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ORIGINAL ARTICLE

Plants extract concoction from *Capsicum annuum, Citrus sinensiss, Tagetes minuta* and *Allium sativum*: A potent killer of bedbugs

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ABSTRACT

Background: *Cimex lectularius* Linnaeus and *Cimex hemipterus* L. (Bedbugs) are hematophagous ectoparasites which are a nuisance and public health menace. In recent past, bed bug infestation has been on the rise which is attributed to the emergence of resistance to commercial insecticides, particularly pyrethroids. Therefore, the aim of this investigation was to evaluate the insecticidal properties of aqueous extracts of different plants on bed bugs.
Methods: Aqueous extracts separately obtained from four different plant materials; ripe fruits of *Capsicum annuum* L. (hot pepper), fruit peelings of *Citrus x sinensis* L. (citrus: orange), leaves of *Tagetes minuta* L. (Mexican marigold) and bulbs of *Allium sativum* L. (garlic**)** were studied against bedbugs in a controlled laboratory setting and their effect based on chemotaxis and mortality observed. The extracts were further tested in a field trial in seven villages randomly selected from Idakho central ward, Ikolomani constituency, Kakamega County, Kenya. The villages were assigned arbitrary identity as V_1, V_2, V_3, V_4, V_5, V_6 and V_7. Twenty households confirmed to be infested with the bedbugs were selected randomly from each sample village. Each village received specific treatment; V_1, V_2, V_3, V_4 and V_5 were treated with 5ml of 10mg/ml of extracts from *Capsicum annuum*, fruit peelings of *Citrus x sinensis*, leaves of *Tagetes minuta*, bulbs of *Allium sativum* and a concoction of the four extracts at the ratio of 1:1:1:1 respectively. Village V_6 was treated with commercial potent synthetic insecticide (Bedlum® 200SL, Jiansu Lanfeng biochemical, China with acetamiprid as active compound) as a positive control and village V_7 was treated with distilled water as a negative control.
Results: The laboratory findings showed that the bed bugs had negative chemotaxis to *T. minuta* and *A. sativum* and also showed some direct mortality. *Capsicum annuum* and *C. sinensis* had a direct killing effect on the bedbugs. The combination of extracts from the four plants showed 100% mortality within the shortest time of 10 seconds as compared to individual plant extracts. The findings from field trials showed higher perception of bedbug management of 90% for a concoction of the four extract as compared to individual plant extracts: 55%, 70%, 40% and 45% for *C. annuum C. sinensis T. minuta* and *A. sativum* respectively. Both laboratory and field trial findings showed that a concoction of the four named plants is a potent insecticide and can be used in bedbug management. There is however need to carry out more research to ascertain other active compounds in the extracts and their mode of action.

Key words: Potent, *Cimex lectularius*, *Cimex hemipterus*, *Capsicum annuum*, *Citrus x sinensis*, *Tagetes Minuta*, *Allium sativum* Bedbug management, Concoction, mortality, Chemotaxis

INTRODUCTION

Bedbugs (*Cimex lectularius* L. and *C. lectularius* L.) are found throughout the world and are blood sucking ectoparasites that feed mostly on human blood but may also attack other animals such as birds and bats[1]. In Africa, tropical bedbugs (*C. hemipterus L.*) are distributed throughout warm zones, whereas temperate ones, (*C. lectularius L.*) are common in the rest of the Africa[2]. They attack normally at night with painless bites but fluids that are injected often produce painful welts on the skin with itching that may be severely irritating[3]. The bedbug may be associated with human pathogens, but no study has clearly proved they can transmit the pathogen to human beings[4,5]. They multiply very quickly as they feed, and the problem become progressively worse. Infestation with bedbugs was initially associated with poverty but has in recent past found their way into social amenities and transport facilities such as hotels, hostels, cruise ships, airplanes, trains, schools, and long- term care facilities making the bedbugs to spread to

