industrial *C. sativa* and specific chemotypes would be preferable. Moreover, it should also be noted that wild hemp would present varying chemical profiles depending on region, soil type, harvesting method, storage conditions, and many other factors. Thus, even when using wild hemp in accordance with ethnobotanical evidence, a remarkable degree of variability should be assumed. This issue has been problematic not only in the field of *C. sativa* research[59,60] but also in research with natural products in general.[61] Metabolic profiling and other validated methods should be performed in studies using complex natural products, although many other approaches are being implemented, such as the use of standardized extracts.

Other sources of heterogeneity include the parts of the plant used and the extraction method. Regarding the former, six studies[38,44,45,49,50,54] used leaves, whereas five studies[40,43,53,55,57] used inflorescences. The remaining studies used combinations of leaves, inflorescences, and other parts of the plant. It should be noted that, generally, the EO is primarily secreted by glandular trichomes present on the surface of plant organs, particularly flowers and leaves. However, in the specific case of *C. sativa*, a greater amount of EO is produced on the inflorescences.[40] Moreover, it has been described that the upper inflorescences have significantly higher amounts of cannabinoids and terpenoids than those lower down the stem.[62] Therefore, the plant parts selected would cause significant variations in the extracted products. Regarding extraction methods, steam distillation and hydrodistillation were the most commonly reported. In that regard, two major approaches can be outlined from the literature on *C. sativa* research. On one side, conventional methods, such as organic solvent extraction, have been extensively used. This process exposes the plant constituents to the combined effects of heat and acid, which may undergo relevant modifications. On the other side, innovative, still marginal methods can be observed, such as superficial fluid extraction (SFE). Carbon dioxide (CO_2), which is quite inefficient at dissolving polar compounds, is usually used as the supercritical solvent. In order to improve the solubility of polar substances, a cosolvent or modifier is often added.[63] To avoid heterogeneity and chemical modifications of EO composition derived from conventional extraction method, future studies should preferentially use improved extraction methods.

Interestingly, one study[15] found greater efficacy in an EO extracted from inflorescences than in the EO extracted from leaves. Moreover, its efficacy was better than those of other EOs considered to be highly promising, such as ones obtained from *Cunninghamia konishii* Hayata or *Corymbia citriodora* Hook.[15] Another study found that the EOs of three different chemovars of *C. sativa* were more effective when they were extracted from fresh parts rather than dried.[57] Considering the well-known loss of terpenes in the drying process, including the more volatile monoterpenes especially,[64] this suggests that terpenes are greatly involved in the pesticide properties of *C. sativa*. In the same study,[57] *N*,*N*-diethyl-meta-toluamide (DEET) was used as a positive control, and it was found that *C. sativa* extracts had an equivalent efficacy. Future studies should also use positive controls in order to compare efficacy.

Although it is generally claimed that natural, so-called green products are safer for the environment than synthetic ones, few studies have assessed the safety of plant EOs in non-target organisms, including humans.[65,66] Among the studies reviewed, two studies assessed the potential toxicity of *C. sativa* against three non-target species. They reported the absence of toxicity for two of them (*Harmonia axyridis* and *Eisenia fetida*) and toxicity against *Cloeon dipterum*, although the toxicity was lower than for target species. Further studies need to test *C. sativa* toxicity against more non-target species of interest. Lastly, other related, potentially fruitful uses in regards to crop protection were not included in the review, but they deserve to be mentioned. First, it seems that the inclusion of *C. sativa* in composting material could entail certain benefits[67] and requires further research, since the enrichment of composting materials is currently under investigation.[68] Additionally, a study that used CBD oil as a post-harvest approach in strawberries is also highly interesting. Despite the scarcity of studies for that indication, we suggest that, given the global and economic interest in post-harvest management, these early, promising results should be taken into account.[68]

