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Research Article

Identification of Local Bioactive Plants as a Source of Natural Insecticides against Fall Armyworm: An Ethno Botanical Approach

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Abstract

Africans have very rich indigenous knowledges of bioactive plants that are important in alleviating problems related to animal and plant diseases, and the pests. The fall armyworm /FAW/ (Spodoptera frugiperda, J.E Smith; Lepidoptera, Noctudea) is an American originated insect pest of food crops that is re-centy widely dispersed almost worldwide. This study aimed at surveying locally grown plants as ecofriendlybotanical solution to the fall armyworm infestation especially in Ethiopia. Ethiopian farmers have very rich indigenous knowledge of alleviating animal and plant pests. In the study, selected bioactive plants were identified by applying rapid ethno-botanical survey method, and simple laboratory tests were conducted using crude extracts to examine the efficacies of the extracts by applying mortality tests on fall armyworm lar-vae. Six local plants were suggested by respondents during the survey. Two of the six suggested plant species (i.e Agarista salicifolia (Ericaceae), and Melia azedarach (Meliaceae) were found to be best bioactive. The study findings proved that the risks associated with use of chemical insecticides to control the FAWs can significantly ($p \leq 0.05$) be minimized by applying extracts from bioactive botanicals.

Keywords: *Fall armyworm; crude extract; Melia azedarach; Agarista salicifolia; Mortality test.*

Introduction

Indigenous knowledge of local people is very important in planning scientific studies of medicinal plants, controlling activities, and preventing the transmission of pests [Misganaw, M. *et al*., 2014]. Ethnobotany tries to get a holistic understanding of indigenous and local knowledge of plants [Mahwasane, S.T. *et al*., 2013]. Ethnobotny generally as a field studies the interactions of local people with plants including medicinal uses of the plants [Martin, G, 1995]. The most commonly used plant parts for ethno medical preparations are the leaves, roots, barks, fruits, and seeds [Campos, E.V.R. *et al*., 2018; Enloe, S.F. *et al*., 2018; & Sharma, D. *et al*., 2013]. Evaluation of the plant extracts for their medicinal efficacy and possible toxicity would also be involved in ethno botanical studies [Phambala, K. *et al*., 2020].

Most African counties including Ethiopia have rich medicinal plant compositions and the associated indigenous knowledge [Mahwasane, S.T. *et al*., 2013]. Ethiopia is especially dominated by a wide range of climate and ecological conditions that possesses enormous diversity of bioactive fauna and flora [Zerabruk, S. *et al*., 2011]. The ethno botanical knowledge of the Ethiopian local people is highly variable between individuals and situational dynamics [Gemeda, N. *et al*., 2015]. The indigenous knowledge is very helpful to effectively heal severe diseases, and pest effects that even cannot be allevi-

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ated by modern medicinal knowledge [Gemeda, N. *et al*., 2015].

Traditional herbal medication was known as the father of scientific medications though still few studies have been conducted on them [Gemeda, N. *et al*., 2015]. The uses of botanical extracts in crop protection can replace the routine uses of synthetic chemical pesticides that are often associated with tremendous risks [Phambala, K. *et al*., 2020]. The botanical pesticides are relatively more advantageous as they are less costly, less toxic, with reduced environmental pollutions, and minimized threats to the public health [NRC, 1993]. The uses of herbal extracts against plant pests, and parasites gaining more popularity nowadays as they are safer, and with fewer side effects compared to synthetic chemicals [Poojary, M.M. *et al*., 2015]. Botanical sources used to control insect pests were suggested to be appealing options in Africa [FAO, 2018]; this rapid ethno-botanical survey was conducted to find such local solutions. The study is aimed at identifying some local plants by applying rapid ethno botanical survey method, and testing the bioactive constitutes thereby.

Materials and Methods

Data Collection and Data Analysis Methods

The study was preliminarily conducted by interviewing traditional herbal medicine users who were also considered to have knowledge about local plants against plant pests. A rapid ethno-botanical survey method was implemented where field surveys undertaken from October 2017 to December 2020. Ethical issues were checked by the Addis Ababa University Department of Microbial, Cellular, and Molecular Biology. The questionnaire for the face to face interview with human subjects was presented to the advisory committee of the department, and was approved before setting out for the data collection. Informed consent was made with the respondents and each of the informants involved on self-volunteer basis in the study. A good rapport was made by continuous communications between the local community members, leaders and the researcher before selecting the informants. After adequate interpersonal rapport was made, then observations and face to face interviews with the informants accompanied by gathering plant specimens were done. Structured questionnaires were used to interview the local informants in Gofa zone, SNNPRS, Ethiopia (Figure 1) in order to identify indignant bioactive plants. The questionnaire consisted of a general introduction including personal information, explaining the purpose of the study, and consent forms. Then open ended questions submitted to the respective informants inquiring the native names of the plants in vernacular tongue /Goffigna Language/, the traditional use of the plants, and uses of the plants against crop pests.