many households especially those in the urban areas [2,6,7]. The bedbugs have given many people sleepless nights and due to social stigma associated with them, many people do not seek professional assistance and try to eliminate them by themselves. This has led to uncontrollable spread of the bedbugs. The bedbugs have developed resistance to many known insecticides especially pyrethroid insecticides proving very hard and costly to manage[8,9]. According to research by a PhD student David Lilly of the university of Sydney, the resistance of the bed bugs is due to development of a thicker cuticle which the insecticide could not penetrate[10]. Currently bedbugs have proven to be a challenging pest to contain or manage and it becomes the subject of significant research and public health concern than ever before[7]. The present study explored the use of cost-effective pest control tools based on new classes of plant-based insecticide with novel mode of action to effectively manage the bedbug infestation in the future. Although the history of using plant extracts as insecticides has not been mapped very well, there are various existing historical sources that in Europe, the use of some plants in protection against insects' dates back more than 3000 years. Primarily, various modified parts of some aromatic plants and their extracts were used, particularly as repellents against troublesome insects such as ectoparasites. Plants were also used to protect stored harvests or foods against storage pests[11,12].

Plants are considered as a rich source of bioactive chemicals and they may be an alternative source of insect pest control agents. Natural products are generally preferred because of their less harmful nature to non-target organisms and due to their ease for biodegradability[13]. The present study used extracts of plants which have history of use as insecticides or pesticides. For instance, *Allium sativum* repels root maggots, cabbage loopers, peach tree borers as well cockroaches. On the other hand, *Tagetes minuta* repels most insects, *C. annuum* has direct killing effect on caterpillars, aphids, flies, ants and other insects while *C. sinensis* peelings have direct killing effect on mosquitoes[14]. However scanty information is available on use of plant extracts on bedbugs. The present study sort to determine whether a concoction of plant extracts with known effects to insect pests may be used to manage bedbugs.