Mechanisms of Cannabis sativa *pesticide activity*

At this point, we can briefly suggest some mechanisms of action through which *C. sativa* may exert its pesticide effects. Studies describing the mechanisms of action of cannabinoids in regard to their insecticidal activity are lacking, but their nematocidal,[46,50] acaricidal,[51] and larvicidal[47] have been reported even in the absence of cannabinoid receptors in insects, which suggests other mechanisms unrelated to those receptors. However, again, we are lacking information about the binding profile of cannabinoids in insects, and comparisons with their binding profile in mammals could not be performed. First, it should be noted that, at least *in vitro*, *C. sativa* exerts a powerful inhibition of acetylcholinesterase (AChE), which is the main effect of insecticides like organophosphates, neonicotinoids, and spinosyns, which eventually cause cholinergic poisoning.[69] In *C. sativa*, both AChE and butyrylcholinesterase (BChE) inhibition seems to be exerted in parallel by different compounds, such as beta-sitoserol, campesterol, apigenin, alpha-bisabolene, Δ^8-tetrahydrocannabinol, THC and other cannabinoids, kaempferol, luteolin, stigmasterol, quercetin, α-guaiene, α-humulene, and caryophyllene oxide.[70] Notably, *C. sativa* EO inhibits AChE in a more effective manner than other EOs.[71] Additionally, both THC and CBD are P-glycoprotein (P-gp) inhibitors.[72] P-gp belongs to an ATP-binding cassette (ABC) transporter subfamily, responsible for the drug efflux from the cytoplasm to the outside of the cell.[73] It is also the main mechanism that has been associated with insecticide-resistant phenotypes.[74] To sum up, it is highly plausible that, along with other mechanisms (e.g., point mutations), insects use ABC transporters for the excretion of plant secondary metabolites or chemical pesticides[74-77] and, therefore, for the development of drug resistance. Then, with P-gp inhibition, not only would higher bioavailability of other insecticidal compounds of *C. sativa* be achieved, but also reduced probability of the development of drug resistance.

Unfortunately, nine studies included in the present review[12,41,44,45,48-51,56] did not perform phytochemical analyses, adding a notable degree of uncertainty that should be avoided in future research. The most commonly reported compounds in the studies reviewed that performed phytochemical analyses[15,38-40,42,43,46,53-56,58] were terpenoids ((E)-caryophyllene, trans-caryophyllene, myrcene, α-humulene, and α-pinene). These volatile compounds are well known for their diverse biological activities against insects, since they can act as insecticides,[78] antifeedants,[79] or repellents,[80] among many other functions. The mechanism of action involved in pesticide effects involves AChE inhibition as well as the blocking of ^{3}H-octopamine or GABA receptors.[81] Notably, certain terpenoids tend to be species-specific[82,83] and, thus, some of them affect only certain insects. The fact that *C. sativa* contains a high number of terpenes is an advantage in that sense.[84] *C. sativa* is also rich in flavonoids, which are involved in several functions mostly related to the plant-environment interaction. They tend to affect the behavior and growth of insects through the inhibition of AChE, the ecdysone receptor (EcR), or alterations in the gustatory, sensile and neuronal responses to food.[85] Remarkably, they are known to also be P-gp inhibitors[86] and, additionally, glutathione S-transferases (GSTs) inhibitors.[87] GSTs are involved in the detoxifying process of insecticides, so one of the putative roles of flavonoids is also to fight against drug resistance mechanisms.

In light of the findings reported in the studies reviewed here, and from a classical pharmacological perspective, it seems advisable to use isolated compounds from *C. sativa* rather than the whole EO, since higher efficacy was observed in the former case in two studies.[55,56] However, although in these assays the isolated compounds exerted enhanced effects, we should consider not only knowledge related to the development of drug resistance over the long term, as occurred with chemical pesticides,[9,88] but also the evidence coming from the polypharmacology paradigm. Mostly developed in the field of human pharmacology, but with obvious connections to phytochemistry and phytotherapy, this paradigm offers a strong body of evidence supporting the use of complex drug products that modulate several targets rather than highly selective ligands.[89-91] One of the main points that justifies this complex approach is that living organisms are complex systems organized within complex networks that, most often, are capable of resisting selective "attacks" through compensatory mechanisms, and thus the modulation of numerous targets is advantageous. In the specific case of pesticides, the argument of the absence of drug resistance in the case of complex products could be more compelling. For instance, the multiple mechanisms described above make the development of resistance extremely unlikely. Moreover, among the hundreds of compounds of different classes within the EO, there are clear interaction effects (additive or synergistic, but potential antagonistic effects should also be identified) that can improve the efficacy of the product.[92-95] In order to obtain these benefits and a suitable product, we would recommend the use of the whole *C. sativa* EO involving appropriate extraction methods and the proper plant varieties and plant parts, which are factors that modify EO composition.