A total of 60 traditional herbal users in Gofa zone, Ethiopia cooperated with us in the study on volunteer basis. They announced that the plants listed were either intercropped with the cereals or chopped, and the juices of which was sprinkled on the surfaces of the leaves, stems or roots of the crops infested with the pests. The herbarium specimens were obtained based on the respondents' information from the study area, and the scientific names were authenticated by a botanist in the National Herbarium at Addis Ababa University. A herbarium specimen from each plant (whole or part) was brought to the National Herbarium to get the scientific names of each plant and facilitate gathering of previous research results. Finally, the crude extracts of the plant specimens were tested as potential anti-pest sources against the insect pest, fall armyworm.

Preparation of the Botanical Crude Extracts

Leaves, fruits, seeds or/and part of the stems freshly cut from candidate plants such as Mani-hot esculenta (cassava), Millettia ferruginea (birbirra), Melia azedarach (chinaberry), Em-belia schimperi (Ekoko), Croton macrostachyus (bissana) and Agarista salicifolia (chanko) were collected from SNNPRS Gofa zone, Gezegofa woreda around Bulki town (Elevation: 2,385 masl; coordinates: 6°17′00″N 36°48′40″E) [CSA, 2012]. The leaves, the fruits, seeds or the stems of the plants were air- dried for 10-15 days

and ground using an electric grinder. The powdered plant parts were mixed with water, acetone, methanol and Chloroform on w/v basis while shaking in a water bath shaker, and left to stand for 72hrs. All the plant extract mixtures were carefully strained before application and 100 g of the ground powder was dissolved in 100ml of solvents (distilled water, acetone, methanol, & chloroform); 500g of powder was dissolved in 250ml; and 750g of powder in 250ml solvents respectively to make crude solutions of varying concentrations. The plant extracts were then filtered with What Man No 1 filter paper and the filtrated extracts were then kept in a refrigerator with plastic bottles until applied to the pest larvae reared in laboratory.

Detail extraction methods:- 1) maceration: Coarsely powdered plant materials were placed in closed containers with the extracting solvents(distilled water, methanol, acetone, and chloroform), and allowed to stand at room temperature for 72 hours with frequent agitation in a water bath shaker until the soluble matter was completely dissolved. The mixture was then filtered, the marc (the damp solid material) was pressed; and the combined liquids were clarified by filtration and decantation. 2) concentration of the filtrate to dryness: the filtered plant extract (liquid portion) was reduced first to paste-like masses, and then to powdered form using lyophilizer (the aqueous, methanol and chloroform extracts); soxhelet extraction (for the acetone extracts) in order to calculate the bioactive ingredients present after the extraction; and the doses of the plant extracts were obtained in milligram that was further reconstituted in measured quantities of 1mg, 2mg, and, 3mg respectively each time in one milliliter of the same solvents for the experimental doses. 3) The percentage yield was calculated using the following formula:

$$\text{Percentage yield} = \frac{\boldsymbol{Weight\ of\ crude\ (g)}}{\boldsymbol{weight\ of\ plant\ material(g)}}\ \boldsymbol{X\ 100}$$

Efficacy Tests by Application of Crude Extracts from the Botanicals

The crude extracts prepared by maceration or soxhlet extraction methods applied to the larvae of FAWs in Petridishes by measuring in milliliters in the biomedical laboratory of Addis Ababa University, Department of Microbial Cellular, and Molecular Biology. The extracts from the candidate plants (leaves, stems, seeds or fruits) assumed to contain the bioactive ingredients were topically applied to the pest larvae. The efficacy calculated after carefully counting the number of larvae killed when application made to the pests.

Efficacy in percent was calculated by:

$$\%\ \text{efficacy} = \frac{\boldsymbol{No\ of\ larvae\ killed\ X\ volume\ of\ extract\ applied}}{\boldsymbol{Total\ no\ of\ larvae\ reared\ in\ the\ labs}}\ \boldsymbol{X\ 100}$$

The effects of the botanical extracts under tests in the labs were compared against that of its effects on the maize crops in the fields assumed to receive the same treatments.