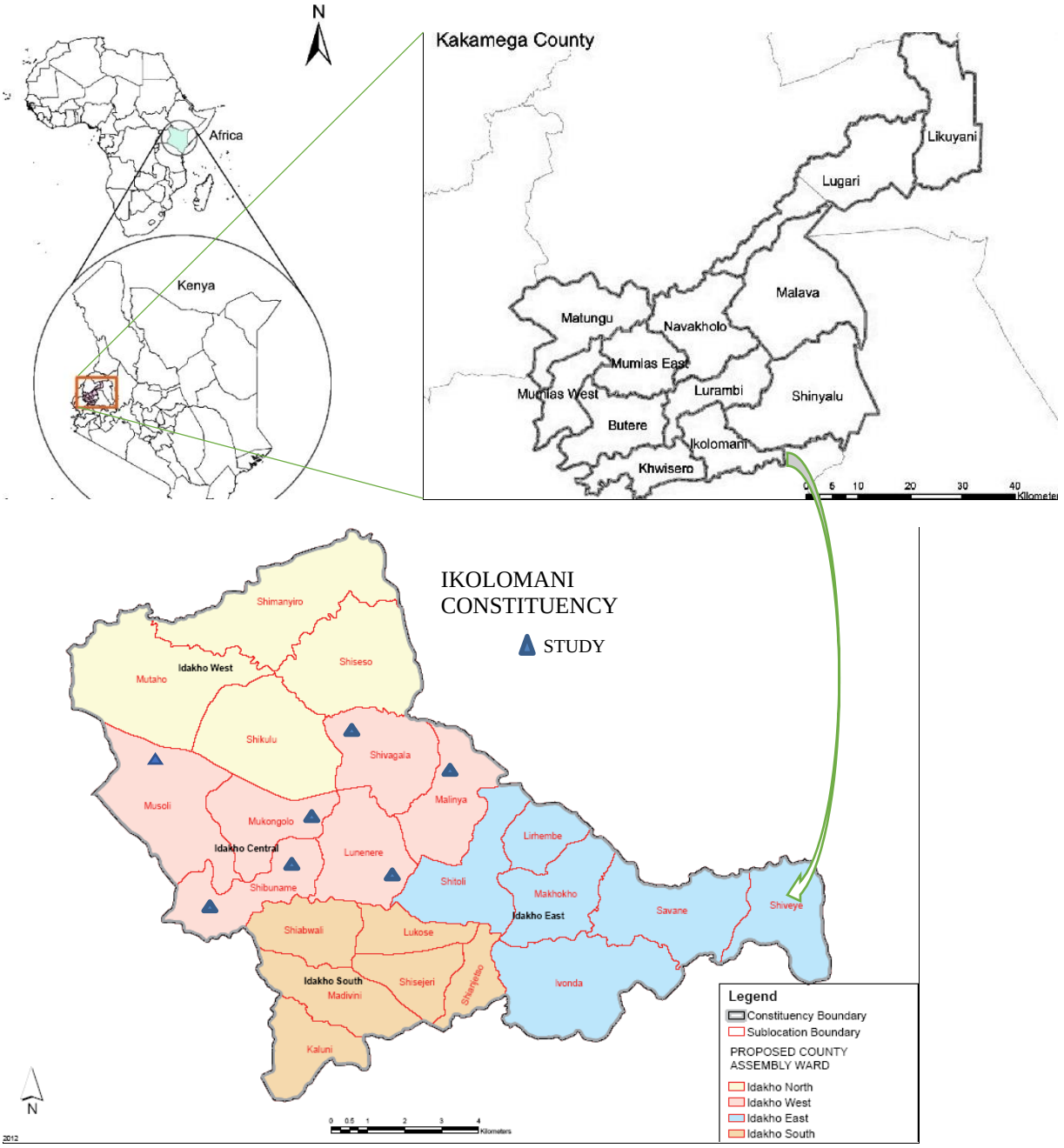
METHODS

The study involved laboratory experiments and field trials of the plant extracts from ripe fruits of *Capsicum annuum*, fruit peelings of *Citrus x sinensis*, leaves of *Tagetes minuta*, bulbs of *Allium sativum* and a concoction of the four extracts on bedbugs.

Study participants

Study participants in the field trials were residents of seven villages in Idakho central, Ikolomani constituency, Kakamega South Sub-county, Kakamega County, Kenya ***(Figure 1)***. Idakho central ward has approximate an area of 39.6 square km and an estimated population of 31,134 people with approximately 3,000 households based on 2009 Kenya national Bureau of statistics (KNBS) census. The ward has 26 villages with an approximate of 200 households each. Houses are commonly two roomed, semi-permanent and mud walled. Recruitment of participants was done by use phone call through a specific line that was displayed on posters throughout the targeted villages. Selected participants were those who made phone calls and were willing individuals who admitted that indeed their households were infested with bedbugs and were staying in 2-roomed and mud-walled semi-permanent houses. This was followed by confirmation by the researcher for bed bug infestation based on inspection for indicators such as detecting the pests themselves or their signs which include; blood spots, fecal spots, egg cases and shed skins (exuviae) on beds, mattress folds furniture and cracks on the walls. The recruited participants signed written consent that they were heads of their households and gave express authority for the study.

FIGURE 1. ***Map of the Study Site: Idakho Central Ward, Ikolomani Constituency, Kakamega County, Kenya***



Map of study site adopted and modified from ikolomanihoney.wordpress.com

Ethical approval

Permission to carry out the study was granted by Public Health Department Kakamega County and National Commission for Science, Technology and Innovation (NACOSTI) Kenya.

Plant material extraction

In the present study four different plants: *Capsicum annuum*, *Citrus x sinensis*, *Tagetes minuta* and *Allium sativum* were used. *C. annuum*, *C. sinensis* and *A. sativum* were bought from Kakamega Municipal Market. Leaves of *T Minuta* collected from Bukura Agricultural Training Centre Farm, Kakamega County, Kenya. Specific parts of each plant were carried to Masinde Muliro University of Science and Technology Biology Laboratory. They were washed with distilled water and processed as explained in the procedures. Extraction was done separately for each of the four plants as follows:

Capsicum annuum: One kilogram of ripe hot pepper were chopped into tiny pieces and boiled in 3 litres of water for 30 minutes. The extract was strained and further processed by soxhlet apparatus to concentrate the extract. The extract was left to cool and 10g of soap was added to improve dispersion as an emulsifier and adherence to surface[15]. The contents were kept in sealed container at 4ºC to be used for further experimental study.

***Tagetes minuta*:** One kilogram of freshly gathered whole plant was crushed using mortar and pestle and boiled in 3 litres of water for 30 minutes and the resultant contents were subjected to similar procedures as in the case of hot pepper.