Notably, in light of the results observed in this review, it seems that most of the experimental studies support the traditional use of *C. sativa* as a pesticide, since varying rates of toxicity against different pest organisms have been systematically reported, albeit sometimes with a certain degree of heterogeneity. To the best of our knowledge, this is the first systematic review assessing this issue, so we hope the information provided here will contribute to other researchers designing further research. The main limitation of this review is the limited number of studies that have addressed the pesticide potential of *C. sativa*. We also found a high heterogeneity of methods used in the studies reviewed and limited knowledge regarding the phytochemical profile of samples used. Despite this, we consider the review presents and organizes relevant information that can be fruitful for future lines of research.

Conclusion

The potential use of *C. sativa* as a pesticide has been highlighted in this review. Despite the high degree of heterogeneity observed in the reviewed studies, excellent results have been observed and certain general recommendations can be extracted. *Cannabis sativa* EO could be a suitable candidate as a greener alternative to current pesticides, due to its eco-friendly cultivation, its numerous endproducts that can be obtained beyond the EO (e.g., fiber, food, etc.), its interesting mechanisms of action, and the renewed interest and recent regulations allowing for and facilitating its large-scale cultivation.

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Figure 1. Flow diagram of the selection of studies for the systematic review.

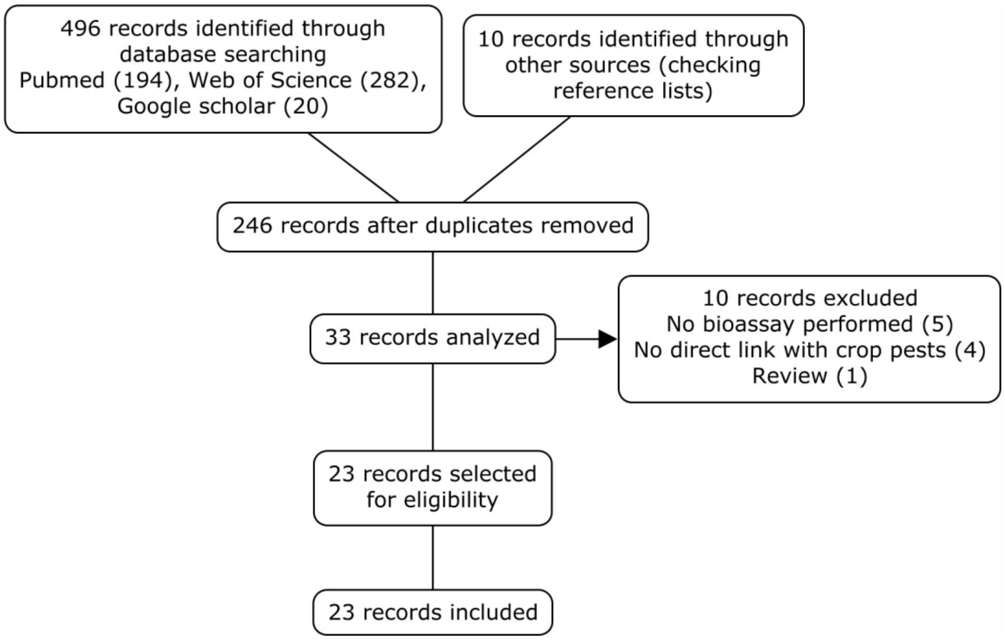

Figure 1. Flow diagram of the selection of studies for the systematic review.