Results

Six locally growing plants were selected by the informants as bioactive against insect pests. Scien-tific names were identified by experts at the National Herbarium of Addis Ababa University. As displayed in the Table 1, we collected responses from 60 different local informants from Gofa zone, SNNPRS, Ethiopia. 47 of the informants (78.3%) suggested Agarista salicifolia (chanqo/Gofa language/) as most bioactive against insect pests; 35 of them (58.3%) suggested Melia azedarach (Lussania); 30 (50%) suggested Millettia ferruginea (Birbirra); 25 (41.7%) suggested Embelia schimperi (Enkoko); 16 (26.7%) suggested Croton macrostachyus (Bisana(Amharic); 7 (11.7%) suggested Manihot esculenta (cassava); and 2 (3.3%) of the respondents suggested no any plants ever known as bioactive against the insect pests of plants (Table 1).

The weighted average mean value from the organized data of result (Table 1) is calculated using the following formula:

$$Xw = \frac{W1X1+w2X2....+WkXk}{W1+W2+\cdots.+Wk}$$

$X_W = 32.5;$

The suggested bioactive botanicals identified with values greater than the weighted average value were considered most bioactive; hence subjected to further tests. Our survey results revealed two plants (i.e A. salicifolia, and M. azedarach) very significantly ($p \leq 0.05$) bioactive against the fall armyworm (Table 1). These results were further subjected to mortality tests on the insect larvae by using crude extracts in order to find more reliable results.

Crude extracts of the identified plants were tested by dissolving into four solvents (distilled water, acetone, methanol, and Chlorofom); and the candidate plants (A. salicifolia, and M. azedarach) found reliably effective .The plants' crude extracts were with good killing effects on the FAW larvae in our test results (Tables 2, 3&4). The other selected plants especially /Bisana/Croton macrostachyus/, Enkoko/Embelia schimperi/, cassava /Manihot esculenta/ resulted in very negligible mortality effects to FAWs larvae. Millettia ferruginea (Birbirra; Amharic) among the ethnobotanically identified plants was moderately active against the insect pest.

Table 1: Identification of local bioactive plants against insect pests; based on the responses from informants (Gofa zone, SNNPRS)

S.N	Plant lists	No of respondents who selected each plant species (R= 60)	percent
1	*Croton macrostachyus*/ Bisana/	16	26.7
2	*Embelia schimperi*/ Enkoko/	25	41.7
3	*Agarista salicifolia*/ Chanqo-Gofa language/	47	78.3
4	*Millettia ferruginea/Birbirra*/	30	50
5	*Manihot esculenta/cassava*/	7	11.7
6	*Melia azedarach*/ Lussania/	35	58.3
7	None	2	3.3

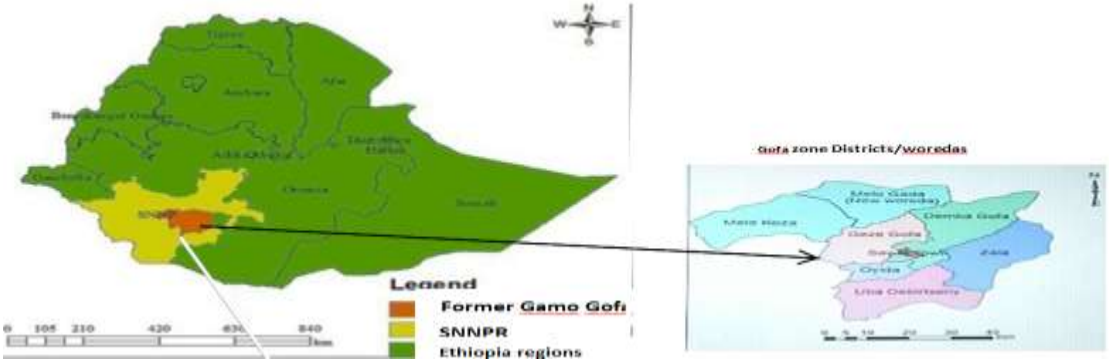

Figure 1: Map of the study area (Photo adapted from www.rippleethiopia.org)

Table 2: The effects of crude extracts of *Agarista salicifolia* on fall armyworm larvae

S.N	Dose of Crude extracts treated (in g /ml)	Total no of larvae reared	No of larvae killed	Percent
1	1.0	30	17	56.6
2	2.0	20	17	85
3	3.0	19	18	95

Table 3: The effects of crude extracts of Melia azedarach on fall armyworm larvae

S.N	Dose of Crude extracts applied (in g/ml)	Total no of larvae reared	No of larvae killed as result of application	Percent
1	1.0	27	12	44.4
2	2.0	25	19	76
3	3.0	15	13	86.6