***Allium sativum*:** Transparent covering were removed from garlic gloves.1kg of garlic cloves (bulbs) were crushed to fine paste and soaked in 3 litres of cold water for 3 days, strained and concentrated. The content was then sealed in a container at 4ºC.

***Citrus x sinensis*:** The fruits were peeled and the rinds (peelings) used for extraction. Much of the white inner portions were removed from the rinds. 1kg of the processed rinds was boiled in 3 litres of water for 10 minutes. The contents were covered and left for 24 hours to allow the rinds to soak creating an oil suspension. Soap was also added as dispersion agent and emulsifier. The extract obtained was also kept in sealed containers at 4ºC.

Laboratory experiments

Adult blood engorged bedbugs were collected from an infested area particularly in the mattress folding and bed joints and identified by an entomologist. The bedbugs were maintained in the laboratory at 25 ± 2ºC and 75 ± 5% RH (Relative Humidity) according to Winston and Bates procedure[16]. Two chambered glass cages (15cm × 10cm × 6cm in length, width and height respectively) with the two chambers separated by a controlled perforated mesh were used to test the action of the plant extracts on the insects. Seven different groups of bed bugs were used in this experiment. Five groups were experimental while two groups were controls i.e. positive and negative controls. Ten (10) bed bugs were put in one chamber of each cage and their movement controlled. In the experimental groups, the bed bugs were treated directly (topically) with approximately 5ml of 10mg/ml of extracts from *Capsicum annuum*, *Citrus x sinensis*, *Tagetes minuta*, *Allium sativum* and a concoction or combination of the four extracts (1:1:1:1) for Group 1, 2, 3, 4 and 5 respectively. Groups 6 and 7 insects were treated topically with 5ml of distilled water and 5ml of 10ml/l of commercial insecticide (Bedlum® 200SL, Jiansu Lanfeng biochemical, China with acetamiprid as active compound) as negative and positive controls respectively. Each treatment was done in duplicates. The action of the extracts and average time taken for 100% chemotaxis or 100% mortality was noted. Chemotaxis was noticed when the bed bugs moved to the second chamber. Mortality was when the insects were immobilized and raised the hind part up.

Field trial study

The extracts were tested in a field trial study in seven villages randomly selected from Idakho central ward, Ikolomani constituency, Kakamega County, Kenya. The villages were assigned arbitrary identity as V_1, V_2, V_3, V_4, V_5, V_6 and V_7 to conceal the village and household identity. Twenty households confirmed to be infested with bed bugs were selected randomly from each sample village. Each village received specific treatments where V_1, V_2, V_3, V_4 and V_5 were treated with 10mg/ml extracts from *C. annuum*, *C. sinensis*, *T. minuta*, *A. sativum* and a concoction or combination of the four extracts respectively. Village V_6 was treated with 5mg/ml of commercial synthetic insecticide (Bedlum® 200SL, Jiansu Lanfeng biochemical, China with acetamiprid as active compound) as a positive control and village V_7 was treated with distilled water as a negative control. The furniture, crevices, mattress folds and beds were thoroughly sprayed with the extracts and entire house fumigated. The treatments were done in the mid-morning and doors and windows closed for 5 hours. A follow up round of treatment was done after 10 days to take care of the bed bugs that may have escaped the first round of treatment. Afterwards, the individuals from the various households were interviewed separately on the perceived effectiveness of the treatment their household received based on the bed bug infestation indicators mentioned earlier. Their response was noted as effective, not effective or not sure.

Data analysis

Data was managed using Microsoft excel (Microsoft company) and data analysis done by Graphpad prism 7 (Graphpad inclusions). Chi-square test was used to analyze the time taken by each treatment to cause mortality or

chemotaxis. Chi-square test was also used to analyze the responses from field trial experiments.

RESULTS AND DISCUSSION

From the laboratory experiments, the time taken for 100% mortality in each treatment was analyzed ***(Table 1)*** and the time taken for 100% movement of the bedbugs to the second chamber (chemotaxisis) was also analyzed ***(Table 2)***.