596x378mm (72 x 72 DPI)

Reference	**Country**	**Use**	**Part of the plant used**	**Product**	**Method of administration**
Ahmad et al. (2015)	Pakistan	Pest control	Leaves	Leave's juice	The leave's juice is used to remove pests.
Bhardwaj et al. (2011)	India	Insect repellent	Leaves	Raw material	Leaves are burnt to generate smoke to repel insects.
Dhale (2013)	India	Against bugs and pests	Leaves and whole plant	NS	NS
Joshi & Joshi (2004)	Nepal	Pest control	Leaves and whole plant	Raw material	Leaves or whole plant are scattered under the bedsheet, which is effective in getting relief from pests
Kantheti & Padma (2017)	India	Insect repellent	Leaves	NS	Fumigants from the leaves act as insect repellent
Mwine et al. (2011)	Uganda	Pest control, coccidiosis, and as antibiotic	Leaves, seeds and inflorescences	Water extract, smoke, trap crop	Extracts, smoke, or trap crop used against storage pests, insect pests, coccidiosis, and as antibiotic
Shah et al. (2016)	Pakistan	Insect repellent	NS	NS	NS
Sharma & Sawant (2012)	India	Against bugs and pests	Leaves and whole plant	NS	NS
Sinha (2010)	India	Insect repellent	Leaves	Raw material	Fresh or dried leaves in and around granaries to repel insects

Table 1. CANNUSE database references informing about the ethnobotanical evidence of the potential use of *Cannabis sativa* as a pesticide. NS= Non-specified

ANIMAL

PHYLUM - CLASS	Order	Species	Reference	Cultivar/chemovarof *C. sativa*	Part of the plant used	Extraction method	Product	Main compounds found	Main findings	Efficacy
ARTHROPODA - ARACHNIDA	Ixodida	*Hyalomma dromedarii* eggs and larvae	Tabari et al. (2020)	'Felina 32'	INF	STD	EO+	(E)-caryophyllene (23.8%), α-pinene (16.4%), myrcene (14.2 %), α-humulene (8.3 %)	Highest ovicidal effect was achieved testing (E)-caryophyllene and α-humulene at 50 µg/mL, which completely inhibited egg hatching. (E)-caryophyllene and α-humulene exerted the highest larvicidal activity (>80%) when tested at 50 µg/mL. At concentrations ranging from 10 to 50 µg/mL, hemp EO led to a significantly lower larvicidal activity compared with (E)-caryophyllene and α-humulene tested at the same concentrations.	E
		Rhipicephalus microplus larvae and adults	Nasreen et al. (2020)	Wild hemp	AP, RT	MEX	EO	NS	Extract from *C. sativa* caused a decrease of *R. microplus* larvae and inhibited egg production. For larval mortality, estimated LC_{50} and LC_{90} values, respectively, were 2.74 and 8.34 mg/mL.	E
	Mesostigmata	*Dermanyssus gallinae*	Tabari et al. (2020)	'Felina 32'	INF	STD	EO+	(E)-caryophyllene (23.8%), α-pinene (16.4%), myrcene (14.2 %), α-humulene (8.3 %).	The highest toxic effect was achieved testing (E)-caryophyllene and α-humulene at 200 µg/mL causing 99.33 and 100 % mortality of *D. gallinae*, respectively. Their toxicities were higher than that of the whole hemp EO tested at the same concentration, which caused 79.26 % mortality.	E
	Trombidiformes	*Tetranychus urticae*	Chermenskaya et al. (2010)	Wild hemp	AP, RT	EEX	EO	NS	*C. sativa* extract exerted toxicity against *T. urticae*	E
			Gorski et al. (2016)	Wild hemp	NS	STD	EO	trans-caryophyllene (35.5%), β-myrcene (18.4%), α-pinene (9.76%), terpinolene (7.40%), ocimene (6.38%)	Essential oil also showed an effect on *T. urticae*. Following the oil application, irrespective of its concentration, a significant effect on mite mortality was observed. Its action was the strongest at its highest concentration, while mortality of the pest at 24, 48 and 72 h after treatment was 83.28, 95.83 and 98.72%, respectively.	E