Table 4: The relative efficacies of the crude extracts of the two plants on fall armyworm larvae (treated dose 3g/ml)

Batch of FAW larvae reared	No of larvae reared	Larvae killed by *M. azedarach*[a]	Larvae killed by *A. Salicifolia*[b]	Relative efficacy (%-a/b)
B-01	32	29	30	2.7/2.8
B-02	30	26	29	2.6/2.9
B-03	28	25	27	2.6/2.9

Data Analysis

The data were analyzed using Excel version 2010. Descriptive data were illustrated; percent calculated and summarized in to tables. The results from application of the extracts, the rate of larval mortality and percent efficacy were subjected to one way ANOVA. Means separated with a significant F value ($\alpha = 0.05$) were evaluated.

Discussion

Shifting the already existing traditional knowledge and experiences of the society with local botanicals to control the invasive pests is simple way of access to specific resources in crop protection for smallholder farmers [Rioba, N.B. *et al*., 2020]. Plants like yams, cassava, and taro contained bioactive constituents of phenolic compounds including saponins, bioactive proteins, glycoalkaloides, and phytic acids [Chandrasekara, A. *et al*., 2016], which were comparable to our study results (Table 1). Plant extracts were more safely applied to kill the fall armyworms (Tables 2&3). There were many local people engaged in traditionally healing humans, and animals from our informants; and in rare cases the plants (Tables 1&4). There is growing evidence that less toxic natural pesticides can facilitate natural pest regulation whilst not significantly sacrificing crop yield [Phambala, K. *et al*., 2020]; we can deduce from this study results (Tables 2 &3) that the infestation of common food crops can be very notably resolved using extracts of local bioactive plants.

Melia azedarach L. (Family: Meliaceae, Chinaberry, cape lilac or pride-of-India) is a deciduous tree plant whose fruits and leaves are poisonous to humans and some other mammals [Enloe, S.F. *et al*., 2018; Sharma, D. *et al*., 2013]. Compounds in the M. azedarach fruits, and leaves were also known to result in insecticidal effects to fruit fly (D. melanogaster) reaching to 90% mortality [Campos, E.V.R. *et al*., 2018]. This same plant extracts containing compounds as flavonoids; catechin and kaempherols were effective insecticides to S. frugiperda reaching to 87% larval mortality (Tables 2 and 3) according to our study results. The effect of Melia azedarach on S. frugiperda was comparable to most of the reports in different literatures except the pest to which we applied in this study [Campos, E.V.R. *et al*., 2018; Chiffelle, G.I. *et al*., 2009; 13; Rioba, N.B. *et al*., 2020].

Several local plants can be suggested as sources of pesticides against insect pests by interviewing more local traditional healers; two of these (Agarista salicifolia, and Melia azedarach) were found to be most active against the fall armyworm larvae with mortality rates 95% and 87% respectively. These botanicals can be suggested as additional potential solutions for the severely damaging economically important insect pest, Spodeptera frugiperda.

Secondary metabolites responsible for pest feeding deterrents, antifeedants, or biotoxicities such as phenolic acids, coumarins, xanthones, flavonoids, anthocyanins, tannins and lignans found in the bioactive local plants [Marianna, P.D. *et al*., 2013]. The plant extracts' chemicals were safer, and less risky than the synthetic hazardous chemicals when used as insecticides, rodenticide, or ayurvedic medicines [Sharma, D. *et al*., 2013]. The use of less risky and safer phytochemicals was suggested to maximize food productivity for rapidly growing human population [Campos, E.V.R. *et al*., 2018]. In a nutshell the use of the plants' extracts to control the prevailing damages due to insect pests such as the fall armyworms is ideal solution for the economic losses , and the associat-ed risks due to use of synthetic hazardous chemicals.

Conclusions

Two of the six identified bioactive plant species (i.e *Agarista salicifolia*, and *Melia azedarach)* were found to have very good effects on fall armyworms reared in the Laboratory. We conclude from this research results that the bioactive local plants such as *Agarista salicifolia*, and *Melia azedarach* can provide ecofriendly, sustainable, and safer pest controlling option of the invasive pest, the fall armyworm. The plants can also be adapted to control other similar insect pests devastating food crops that result in notable food insecurities. The plants can be sustainably supplied as they are trees in their growth habit and versatile in existence in the tropical climate of Ethiopia. We further recommend additional researches to confirm the chemical compositions in the plant extracts' that resulted in killing the pest larvae as we conducted on crude extracts.

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