TABLE 1. ***Time of action for plant extracts to cause 100% mortality on bedbugs***

Groups	Name of plant/treatment	Part of plant used	Quantity and Concentration in mg/ml	Time for 100 % mortality (in seconds)
Group 1	*Capsicum annuum*, ,	Ripe fruits	5ml of 10mg/ml	23± 0.011
Group 2	*Citrus x sinensis*,	Rinds/peelings	5ml of 10mg/ml	15± 0.004
Group 3	*Tagetes minuta*	Whole plant	5ml of 10mg/ml	320± 0.033
Group 4	*Allium sativum*	Bulbs (gloves)	5ml of 10mg/ml	300 ± 0.027
Group 5	*Capsicum annuum* + *Citrus x sinensis* + *Tagetes minuta* + *Allium sativum*	Mixture/concoction of the extracts	5ml of 10mg/ml in the ratio 1:1:1:1	10± 0.001
Group 6	Distilled water	Negative control	5ml	-
Group 7	Bedlum® 200SL insecticide with acetamiprid as active compound	Positive control	5ml of 10ml dissolved in a litre of distilled water	9± 0.002

TABLE 2. ***Time of action for plant extracts to cause 100% motility (Negative chemotaxis) on bedbugs***

Groups	Name of plant/treatment	Part of plant used	Quantity and Concentration in mg/ml	Time for 100 % motility (in seconds)
Group 1	*Capsicum annuum*	Ripe fruits	5ml of 10mg/ml	00± 0.011
Group 2	*Citrus x sinensis*	Rinds/peelings	5ml of 10mg/ml	00± 0.004
Group 3	*Tagetes minuta*	Whole plant	5ml of 10mg/ml	15± 0.035
Group 4	*Allium sativum*	Bulbs (gloves)	5ml of 10mg/ml	13 ± 0.021
Group 5	*Capsicum annuum* + *Citrus x sinensis* + *Tagetes minuta* + *Allium sativum*	Mixture/concoction of the extracts	5ml of 10mg/ml in the ratio 1:1:1:1	00± 0.001
Group 6	Distilled water	Negative control	5ml	-
Group 7	Bedlum® 200SL insecticide with acetamiprid as active compound	Positive control	5ml of 10ml dissolved in a litre of distilled water	0± 0.002

The laboratory findings showed that the bed bugs had negative chemotaxis to *T. minuta* and *A. sativum* and also showed some direct mortality with *A. sativum* taking the shortest time of 13 seconds compared to 15 seconds taken by *T. minuta* for chemotaxis. Extracts from *C. annuum*, *C. sinensis* and the concoction of the extracts did not cause chemotaxis. On the other hand, the concoction of the four extracts killed the bedbugs within the shortest time of 10 seconds as compared to time taken for individual extracts; 23 seconds, 15 seconds, 320 seconds and 300 seconds for *C. annuum*, *C. sinensis T. minuta* and *A. sativum* respectively. The results obtained in the field trial experiments were based on individual responses based on the effectiveness of the plant extract treatment given to their household in managing the bed bugs ***(Table 3)***.

TABLE 3. ***Responses on interviews from field study on the on the perception of effectiveness of the plant extracts on management of bedbugs***

Village	Name of plant/treatment	Number of households	response			
			Effective	Not effective	Not sure	% response on effectiveness of the treatment
V_1	*C. annuum*	20	11	9	0	55
V_2	*C. sinensis*	20	14	2	4	70
V_3	*T. Minuta*	20	8	4	8	40
V_4	*A. sativum*	20	9	5	6	45
V_5	*C. annuum* + *C. sinensis* + *T. Minuta* + *A. sativum*	20	18	0	2	90
V_6	Bedlum® 200SL insecticide with acetamiprid as active compound	20	17	0	3	85
V_7	Distilled water	20	0	19	1	0