ARTHROPODA - INSECTA	Blattodea	*Reticulitermes virginicus*	Satyal & Setzer (2015)	Wild hemp	L	HD	EO	(E)-caryophyllene (20.4%), α-humulene (7.0%), α-bisabolol (5.8%), caryophyllene oxide (3.8%)	Essential oil exerted marginal toxicity against *R. virginicus*	IN
	Coleoptera	*Brassicogethes aeneus*	Willow et al. (2020)	Wild hemp	L, INF	STD	EO	α-myrcene (45%), α-pinene (38%), D-limonene (5%), α-ocimene (3%)	No significant effect on survival or mobility was observed	IN
		Oryzaephilus surinamensis larvae	Mantzoukas et al. (2020)	-	-	-	CBD oil	-	*O. surinamensis* larvae suffered mortality between 17% and 100% on wheat, 36% and 96% on corn, and 67% and 100% on rice. At the higher dose of CBD oil, the lowest pupation and adult emergence were 3.71% and 1.89%	E
		Tribolium confusum larvae	Mantzoukas et al. (2020)	-	-	-	CBD oil	-	*T. confusum* larvae suffered mortality between 17% and 100% on wheat, 17% and 93% on corn, and 26% and 83% on rice. Mortality was always significantly increased as the dose increased. At the higher dose of CBD oil, the lowest pupation and adult emergence were 5.75% and 3.13%	E
		Trogoderma granarium	Kavallieratos et al. (2020)	‘Felina 32’	INF	HD	EO	(E)-caryophyllene (23.8%), α-pinene (16.4%), myrcene (14.2%), terpinolene (9.6%), α-humulene (8.3%)	The EO of *C. sativa* led to the lowest mortality when compared to the other EOs used in the study.	IN
	Diptera	*Aedes aegypti* larvae and adults	Wanas et al. (2020)	Three chemotypes (high THC, THC/C	INF	HD	EO	High THC fresh= β-Caryophyllene (16.5%), myrcene (10.3%); dried= β-Caryophyllene (20.7%), selina-	Biting deterrent activity of EOs from the varieties high THC fresh, high CBD fresh, and THC/CBD fresh were similar to DEET. The oil obtained from the fresh and dried high CBD cannabis showed good biting deterrent and larvicidal activity at 10 μg/cm^2.	E

		BD, and high CBD). Fresh and dried				3,7(11)-diene (13.2%). THC/CBD fresh= limonene (33%), α-pinene (15.4%); dried= limonene (27.1%), α-pinene (15.6%). High CBD fresh= myrcene (27.5%), limonene (14%); dried= myrcene (17.1%), limonene (17%).		
Aedes albopictus	Bedini et al. (2016)	NS	AP	NS	EO	Myrcene (22.9%), caryophyllene (18.7%), terpinolene (12%)	*C. sativa* EO showed toxicity against *A. albopictus*. LC$_{50}$ values were 301.5 μL/L^{-1} .	E
Anopheles gambiae larvae and pupae	Rossi et al. (2020)	'Felina 32' Carmagnola Selezionata (CS)	INF (♂, ♀)	STD HD	EO	'Felina 32'= (E)-caryophyllene (34.8%), α-pinene (15.1%), myrcene (11.8%). CS male= (E)-caryophyllene (47.2%), α-humulene (15.1%), myrcene (10.6%). CS female= myrcene (24.3%), (E)-caryophyllene (19.3%), terpinolene (13.5%).	EOs were highly active against *A. gambiae* for both stages, with LC$_{50}$ values lower than 80 ppm.	E

	Anopheles stephensi larvae and pupae	Rossi et al. (2020)	'Felina 32' Carmagnola Selezionata (CS)	INF (♂, ♀)	STD HD	EO	'Felina 32'= (E)-caryophyllene (34.8%), α-pinene (15.1%), myrcene (11.8%). CS male= (E)-caryophyllene (47.2%), α-humulene (15.1%), myrcene (10.6%). CS female= myrcene (24.3%), (E)-caryophyllene (19.3%), terpinolene (13.5%).	EOs were highly active against *A. stephensi* for both stages, with LC_{50} values lower than 80 ppm. Mortality caused by hemp EO at 100 ppm was 82.7% and 100% ('Felina 32'), 90.2% and 94.2% (CS female), and 89.8% and 90.5% (CS male) on larvae and pupae, respectively.	E
	Chaoborus plumicornis	Satyal & Setzer (2015)	Wild hemp	L	HD	EO	(E)-caryophyllene (20.4%), α-humulene (7.0%), α-bisabolol (5.8%), caryophyllene oxide (3.8%)	Essential oil exerted marginal toxicity against *C. plumicornis*	IN
	Culex quinquefasciatus larvae and adults	Benelli et al. (2018a) Benelli et al. (2018b) Maurya et al. (2008)	'Felina 32' 'Futura 75' Wild hemp	INF L, INF L	STD MEX PE, CT, MEX	EO EO LEX	(E)-caryophyllene (23.8%), α-pinene (16.4%), myrcene (14.2%) Essential oil from leaves= (E)-caryophyllene (26.1%), α-humulene (8.9%) and caryophyllene oxide (10.5%). Essential oil from inflorescences=	Toxicity of the essential oil was scarce towards *C. quinquefasciatus* larvae (LC_{50} of 252.5 mL/L^{-1}) and adults (LC_{50} > 500 µg/cm^{-2}). In the toxicity experiments, the LC_{50} values of the essential oil against *C. quinquefasciatus* larvae were 152.3 and 124.5 µL/L for the leaf and inflorescence essential oil, respectively. Carbon tetrachloride extract was more E as compared to petroleum ether and methanol extracts after 24 and 48h (LC_{50} 88.51 and 68.69 ppm).	IN E E

							(E)-caryophyllene (21.4%), myrcene (11%), cannabidiol (11%), α-pinene (7.8%). NS		
	Drosophila melanogaster	Satyal & Setzer (2015)	Wild hemp	L	HD	EO	(E)-caryophyllene (20.4%), α-humulene (7.0%), α-bisabolol (5.8%), caryophyllene oxide (3.8%)	Essential oil exerted marginal toxicity against *D. melanogaster*	IN
	Musca domestica	Benelli et al. (2018a) Benelli et al. (2018b)	'Felina 32' 'Futura 75'	INF L, INF	STD MEX	EO EO	(E)-caryophyllene (23.8%), α-pinene (16.4%), myrcene (14.2%) Essential oil from leaves= (E)-caryophyllene (26.1%), a-humulene (8.9%) and caryophyllene oxide (10.5%). Essential oil from inflorescences= (E)-caryophyllene (21.4%), myrcene (11%), cannabidiol (11%), α-pinene (7.8%).	The essential oil was highly toxic to *M. domestica* flies ($LD_{50(90)}$=43.3(213.5) µg adult^{-1}). The LD_{50} values estimated for *M. domestica* flies were 305.2 and 122.1 µg/adult for the leaf and inflorescence essential oil, respectively. As a general trend, the essential oil from inflorescences, mainly composed by (E)-caryophyllene, myrcene, CBD, α-pinene, terpinolene, and α-humulene, was more E than leaf essential oil.	E
Hemiptera	*Aulacorthum solani*	Gorski et al. (2016)	Wild hemp	NS	STD	EO	Trans-caryophyllene (35.5%), β-myrcene (18.4%), α-pinene (9.76%), terpinolene	At 24h after the application of the essential oil at a concentration of 0.1% mortality rate of the pest was 98.20%, while after 48 h it reached 100%. A significant, although much lesser effect of the oil on the population size of the aphid was recorded when it was applied at a concentration of 0.05%.	E