The findings from field trials showed higher perception of effectiveness of bedbug management of 90% (18 out of 20 households) when a concoction of the four extract was used as compared to individual plant extracts with perception of effectiveness of 55% (11 out of 20 households), 70% (14 out of 20 households), 40% (8 out of 20 households) and 45% (9 out of 20 households) when *C. annuum C. sinensis T. minuta* and *A. sativum* were used respectively. Results from laboratory experiments revealed that extracts from *C. annuum, C. sinensis* have direct killing effect. This could be strongly attributed to active compounds they possess. Extracts from *C. sinensis* rinds contain limonene as the main component which is a strong insect repellant and also paralyses insects like fleas[17]. It is further documented that its neurotoxic effect characterized by hyperactivity followed by hyperexcitation leads to rapid knock down and immobilization of the insects. Furthermore, extracts from *C. sinensis* rinds contains linalool which is a pest killer identified as an inhibitor of acetylcholinesterase[18]. However, their effect on bedbugs is not well documented and therefore this study provides the basis of use of extract from *C. sinensis* rinds as an ingredient in management of bedbugs. *C. annuum* on the other hand contain capsaicin (8-Methyl-N-vanithyl-6-nonenamide) and other several compounds called capsaicinoids as active compound which are irritants with chocking effect to most animals including insects[19]. In insects, capsaicin causes metabolic disruption, membrane damage and nervous system failure with physical repellent action[18]. According to previous reports, capsaicin has broad-spectrum insecticidal activity against many species of insects, e.g., stored product beetles (*Sitophilus zeamais* and *Tribolium castaneum*) as well as *Bemisia tabaci*[20]. The present study documents that *C. annuum* cause direct killing effect to the bedbugs as observed in the laboratory experiment. The present study revealed that bedbugs are negatively chemotactic to *A. sativum* and *T. minuta* indicating that the extracts have substances that are repulsive to bedbugs. When crushed chopped or blended, garlic bulbs contain an amino acid which undergo biotransformation to a substance called allicin which has a characteristic odor that repel insects[21]. This could probably be the cause of movement of the bedbugs away from the point of application of the extract as observed in the laboratory experiment. Notable activity of *A. sativum* has been reported against a number of insects including dipteran[22], lepidopteran[23, 24] and hemipteran pests amongst others[25]. *Tagetes minuta* on the other hand contains chemical compounds such as thiopenes, flavonoids, carotenoids, and triterpenoids in their seeds, leaves, roots and other parts of the plant which are offensive to insects as well as nematodes[26]. Efficacies of *Tagetes sp.* extract have been reported against blood-sucking parasites such as *Tribolium castaneum*[27]. This effect could probably be responsible for negative chemotaxis to bedbugs in the observed in laboratory experiment. When all the four extracts were mixed to form a concoction, it had direct killing effect which took the shortest time possible as compared to extracts from individual plants. This could be as a result of combined effect of the active compound in the extracts of the four plants. Previous studies have reported effectiveness of a combination of plant extracts in management of bedbugs. For instance, a mixture of extracts from *Azadiracta indica, Mangifera indica, Polyalthia longifolia, Annona squamosa, and Ficus bengalensis* was reported be an effective killer of bedbugs within the shortest time possible[13]. The efficiency of an insecticide is determined by time of action and therefore the effect observed with the concoction plant extracts suggests that it is an effective plant-based insecticide in the management of bedbugs. The results from the field trial experiment also reveal that a concoction (combination) of the extracts from the four plants is most effective in management

bedbugs as opposed to single plant extract. Therefore, based on the findings from the laboratory experiments and field trials, the present study documents that a concoction (combination) of plant extracts of *Capsicum annuum*, *Citrus sinensis*, *Tagetes minuta* and *Allium sativum* is effective in management of bedbugs.

CONCLUSION

Plants contain numerous primary and secondary bio-active molecules used to protect them from their natural enemies predominantly insects and pathogens. Plant extracts are often easily accessible, affordable, user and eco-friendly in nature, and they have become more attractive alternative insecticides to resource-limited settings[28]. The results of the present study apparently reveal that a combination of the candidate plants extracts has remarkable insecticidal activity against bedbugs. Bedbugs have since developed resistance against most of the conventional insecticides, particularly of pyrethroid origin which is considered to be one of the main factors in the recent resurgence of bedbugs. In this context a concoction of plant extracts from *Capsicum annuum*, *Citrus sinensis*, *Tagetes Minuta* and *Allium sativum* has proved effective and could play a pivotal role as a potent bedbug control agent in the near future. However, this finding warrants further investigations to elucidate the responsible bio-active principles, mode of action and safety. Furthermore, adequate efforts have to be made by involving pertinent stakeholders in order to formulate and commercialize the plant extract concoction-based insecticide.

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