							(7.40%), ocimene (6.38%)	In that combination mortality rate of the true bug after 72 h was 57.33%. No significant effect of hemp oil was found on survival rates of the foxglove aphid at its application at the lowest concentration (0.02%).	
	Brevicoryne brassicae	Ahmed et al. (2020)	Wild *C. indica*	L	EEX	LEX	THC (57.29%), 4-nitrobenzenesulfonic acid (25.54%), cannabinol (2.29%), cyclobarbital (2.19%)	Extract showed toxicity with an LC$_{50}$ of 10.04 mg/mL^{-1} in the residual assay. In the contact assay, the extract showed toxicity with a LC$_{50}$ of 1.96 mg/mL^{-1}. Sesquiterpenes, α-bisabolol, and THC exhibited insecticidal properties.	E
	Myzus persicae	Benelli et al. (2018a)	'Felina 32'	INF	STD	EO	(E)-caryophyllene (23.8%), α-pinene (16.4%), myrcene (14.2%)	The essential oil from inflorescences of industrial hemp 'Felina 32' was highly toxic to *M. persicae* aphids (LC$_{50(90)}$ of 3.5(6.2) mL/L^{-1})	E
	Schizaphis graminum	Chermenskaya et al. (2010)	Wild hemp	AP, RT	EEX	EO	NS	*C. sativa* extract exerted toxicity against *S. graminum*	E
Lepidoptera	*Manduca sexta*	Park et al. (2019)	-	-	-	CBD oil	-	CBD acts as a feeding deterrent against pests. The CBD ingested Ely inhibited the larval growth and development, resulting in high mortality. The results highlight the potential use of CBD-rich hemp extract as a repellent and/or companion crop. Lethal amounts of CBD function differently in the presence of EtOH stress, becoming protective and reducing 40% the EtOH-related mortality.	E
	Plodia interpunctella	Mantzoukas et al. (2020)	-	-	-	CBD oil	-	*P. interpunctella* larvae suffered mortality between 16% and 76% on wheat, 13% and 60% on corn, and 33% and 63% on rice.	E
	Spodoptera littoralis larvae	Benelli et al. (2018a) Benelli et al. (2018b)	'Felina 32' 'Futura 75'	INF L, INF	STD MEX	EO EO	(E)-caryophyllene (23.8%), α-pinene (16.4%), myrcene (14.2%) Essential oil from leaves= (E)-caryophyllene	Essential oil toxicity was moderate towards *S. littoralis* larvae (152.3 µg larva−1). Insecticidal experiments carried out on *S. littoralis* larvae showed LD$_{50}$ values of 112.8 and 65.8 µg/larva for the leaf and inflorescence essential, respectively.	E E

								(26.1%), α-humulene (8.9%) and caryophyllene oxide (10.5%). Essential oil from inflorescences= (E)-caryophyllene (21.4%), myrcene (11%), cannabidiol (11%), α-pinene (7.8%).		
	Thysanoptera	*Frankliniella occidentalis* larvae	Chermenskaya et al. (2010)	Wild hemp	AP, RT	EEX	EO	NS	*C. sativa* extract did not exert toxicity against *F. occidentalis*	IN
MOLLUSCA - GASTROPODA	*Incertae sedis* (superorder Hygrophila)	*Physella acuta*	Bedini et al. (2016)	NS	AP	NS	EO	Myrcene (22.9%), caryophyllene (18.7%), terpinolene (12%)	*C. sativa* EO showed toxicity against *P. acuta*. LC$_{50}$ value was 301.5 µL/L^{-1}.	E
NEMATODA - CHROMADOREA	Rhabditida	*Caenorhabditis elegans*	Satyal & Setzer (2015)	Wild hemp	L	HD	EO	(E)-caryophyllene (20.4%), α-humulene (7.0%), α-bisabolol (5.8%), caryophyllene oxide (3.8%)	Essential oil exerted marginal toxicity against *C. elegans*	IN
	Rhabditida	*Heterorhabditis bacteriophora*	Laznik et al. (2020)	'Tiborszallasi' 'Futura 75'	INF, L, RT	EEX	EO	'Tiborszallasi'= Trans-caryophyllene (39.9%), nerolidol (13.9%), α-pinene (12.2%).	The extract from inflorescences regardless of cannabis genotype had the strongest chemotropic effects on nematodes, followed by leaves and roots.	E

			MX-CBD-11 MX-CBD-707				'Futura 75'= Trans-caryophyllene (36.5%), nerolidol (11.5%), α-pinene (11.4%)		
Rhabditida	*Steinernema carpocapsae*	Laznik et al. (2020)	'Tiborszallasi' 'Futura 75' MX-CBD-11 MX-CBD-707	INF, L, RT	EEX	EO	'Tiborszallasi'= Trans-caryophyllene (39.9%), nerolidol (13.9%), α-pinene (12.2%). 'Futura 75'= Trans-caryophyllene (36.5%), nerolidol (11.5%), α-pinene (11.4%)	The extract from inflorescences regardless of cannabis genotype had the strongest chemotropic effects on nematodes, followed by leaves and roots.	E
Rhabditida	*Steinernema feltiae*	Laznik et al. (2020)	'Tiborszallasi' 'Futura 75' MX-CBD-11 MX-CBD-707	INF, L, RT	EEX	EO	'Tiborszallasi'= Trans-caryophyllene (39.9%), nerolidol (13.9%), α-pinene (12.2%). 'Futura 75'= Trans-caryophyllene (36.5%), nerolidol (11.5%), α-pinene (11.4%)	Cannabis inflorescences of medical cannabis 'MX-CBD-707', which contains the highest concentration of measured cannabinoids, caused the highest CI value of 0.37 [0.21, 0.53] for *S. feltiae* at 25 °C.	E
Rhabditida	*Strongyloides papillosus*	Boyko & Brygadyrenko (2019)	Wild hemp	L, TS, INF, S	NS	ASO	NS	Aqueous extract of *C. sativa* did not show significant nematocidal activity. LC_{50} value was not calculated.	IN

	Strongylida	*Haemonchus contortus*	Boyko & Brygadyrenko (2019)	Wild hemp	L, TS, INF, S	NS	ASO	NS	Aqueous extract of *C. sativa* did not show significant nematocidal activity. LC_{50} value was not calculated.	IN
	Tylenchida	*Meloidogyne incognita*	Kayani et al. (2012)	NS	L	-	PL	-	*C. sativa* caused reduction in nematode infection and reproduction and improved plant growth as compared to control. Generally, *C. sativa* was significantly better as compared *Z. alatum*.	E
			Mateeva (1995)	NS	-	-	-	NS	*C. sativa* decreased the nematode development and the subsequent damage to plants. The nematocidal effect was weaker than *Tagetes patula*.	E
			Mukhtar et al. (2013)	Wild hemp	L	HD	EO	NS	Mortality caused by *C. sativa* on *M. incognita* was significantly higher than *Zanthoxylum alatum*. Concentration had significant effects on mortality.	E
FUNGI										
PHYLUM - CLASS	**Order**	**Species**								

BASIDIOMYCOTA - AGARICOMYCETES	Atheliales	*Sclerotium rolfsii (Athelia rolfsii)*	Khanzada et al. (2006)	Wild hemp	L	M	AEX	NS	The effect of the aqueous leaf extract on mycelial growth was significant, especially at 2% concentration.	E
BASIDIOMYCOTA (DIVISION) - TREMELLOMYCETES	Tremellales	*Cryptococcus neoformans*	Wanas et al. (2016)	CHPF-01	INF	HEX	EO+	NS	The oil showed modest antifungal activity with an LC_{50} value of 33.15 µg/mL against *C. neoformans*. α-humulene isolated showed potent antifungal activity.	E

Table 2. Experimental studies assessing the potential of hemp as a pesticide.

THC= tetrahydrocannabinol; LC= lethal concentration; EO= essential oil; E= effective; IN= Ineffective; LD= lethal dose; CBD= cannabidiol; DMSO= dimethyl sulfoxide; NS= Non-specified; AP= aerial parts; RT= roots; INF= inflorescences; L= leaves; TS= thin stems; S= seeds; EEX= ethanol extraction; HEX= hexane extraction; HD= hydrodistillation; M= maceration; MEX= methanol extraction; PE= petroleum ether; CT= carbon tetrachloride; STD= steam distillation; AEX= aqueous extract; ASO= aqueous solution; EO+= Essential oil and isolated compounds; LEX= leaf extract; PL= pulverized leaves

Figure 1. Flow diagram of the selection of studies for the systematic review.